UNIVERSITY OF OVIEDO

DEPARTMENT OF CHEMICAL AND ENVIRONMENTAL ENGINEERING

HYDROTHERMAL TREATMENT OF SEWAGE SLUDGE UNDER OXIDATIVE AND NON-OXIDATIVE CONDITIONS

DOCTORAL THESIS

PROGRAM IN CHEMICAL, ENVIRONMENTAL AND BIO-FOOD ENGINEERING

BY

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RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Tratamiento hidrotérmico de lodos de depuradora bajo condiciones oxidativas y no-oxidativas.	Inglés: Hydrothermal treatment of sewage sludge under oxidative and non-oxidative conditions.

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RESUMEN (en español)

En esta tesis doctoral se ha estudiado la aplicación de las técnicas de tratamiento hidrotérmicas de tipo oxidativa (oxidación húmeda) y no-oxidativa (hidrólisis térmica) para reducir el volumen de lodos activados. El propósito de este trabajo consistió en analizar el efecto de estos tratamientos sobre las propiedades químicas, biológicas y reológicas del lodo. Además, la oxidación húmeda fue empleada para tratar tanto el lodo crudo, como sus componentes estructurales con el fin de investigar en mayor profundidad las reacciones envueltas en este proceso.

Preliminarmente, se llevó a cabo una revisión bibliográfica en la cual se encontró que existen numerosos datos sobre productos de valor industrial formados durante los tratamientos hidrotérmicos del lodo, pero que se encontraban muy dispersos en la amplia literatura del tema. Por lo tanto, se preparó un documento tipo review, en el cual se abordó esa temática, y que a su vez sirve como parte complementaria a la introducción de la tesis.

Posteriormente, se analizó el efecto de las variables de operación de ambos tratamientos sobre las propiedades físicas, químicas y reológicas de los lodos. Se establecieron ecuaciones empíricas para modelar los efectos de la solubilización de sólidos sobre las propiedades reológicas del lodo cuando fue aplicada la hidrólisis térmica. Asimismo, se propuso un mecanismo de reacción para la oxidación húmeda del lodo, el cual incluyó dos etapas en serie: primero la solubilización del material orgánico sólido y segundo la oxidación del material solubilizado.

El siguiente paso fue comparar y analizar las diferencias entre ambas técnicas hidrotérmicas aplicadas al tratamiento del lodo. Los resultados mostraron que la oxidación húmeda representa una técnica más efectiva para el tratamiento de los lodos, aportando mejores resultados en términos de solubilización, sedimentabilidad y mineralización.

La parte restante de la investigación se centró en estudiar en más detalle el tratamiento por oxidación húmeda del lodo. Para tal fin, los lodos fueron separados en sus componentes estructurales correspondientes a: productos microbianos solubles, sustancias poliméricas extracelulares débilmente ligadas, sustancias poliméricas extracelulares fuertemente ligadas y células. Cada fracción por separado fue sometida a tratamiento por oxidación húmeda y los resultados se compararon con los obtenidos del lodo crudo. De esta forma, se identificó que la oxidabilidad de los biopolímeros que componen el lodo, es dependiente de la localización de estos en la estructura flocular. Por lo tanto, mayor refractariedad a la oxidación fue observada en las fracciones localizadas más externamente de las células. Además, la distribución del tamaño molecular de los biopolímeros durante la oxidación húmeda, mostró que las moléculas de mayor tamaño fueron degradadas tanto por reacciones de hidrólisis térmica como de

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oxidación, obteniéndose al final del tratamiento un efluente compuesto principalmente de polímeros de bajo peso molecular (<35 kDa).

En la etapa final se analizó el efecto de la oxidación húmeda sobre los principales biopolímeros del lodo, llevando a cabo este estudio para cada componente estructural del lodo. A partir de estos resultados fue propuesto y validado un modelo cinético que incluye los efectos de la concentración de oxígeno y de la temperatura del proceso, sobre las velocidades de solubilización de los sólidos volátiles, así como en las velocidades de formación y de reacción de los principales biopolímeros solubilizados.

Adicionalmente, se analizó el efecto causado por la oxidación húmeda sobre las propiedades físico-químicas y la composición de tres muestras de lixiviados de vertedero (joven, maduro y estabilizado biológicamente). La identificación de los compuestos orgánicos presentes tanto en las muestras iniciales como en las tratadas por oxidación húmeda se realizó por medio de la técnica de Cromatografía Líquida-Espectrometría de Masas.

RESUMEN (en Inglés)

In this doctoral thesis, the application of hydrothermal treatments both oxidative (wet oxidation) and non-oxidative (thermal hydrolysis) to stabilize activated sludge has been studied. Specifically, the purpose of this work was to analyse the effect of these treatments on the chemical, biological and rheological properties of sludge, in order to investigate in more depth the reactions involved in this process.

As a preliminary part of this study, an extensive literature review of the research works related to the topic was carried out. Consequently, it was observed that there are numerous published data on valuable industrial products formed during the hydrothermal treatments of sludge, but those were too scattered in the wide literature about the subject. Therefore, a review article dealing with this topic was prepared, which in turn will serve as a complementary part to the introduction section.

Afterwards, the effect of the operating variables for both treatments on the physical, chemical and rheological properties from sludge was analysed. Empirical equations to model the effects of solubilisation of solids by thermal hydrolysis on the rheological behaviour of the sludge were stablished. Likewise, a reaction mechanism was proposed for sludge wet oxidation, which included two stages in series: firstly, the solubilisation of the solid organic matter; secondly, the oxidation of the solubilised material.

The next step was to compare and analyse the differences between both hydrothermal techniques applied to the sludge treatment. The results showed that wet oxidation represents a more effective technique to treat sewage sludge, providing better results in terms of solubilisation, settleability and mineralization than thermal hydrolysis.

According to the above mentioned, the following part of the research was focused on studying in more detail the treatment of the sludge by wet oxidation. For that purpose, the sludge was separated into its structural components, which correspond to: soluble microbial products, loosely bound extracellular polymeric substances, tightly bound extracellular polymeric substances and naked cells. Each fraction was subjected separately to treatment by wet oxidation and the results were compared with those obtained from the treatment of the raw sludge. In this way, it was identified that the oxidability of the biopolymers that make up the sludge depends on the location of these in the floc structure. Therefore, greater refractoriness to the oxidation was observed on those fractions located more externally to the cells. Moreover, the analysis of the molecular size distribution of the biopolymers during wet oxidation showed that the larger molecules were degraded by thermal hydrolysis and oxidation reactions, obtaining an effluent composed mainly of low molecular weight polymers (<35 kDa) at the end

of the treatment.

In the final stage of the research, the effect of wet oxidation on the main biopolymers of the sludge was analysed, carrying out that study for each structural component of the sludge. From these results, a kinetic model that includes the effects of oxygen concentration and temperature of the process, as well as the rates of formation and reaction of the main solubilised biopolymers was proposed and validated.

In addition, a study addressed to analyse the effect of wet oxidation on physicochemical characteristics and composition of different landfill leachates (young, old and biologically stabilised) was carried out. Liquid Chromatography Mass Spectrometry was used to identify the organic compounds present in the leachates and their transformations during the wet oxidation treatment.

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TRATAMIENTO HIDROTÉRMICO DE LODOS DE DEPURADORA BAJO CONDICIONES OXIDATIVAS Y NO-OXIDATIVAS

TESIS DOCTORAL

PROGRAMA EN INGENIERÍA QUÍMICA, AMBIENTAL Y BIOALIMENTARIA

POR

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SUMMARY

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La parte restante de la investigación se centró en estudiar en más detalle el tratamiento por oxidación húmeda del lodo. Para tal fin, los lodos fueron separados en sus componentes estructurales correspondientes a: productos microbianos solubles, sustancias poliméricas extracelulares débilmente ligadas, sustancias poliméricas extracelulares fuertemente ligadas y células. Cada fracción por separado fue sometida a

tratamiento por oxidación húmeda y los resultados se compararon con los obtenidos del lodo crudo. De esta forma, se identificó que la oxidabilidad de los biopolímeros que componen el lodo, es dependiente de la localización de estos en la estructura flocular. Por lo tanto, mayor refractariedad a la oxidación fue observada en las fracciones localizadas más externamente de las células. Además, la distribución del tamaño molecular de los biopolímeros durante la oxidación húmeda, mostró que las moléculas de mayor tamaño fueron degradadas tanto por reacciones de hidrólisis térmica como de oxidación, obteniéndose al final del tratamiento un efluente compuesto principalmente de polímeros de bajo peso molecular (<35 kDa).

En la etapa final se analizó el efecto de la oxidación húmeda sobre los principales biopolímeros del lodo, llevando a cabo este estudio para cada componente estructural del lodo. A partir de estos resultados fue propuesto y validado un modelo cinético que incluye los efectos de la concentración de oxígeno y de la temperatura del proceso, sobre las velocidades de solubilización de los sólidos volátiles, así como en las velocidades de formación y de reacción de los principales biopolímeros solubilizados.

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1. OBJECTIVES

The general aim of this thesis was focused on studying and analysing the effect of hydrothermal treatment techniques (thermal hydrolysis and wet oxidation) on the properties of sewage sludge. In addition, differences on the characteristics of sludge treated under inert or oxidising conditions were established. In basis to the results of treatment achieved, the best technique was selected to carry out a study in greater depth about its effect on the structural components of sludge. For the development of this work, the following specific objectives were proposed:

- To analyse the effect of operating conditions (temperature, pressure and reaction time) of thermal hydrolysis and wet oxidation on physical, chemical and rheological properties of the sludge.
- To establish a reaction mechanism and propose a kinetic model for sludge wet oxidation.
- To study and compare the effect of an inert or oxidising atmosphere on solubilisation, mineralisation and settleability of sludge, as well as on the composition and molecular weight sizes of the biopolymers solubilised during the treatment.
- To choose the more effective hydrothermal treatment in order to investigate and analyse in more detail the changes in both the structural components and the main species of polymeric substances from sludge during the treatment.

2. INTRODUCTION

2.1 ORIGIN AND CURRENT PANORAMA ON THE PRODUCTION OF BIOLOGICAL SLUDGE

Treatment biological systems are a method applied commonly for treating wastewater with biodegradable characteristics. Success of these systems is mainly due to its simplicity and low operation costs when it is compared to other physical, chemical or physicochemical processes.

Removal and stabilization of organic matter in wastewater by biological processes are achieved by the action of a variety of microorganisms, mainly bacteria. These microorganisms use biodegradable compounds as a substrate to carry out their metabolism and to synthesize new cellular material, the latter causing an increase in sludge volume in the system (Metcalf & Eddy Inc. et al. 2003).

The biological treatment system more commonly applied at industrial scale is activated sludge, which basically consist of an aerobic treatment process by a suspended culture of microorganisms (Hreiz et al. 2015). In this work, activated sludge was used as study material during all the research since this is the most generated sludge by wastewater treatment plants (WWTPs). The typical characteristics reported in the literature for activated sludge are presented in Table 2.1.

Table 2.1. Typical characteristics of activated sludge. (Metcalf & Eddy Inc. et al. 2003).

Characteristics	Range
Total dry solids (TS), %	0.83-1.16
Volatile solids (% of TS)	59-88
Proteins (% of TS)	32-41
Nitrogen (N, % of TS)	2.4-5.0
Phosphorus (P_2O_5, % of TS)	2.8-11.0
pH	6.5-8.0

Management of this sludge has been and continues to be one of the most complex and costly problems in WWTPs, representing between 50-60% of total cost of operation (Yang et al. 2011). The difficult management of sludge is caused by the high volume produced, as well as its difficult dewaterability.

In the last decades, the construction of new WWTPs due to the application of the Urban Waste Water Treatment Directive 91/271/EEC has led to a considerable increase in the sludge production in the European countries (Pérez-Elvira et al. 2006).

According to the published data by the Eurostat, the sludge production total for the year 2010 in the European Union (EU) was about 9.2 million tons, this being the last year in which were collected the data for almost all the state members. As it can be seen in the Figure 2.1, the largest sludge producers were Germany, United Kingdom, Spain, Italy and France, achieving the 72% of the total produced by the EU. Moreover, the collection of data for the state members has not been consistent from the year 2010, especially for the largest sludge producers. Germany is the only large producer of which there are annual data until the year 2015. In fact, the sludge production total for the year 2015 in the EU is only 3.5 million tons according to the existent data (collected mainly for smaller producers), in contrast to the 9.2 million tons corresponding for the year 2010.

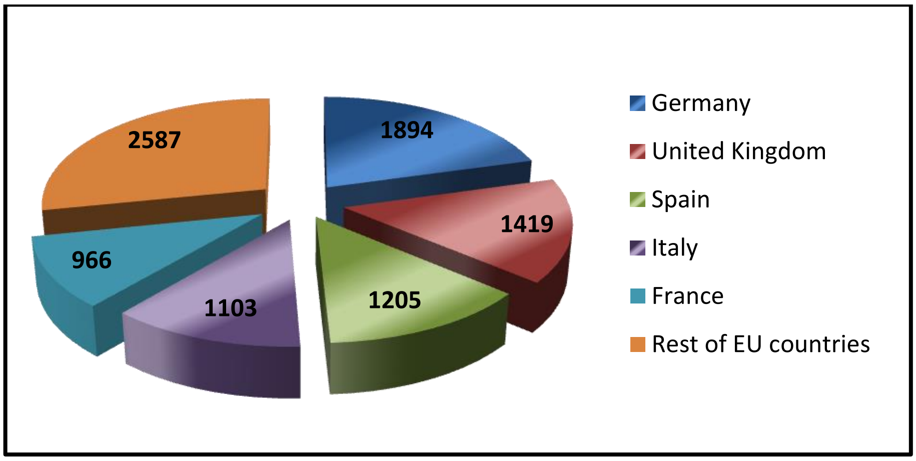


Figure 2.1. Distribution of the sludge production (thousand tons) in the states of the European Union for the year 2010. (Eurostat 2018).

Regarding to the sludge production in Spain, the most recent data published show that the amount of sludge generated has followed a tendency to rise since the year 2001, reaching an increase of 35% for the year 2009 (Figure 2.2). However, the amount of sludge produced decreased slightly in the following years, remaining in more stable values.

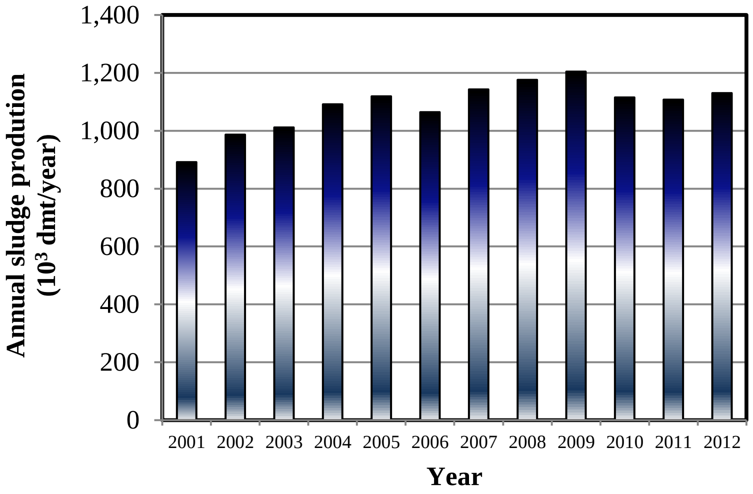


Figure 2.2. Sludge production generated in Spain in the last years (MAPAMA 2018).

This marked increase in the sludge production from 2001 in Spain was the result of taking measures derived from compliance with the Directive 91/271/CEE of the European Union. This fact led to the construction of new WWTPs in accordance with the National Sanitation and Treatment Plan corresponding to the period between 1995 and 2005. In this National Plan, it was established the wastewater treatment of all urban areas with more of 2000 equivalent habitants as a target to achieve. Subsequently, the National Water Quality Plan: Sanitation and Treatment 2007-2015 was created in Spain in order to respond to both to the objectives not reached during the previous plan, and to the new necessities established by the Water Framework Directive and by the AGUA program (MAPAMA 2018).

According to the data reported by DBK, a Spanish company specialized in the elaboration of sectoral analysis and competition studies, there were approximately 2350 WWTPs operating in Spain for the year 2004. In the year 2007, that number was increased to 2500, reaching a total of 2940 at the end of 2015. Therefore, it is evident that while the number of households discharging in WWTPs continues increasing, the forecast for the coming years is an upward trend in sludge production.

Some methods that have been commonly used for sludge removal, such as incineration, disposal on land as fertilizer and evacuation in landfill, are currently being more controlled. This fact is due to pollution problems that these methods can cause on air, ground and groundwater (Guo et al. 2013).

In basis to the last data reported by the National Register of Sludge, 1.13 million of dry matter tons (dmt) of sewage sludge were generated in Spain in the year 2012. As can be observed in the Figure 2.3, the most part of the sludge produced during that year was destined for agricultural use, while less than 20% was removed by landfilling, incineration and other alternatives.

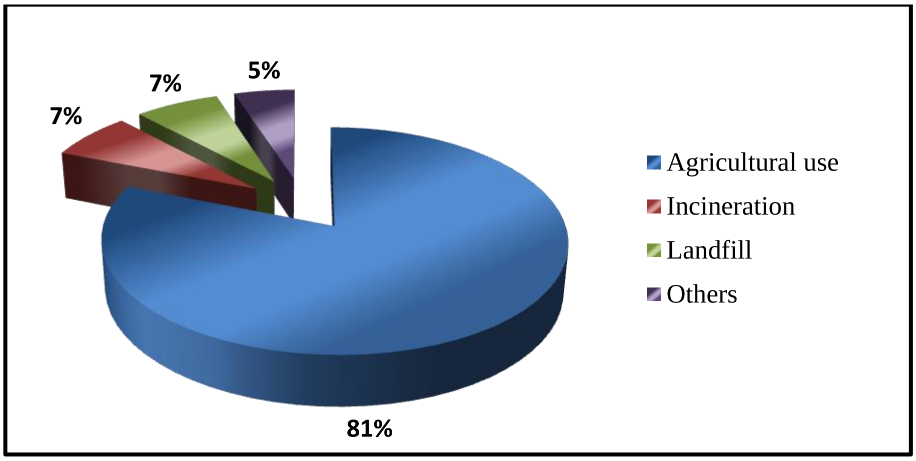


Figure 2.3. Final destination of the sludge produced in Spain in the year 2012 (MAPAMA 2018).

Nevertheless, the direct use of sludge as fertilizer leads to a series of contaminating risks, which makes it necessary to strictly control the composition of sludge and its physical, chemical and biological characteristics previously to its application on ground. This fact as well as the application of more restrictive standards has made it necessary to search for new alternatives of treatment or to improve the existing technologies, in order to reduce the volume of sludge, to reach its stabilization and to improve its manageability without compromising the health of the population or contaminating natural resources.

2.2 STRUCTURE AND COMPOSITION OF BIOLOGICAL SLUDGE

Biological sludge is a complex mixture formed by an ecosystem of microorganisms surrounded by extracellular polymeric substances (EPS) and divalent cations. Amongst the microorganisms that make up the sludge, bacteria play a fundamental role at biological treatment (Mesquita et al. 2013). In the Figure 2.4, it is shown an image taken through a scanning electron microscope, in which the presence and morphology of some cellular organisms from sludge can be observed.

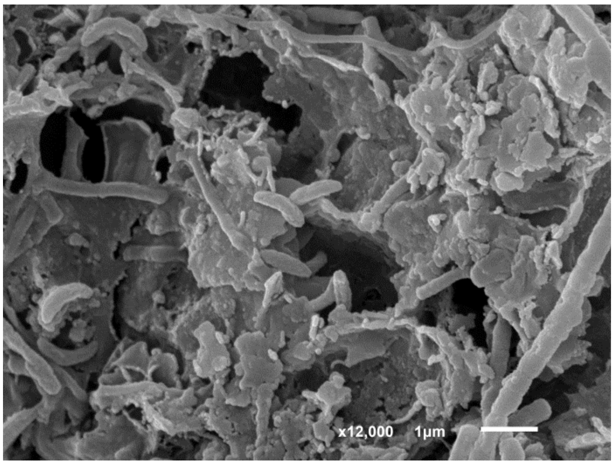


Figure 2.4. Morphology of an activated sludge floc.

EPS have great relevance on the conformation of microbial aggregates and represent the main organic fraction that determines the structure, integrity and strength of the floc (Eskicioglu et al. 2006). Furthermore, these substances have been identified as responsible for the difficult dewaterability of sludge. According to Neyens et al. 2004, EPS can reach up to 80% of the overall mass of activated sludge, when they are considered together with the water retained within its structure. For that reason, some research works have been focused on studying the effects of different treatment techniques of sludge on the EPS fractions specifically.

2.2.1 EPS. Composition and classification

EPS are formed by polymers of high molecular weight that come from bacteria metabolism, cell lysis and compounds present in wastewater (Frølund et al. 1996, Sheng et al. 2013). Their composition includes a variety of organic substances, mainly proteins, humic acids and carbohydrates. Likewise, other type of substances such as uronic acids and DNA are present, although at lower quantities (Zuriaga-Agustí et al. 2013).

The functions of EPS in microbial aggregates are numerous. On the one hand, they are responsible for keeping the organisms gather, forming a highly hydrated matrix by means of weak physical-chemical interactions, thus preventing their desiccation and controlling the movement of ions in the solution (Neyens and Baeyens 2003, Simon et al. 2009). On the other hand, EPS form a protective layer for cells against the harsh conditions of the external environment and even serve as a reserve of carbon and energy for cells during starvation stages (Liu and Fang 2002).

Based on their physical state, EPS have been classified into soluble microbial products (SMP) and bound EPS. SMP are defined as the group of organic compounds that are dissolved into solution, which are originated from the metabolism of substrate and the decomposition of biomass (Kunacheva and Stuckey 2014). With regard to bound EPS, these are in turn classified into two fractions, depending on their degree of bounding to cells. Loosely bound EPS (LB-EPS) are those that form a diffuse layer of slime, which is loose and disperse, surrounded the cells, whilst tightly bound EPS (TB-EPS) are firmly bound to the surface of the cells (Sheng et al. 2010). In the Figure 2.5, it is showed a scheme of the distribution of the EPS fractions in microbial aggregates.

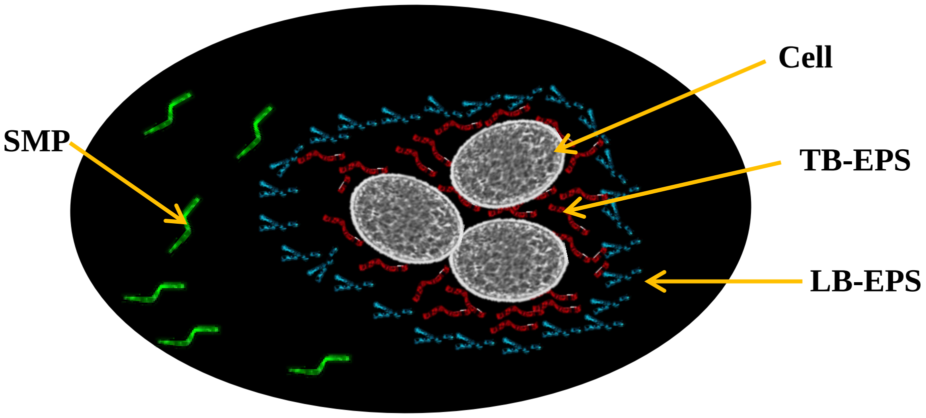


Figure 2.5. Distribution of EPS in microbial aggregates.

2.3 TREATMENT METHODS OF SEWAGE SLUDGE

Currently, there is a wide variety of treatment techniques that have been applied to sludge in order to reduce its volume and facilitate its subsequent management. These techniques can be used as methods of pretreatment to improve the characteristics of dewaterability and/or digestibility of sludge, or as final methods of treatment before sludge disposal. Moreover, these technologies have been studied under different configurations, thus the available information in the bibliography regarding to those is very extensive. For that reason, only a brief description of the most known and used techniques for the sludge treatment is presented in this section. A more detailed list of treatment techniques has been included in Table 2.2.

Table 2.2. Treatment and pretreatment methods to reduce and/or remove sewage sludge (Fytili and Zabaniotou 2008, Tyagi and Lo 2011).

Method type	Treatment methods
1. Biological	1.1. Anaerobic digestion 1.2. Aerobic digestion 1.3. Enzymatic lysis
2. Mechanical	2.1. High pressure homogenizer 2.2. Ultrasonication 2.3. Electric pulse power method 2.4. Gamma irradiation 2.5. Stirred ball mills 2.6. Lysate-thickening centrifugal technique 2.7. Jetting and colliding method 2.8. Rapid non-equilibrium decompression 2.9. ‘‘Bug Buster’’ technology High speed rotary disc process 2.10. Electron beam pretreatment
3. Chemical	3.1. Acidic and alkaline 3.2. Ozonation. 3.3. Peroxidation 3.4. Fenton 3.5. Peracetic acid
4. Thermal	2.1. Combustion 2.2. Co-combustion 2.3. Pyrolysis 2.4. Gasification 2.5. MW irradiation 2.6. Hydrothermal treatment 2.6.1. Thermal hydrolysis 2.6.2. Wet oxidation
5. Hybrid methods	5.1. Chemically enhanced thermal process 5.1.1. Conventional heating-Alkali/acid treatment 5.1.2. Microwave-alkali treatment 5.1.3. Microwave enhanced advanced hydrogen peroxide oxidation process 5.2. Mechanical-chemical methods 5.2.1. Alkali-ultrasonic treatment 5.2.2. Ultrasonication-ozonation 5.2.3. Alkali-high pressure homogenizer 5.3. Thermo-mechanical 5.3.1. Combination of thermal, explosive decompression and shear forces 5.4. Chemical-chemical 5.4.1. Alkali-hydrogen peroxide

2.3.1 Biological treatments

The conditioning and treatment of sludge by biological processes is carried out through aerobic and anaerobic digesters. Treatment by anaerobic digestion is the process most widely used for sludge stabilization. Its ability to remove odours and pathogens, to stabilize organic matter and to produce renewable energy in form of methane has made it still remains as a basic process in the line of sludge treatment in a large number of WWTPs. In the case of aerobic digestion, this process presents as advantage the use of hydraulic retention time (HRT) lower than those of anaerobic digestion, achieving similar results in the reduction of solids. Nevertheless, the energy cost associated to the oxygen supply has led to its use is limited to small WWTPs (Metcalf & Eddy Inc. et al. 2003).

It is well known that the prolonged hydrolysis step of sludge, especially under anaerobic conditions, leads to long HRT, which in turn affects to the size of the digester. Therefore, the anaerobic digestion is being coupled to different pretreatment techniques in order to accelerate the hydrolysis of the sludge and improve the performance of the process (Zhen et al. 2017). There is a great variety of pretreatment techniques applied to solubilise the sludge for its subsequent digestion. These include mechanical, thermal, enzymatic, ultrasonication and chemical pretreatments, the latter includes in turn both oxidation and acid or alkaline hydrolysis (Appels et al. 2008).

These techniques have been object of study in several works. For instance, Salsabil et al. 2010 analysed the effect of different pretreatments on the anaerobic or aerobic digestion of a sludge. They determined that the pretreatment by ultrasonication favoured to a major sludge removal during its subsequent digestion (61 and 54% under aerobic and anaerobic conditions, respectively). For thermal treatment, the sludge removal was higher when the temperature was increased, whilst at 90 ºC it achieved values of sludge removal (20%) similar to those obtained with ozone fed on a ratio of 0.1 g O_3 gTS^{-1}. Nah et al. 2000 reported a HRT reduction from 13 to 6 days during the anaerobic digestion of a pretreated sludge by mechanical injection (30 bars). Likewise, Knezevic et al. 1995 determined that after a sludge was pretreated by alkaline method, the HRT during its anaerobic digestion decreased from 25 to 10 days. However, it was not observed major effects of this treatment on the methane yield or the quality of the effluent.

2.3.2 Mechanical treatments

Mechanical methods of sludge disintegration involve the action of externally applied stress or pressure on the cells. Cells are disrupted when the external pressure exceeds the internal cell pressure (Tyagi and Lo 2011). The most widely studied mechanical method has been ultrasonication. The main effect of ultrasonic pretreatment is particle size reduction at low frequency (20–40 kHz) sound waves. Furthermore, high-frequency sound waves also cause the formation of radicals, which produce the oxidation of organic material (Ariunbaatar et al. 2014). The duration of irradiation is the key factor to consider in this process. A short length of exposure can only break the microbial flocs, without destroying the cell wall, whereas a longer duration can completely destroy the bacterial cells (Anjum et al. 2016). Bougrier et al. 2006 determined that sonication led to a decrease in particles size, in apparent viscosity and in filterability of sludge, as well as, an enhancement of biogas production during its anaerobic digestion.

On the other hand, high pressure homogenization is another well-known mechanical method used for cell disruption. Recently, this technique has been employed as a sludge pretreatment method, which changes both the rate and extent of sewage sludge degradation in anaerobic digestion processes. The disruption of microbial cell walls and the release of cytoplasm into the liquid phase are achieved by the effect of pressure gradient, cavitation, turbulence, impingement, shear stresses, and extensional shear (Zhang et al. 2012). Fang et al. 2015 studied the effect of high pressure homogenization treatment on the physicochemical properties of a secondary sludge. They found that the disintegration degree and soluble chemical oxygen demand increased with the increment of homogeneous pressure and cycle, showing effective organic release from sludge solid to liquid after treatment.

2.3.3. Chemical treatments

The chemical treatment methods most commonly used to reduce the sludge volume are ozonation, peroxidation and Fenton. These treatments of oxidative type, destroy the cell membranes of the microorganisms, releasing their cytoplasmic content, and consequently, improving the sludge dewaterability. Furthermore, the oxidation degree of the organic matter depends on the amount of oxidant fed (Zhen et al. 2017).

The Fenton process is a technique that has been intensively applied to improve the dewaterability of sludge. It consists on the reaction of hydrogen peroxide with iron ions (Fe^{2+}), which act as catalysts for producing hydroxyl radicals ($OH^{\bullet}$). The hydroxyl radicals have an oxidation potential of +2.80 (in acid conditions), being much higher than that for hydrogen peroxide (+1.36) and ozone (+2.07) (Zhen et al. 2017).

Therefore, the Fenton reaction is more effective when the oxidation of refractory pollutants is required.

Erden and Filibeli 2011 studied the effects of a Fenton pretreatment on the properties of an activated sludge. They determined that this method was effective to enhance the biodegradability and the filterability of the sludge. Furthermore, the production of methane increased 19.4% when doses of 4 g Fe^{2+}/kg total solids (TS) and 60 g H_2O_2/kg TS were applied. Likewise, Déléris et al. 2000 studied the potential of the ozonation on an activated sludge (0.2-2.0 g suspended solids), employing high doses of ozone (>10 g O_3/g VSS). It was reported that between 40 and 90% of the organic carbon, depending on the ozone dose, was mineralized.

Moreover, an ozonation process modified by introducing hydrogen peroxide was tested in a sewage sludge by Yin et al. 2008. The treatment caused a reduction in the volume and water content of the sludge, as well as an improvement of its settleability. However, the CST (capillary suction time) increased from 17.2s to 120.4s, which meant that the sludge filterability was deteriorated.

Abelleira et al. 2012 proposed a variant of the peroxidation through a thermo-chemical process in which thermal hydrolysis was combined to peroxidation to treat a secondary sludge with a content of total solids of 50 g/L. The treatment at 138 ºC and a 0.9 ratio of oxidant with respect to the stoichiometric oxygen caused a reduction of 50-60 of organic matter from sludge.

2.3.4 Thermal treatments

Thermal treatments can be categorized in three groups: incineration, co-combustion and alternative processes such as pyrolysis, gasification and wet oxidation (Fytili and Zabaniotou 2008). Incineration is one of the most used methods for sludge disposal. This technique is based on the complete combustion of the sludge, producing in turn energy from the heat of the gases generated. On the other hand, co-combustion has emerged as an alternative method to incineration. In this process, the sludge mixed in low proportions is burned with other combustible materials such as solid wastes, coal, etc. (Lin and Ma 2012).

Pyrolysis is defined as the thermal decomposition of sludge in absence of oxygen and under a range of temperatures that goes from 300 to 900 ºC. The obtained products are;

pyrolysis gas, char (a carbon-rich solid) and an oil composed by organic acids. In contrast, the thermal decomposition by gasification is carried out in presence of oxygen, although in conditions of deficit to reach only a partial oxidation. The obtained products are mainly a synthesis gas that can be utilized to produce fuel or energy, and a small quantity of char (Libra et al. 2011, Samolada and Zabaniotou 2014).

The thermal reduction of the sludge has the ability of removing large volume of this, achieving in turn the destruction of pathogens and toxic organic compounds (Li et al. 2014). In addition, it is possible recover energy from the process. In the case of the incineration, the derived problems from the generation of pollutant gases and the management of ash, the latter considered as a hazardous waste, have made its use can be complicated and expensive. In spite of this, the incineration can be considered an attractive option for urban areas where large volumes of sludge are generated and which, in turn, do not have conditioned zones for their disposition. Furthermore, technological advances have significantly improved this process, contributing to a major control on its gas emissions and reducing its operating costs (Werther and Ogada 1999).

An example of the above mentioned is the work performed by Li et al. 2014, in which an integrated drying and incineration technique was proposed and tested with a sludge with a moisture content of about 80%. This system combines a bubbling fluidized bed dryer with a circulating fluidized bed incinerator. The advantage of this design is that the dried sludge is transported directly to the incinerator, eliminating any period of storage. According to the authors, this system allows a highly efficiency combustion with low pollutant emissions, in addition to low investment and operating costs. Moreover, Murakami et al. 2009 proposed a new type of incinerator that combines a pressurized fluidized bed combustor and a turbocharger driven by flue gas. This design contributed to reduce at 50% the emissions of CO, NOx, and N_2O of the combustion gases with respect to a conventional plant. Furthermore, the data showed that this system could reach energy saves of up to 50%. Otero et al. 2002 studied the behaviour of the co-combustion of sludge with coal. They determined that using mixtures of sludge and coal, the combustion starts at lower temperatures than that for only coal. This performance associated with the releasing of volatile compounds from sludge, could represent a great advantage to initiate the combustion in the process.

2.3.5. Hydrothermal treatments

The hydrothermal technologies are well established treatment methods that are used to improve the dewaterability characteristics of the sludge and to reduce its volume to disposal. These technologies, which have been classified into oxidative (wet oxidation) and non-oxidative (thermal hydrolysis) techniques, involve high temperature and pressure conditions. A brief description of the most relevant aspects with regarding the theoretical fundaments of both processes is performed below. A more detailed bibliographical revision of the research works involving these technologies is collected in the article “Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review” located in the section 4.1.

2.3.5.1 Thermal hydrolysis

Thermal hydrolysis is a technique that has been implemented as a pretreatment for sludge before being introduced into an anaerobic digester. Thermal hydrolysis has as a goal to increase the solubilisation of organic matter from sludge. This is achieved by the partial or total destruction of the cellular structures from bacteria caused by the heating effect. In this way, the rate of sludge digestion is improved because its solubilised components are more easily assimilate by the microorganisms (Hii et al. 2014). Consequently, the anaerobic digestion of the hydrolysed sludge could produce an increase in the production of biogas of up to 40%, in approximately half of the residence time of a conventional anaerobic digestion (Pérez-Elvira et al. 2008). In addition, thermal hydrolysis improves the fluidity of the sludge, thereby encouraging its subsequent handling in operations of pumping and transport (Urrea et al. 2015).

On the other hand, thermal hydrolysis can be used to treat any type of sludge; however, the best results are achieved when operating with secondary sludge (Abelleira et al. 2012). The operating conditions generally include temperatures that are between 160 and 180 ºC, pressures below 13 bar and residence times from 30 to 60 min (Laurent et al. 2011). The temperature is considered the most important factor of the process, since it has the major influence on the degree of disintegration of the sludge. Most studies have demonstrated an optimum range of 160-180 ºC for the thermal hydrolysis of the sludge, being obtained, to those conditions, better yields of methane during its subsequent anaerobic digestion. The use of higher treatment temperatures has been associated with the formation of biorefractary compounds, which affect negatively the yield of the production of biogas (Dwyer et al. 2008).

Commercial thermal hydrolysis processes

Currently, there are available several commercial processes in which thermal hydrolysis is used as sludge pretreatment, such as Cambi®, Exelys® and Biothelys®, LysoTherm®, Haarslev's Continuous Hydrolysis System (HCHS), Turbotec®, Aqualysis® and TH_4^+. These technologies present differences in terms of operation (batch or continuous), reactor configuration, operation conditions (temperature, pressure and residence time) and energy integration scheme (Lema and Suarez 2017). A comparison of the general operating conditions for the thermal hydrolysis processes is summarized in Table 2.3, and a brief description of the most commercialised processes is presented subsequently.

Table 2.3. Typical operating conditions and performance reported for the commercial thermal hydrolysis processes (Lema and Suarez 2017).

Company	Cambi	Veolia		Lysotherm	Haarslev	Sustec	Aqualogy	$teCH_4$
Technique	THP	Exelys	Biothelys	SH + E	HCHS	Turbotec	Aqualysis	tH_4^+
Operation	Batch	Cont.	Batch	Cont.	Cont.	Batch/ Cont.	Cont.	Cont.
% TS in feeding	12-16	>20	10-15	6-8	17-22	10	8-12	6-15
T (ºC)	160-180	150-170	150-170	175	150-170	140-190	165-180	220
P (bar)	6-9	7-9	7-9	5-15	7-8	4-8	7-10	12-14
T (min)	20-30	30-60	30-60	30-60	20	30-75	15-30	<5

N.A.: not available

Cambi®

The Cambi® technique is the most widely thermal hydrolysis process used at present. Sludge is pretreated at temperatures between 150 and 180 ºC under a pressure of 4.5 bar and a residence time of 30 min. Firstly, the sludge is dewatered to 17% dry solids before it is heated using recycled steam. Afterwards, the treated sludge is flashed into a flash tank once the reaction has finished (Hii et al. 2014). The process improves the dewaterability and digestibility of the sludge, and thus enhances biogas production and contributes to reduce anaerobic digester size (Aggrey et al. 2011).

Exelys® and Biothelys®

Exelys® and Biothelys® are two thermal hydrolysis processes combined with anaerobic digestion, which have been developed by Veolia Water. The first one works in continuous mode, whereas the second one operates in batch mode (Hii et al. 2014). The thermal hydrolysis phase in Biothelys® treatment is carried out at 165 ºC and 9 bar for approximately 30 min. Moreover, the continuous thermal hydrolysis in Exelys® is performed at the same operating conditions as Biothelys®, working 24 hours a day with feed and removal levels that are adjustable in real time. Both techniques offer enhanced performance over conventional digestion of sludge by producing no odours, 25-35% less dry solids, 30-50% more biogas and a pasteurised digestate, for safe reuse (Veolia 2018).

Reaction mechanisms

The solubilisation of organic compounds by thermal hydrolysis occurs by the breakdown of bonds of macromolecules, which results in the formation of lower molecular weight compounds that are more soluble. It is known that hydrolysis reactions occur when the organic compounds react with water (Hii et al. 2014).

These reactions are characterized by the division of the molecule of water, giving way to the formation of the hydroxyl (OH^-) and hydronium (H_3O^+) ions (Equation 1).

$$2H_2O \rightarrow H_3O^+ + OH^- \qquad (1)$$

The formed ions react with the organic compounds, producing the rupture of their bonds and giving origin to other compounds of lower complexity and size (Equation 2) (Hii et al. 2014).

$$RX + H_3O^+ + OH^- \rightarrow ROH + HX + H_2O \qquad (2)$$

Where: X represents a leaving group (an atom much more electronegative than carbon)

Afterwards, the hydrolysis reactions continue to develop until the energy necessary to break the next molecular bond is larger than that available in the reaction medium, thus the hydrolysis of that compound is finished. As it is required enough energy to break the bonds of the compounds, the degree of hydrolysis of molecules and its rate depends mainly on the temperature of the process (Li and Noike 1992).

2.3.5.2. Wet oxidation

Wet oxidation is a technique that has been widely used in the treatment of effluents, especially those of industrial character, in which there are present large concentrations of biorefractary organic matter (Mishra et al. 1995). Nevertheless, this process has been adapted for the treatment of sewage sludge whose concentration is too high to be treated by a biological process, or as a less contaminant alternative to the incineration (Mucha and Zarzycki 2008). Wet oxidation has as target to improve the settleability and dewaterability from sludge, making easier its subsequent management in the WWTPs (Khan et al. 1999).

The organic compounds present in the sludge are oxidised in aqueous phase by the injection of an oxidant, oxygen or air, in a reactor designed to operate at high temperatures and pressures. The oxidation reactions that occur in the process are generally exothermic. This fact contributes to that the reactions provide part or all the heat necessary to self-sustain the high temperatures of the process (Debellefontaine and Foussard 2000). Typical operating conditions include temperatures at 125-320 ºC, pressures of 0.5-20 MPa and residence times between 15 and 120 min (Luan et al. 2017). However, wet oxidation has been classified in two categories in function to the operating conditions used. On the one hand, subcritical wet oxidation is called when the process is operated below the critical point of water (374 ºC temperature and 220 bar pressure). On the other hand, supercritical wet oxidation refers to the use of operating conditions above critical point of water (Yousefifar et al. 2017).

Initially, wet oxidation causes the lysis of cells walls of the microorganisms present in the sludge, leading to the releasing of the intracellular material. Higher temperatures and longer retention times increase the cell rupture. Furthermore, some insoluble proteins are broken down into their more soluble constituent-amino acids. Likewise, partial or complete solubilisation of non-microbial organic matter also occurs during the process (Genç et al. 2002). The basic principle of the process is to enhance the contact between oxidant and aqueous medium where the organic matter is present. High temperature conditions lead to solubilisation of pollutants by effect of hydrolysis reactions, leaving these ready for their subsequent oxidation. The liquid phase is maintained by a high pressure which also increases the concentration of dissolved oxygen, thus favouring the oxidation rate (Debellefontaine and Foussard 2000). The reaction products are inorganic salts, carbon dioxide and water when the oxidation is complete, or simple biodegradable (organic acids of low molecular weight) and non-biodegradable compounds when it is partial. In addition, wet oxidation does not produce emissions of pollutants, such as NOx, SO_2, HCl, dioxins, furans, fly ash, etc (Luck 1999).

The use of catalyst in the wet oxidation of different effluents has been addressed in several studies. The main objective of adding catalyst is to reduce the high temperatures, pressures and reaction times necessary to achieve the complete oxidation of organic compounds (Bhargava et al. 2006). Although catalytic wet oxidation provides lower energy requirements and much higher oxidation efficiencies, it must be taken into account the cost that would suppose the implementation of a subsequent operation to recover the catalyst, as well as the regeneration and replacement of this. Furthermore, for the case of the sludge, wet oxidation is applied commonly as a pretreatment technique instead of being used for its complete oxidation (Abe et al. 2011). Therefore, the use of catalyst in the wet oxidation of sludge has not been studied in much detail in research works (Bernardi et al. 2010).

Commercial wet oxidation processes

Zimpro process

The Zimpro process, developed in the 1930s by Mr. F.J. Zimmermann, was the first commercial wet oxidation process. However, it was not until the late 1950s that the installation of the first units was carried out (Kolaczkowski et al. 1999). Several plants were built in the early 1960s for the treatment of municipal sludge in order to improve its dewaterability and remove matter organic, with different results achieved in basis to changes in the operating conditions (Debellefontaine and Foussard 2000). Temperatures between 210-240 ºC are used for sludge conditioning, whilst for sludge destruction, temperatures of 250-270 ºC and pressures of 85-120 bar are applied, being fed air as oxidant (Hii et al. 2014). The Zimpro reactor is a co-current bubble column, with or without internal baffling depending on the desired mixing conditions. By 1996, approximately 200 commercial units had been installed, over half for sludge treatment (Kolaczkowski et al. 1999).

Athos process

The Athos process is one of the main wet oxidation technologies to treat sewage sludge. This technology, commercialised by Veolia (France), is able to reduce up to 98% of sludge volume and remove more than 85% of COD (Aggrey et al. 2011).The process operates at temperatures between 250-300 ºC, using air or pure oxygen as oxidant, and copper salts as catalyst. This treatment is usually used as a final sludge destruction process, in which mineral products, clean gas emissions and biodegradable liquids are produced. In addition, the reaction is exothermic and becomes energetically self-sufficient after start-up (Hii et al. 2014).

Wetox process

The main feature of this process is the design of the reactor which is a horizontal autoclave, comprising four to six compartments that essentially act as a series of continuous stirred tank reactors. The Wetox reactor typically operates at temperatures between 210 and 250 ºC, with the temperature successively increasing in each compartment due to the heat of oxidation of the waste. The operating pressure is about 40 bar and the retention time goes from 30 to 60 min (Kolaczkowski et al. 1999).

Recently, the Wetox process has been modified to be much more affordable. The variant has been developed in Victoria University of Wellington, New Zealand. The aim of this process is to produce useful chemical by-products and recover valuable minerals and metals from sludge. Sludge is pumped to a pre-treatment tank where the catalyst and some chemicals are added. Afterwards, the mixture is pumped into a reaction chamber with air under pressure, where the reaction is carried out. Heat is given off by the process, which means that the reaction is self-sustaining. The steam containing carboxylic acid is condensed and recovered. Further, the steam can also be used to generate electrical power through a steam turbine. Heat from the condensed steam is returned to pre-treat the incoming sludge. Periodically, the reactor is drained and unreacted materials and catalyst are separated, the latter being recycled back into the process. Likewise, nutrients, predominantly mineral phosphates, and metals are separated and recovered. The process is effective to reduce up to 90% of sludge volume (Wetox 2018).

Loprox process

The Loprox process (low pressure wet oxidation), patented by Bayer AG and licensed to Bertrams AG, has as main aim the biodegradability improvement of wastewaters and sludge, leaving the degradation of contaminants to a downstream biological treatment unit. The process works preferably under acidic conditions, at pressures and temperatures lower than 35 bar and 230 ºC, respectively, and residence times of between 1 to 3 hours (Debellefontaine and Foussard 2000). The oxidation reaction is catalyzed by the addition of Fe^{2+} ions and organic quinone forming substances (Kolaczkowski et al. 1999).

VerTech process

The VerTech process uses a vertical sub-surface reactor, comprising two concentric tubes (downcomer and upcomer), which descend in a shaft to a depth of 1200 to 1500 m. This design has as advantage the use of gravity to produce the high pressures required for the reaction. The depth of the shaft leads to a pressure at the bottom of the reactor of approximately 85 to 110 bar, whilst the temperature is about 280 ºC (Kolaczkowski et al. 1999). The first commercial VerTech wet oxidation plant began operation in 1994 in Apeldoorn, the Nederlands, to treat 460 thousand tons per annum of sewage sludge. Moreover, serious problems of scaling and sedimentation of solids during operation have been reported (Debellefontaine and Foussard 2000).

Kenox process

The Kenox process was developed in the 1980s by the Kenox Corporation of Canada. This technology is used to treat municipal and industrial waste water, as well as sewage sludge. The reactor is formed by two concentric shells where influent (acidified at pH around 4) and air flowing down through the inner cylinder. Subsequently, those flow upward through the space between the inner and outer cylinders. The static mixer can be impregnated onto the surface with heterogeneous catalysts in order to improve the oxidation degree of pollutants. The typical operating conditions are temperatures of 200-240 ºC, pressures of 40-50 bar and treatment time of about 40 min (Kolaczkowski et al. 1999).

Osaka Gas process

The Osaka Gas process has been tested to treat industrial wastewater, sewage sludge and, domestic and pharmaceutical wastes. The reaction is carried out adding a catalyst based on a mixture of precious and base metals on titania or titania–zirconia carriers (honeycomb or spheres) (Luck 1999). The operating conditions are established according to the composition of the waste and the required removal performance (Kolaczkowski et al. 1999).

Reaction mechanism

The removal of organic compounds by wet oxidation is known to be by means of free radical chain reactions. Free radicals in the absence of initiators are commonly formed by the reaction of oxygen with the weakest bonds of carbon and hydrogen of the oxidised organic compounds as shown in Equations 3 and 4 (R represents the functional organic group) (Collyer 1995).

$$\mathrm{RH} + \mathrm{O_2} \rightarrow \mathrm{R^\bullet} + \mathrm{HO_2^\bullet} \tag{3}$$

$$\mathrm{RH} + \mathrm{HO_2^\bullet} \rightarrow \mathrm{R^\bullet} + \mathrm{H_2O_2} \tag{4}$$

$$\mathrm{H_2O_2} + \mathrm{M} \rightarrow \mathrm{R^\bullet} + 2\mathrm{OH^\bullet} \tag{5}$$

$$\mathrm{ROO^\bullet} + \mathrm{RH} \rightarrow \mathrm{R^\bullet} + \mathrm{ROOH} \tag{6}$$

By effect of the temperature, the hydrogen peroxide obtained decomposes rapidly to hydroxyl radicals (Equation 5). The Equation 6 corresponds to a propagation step leading to organic hydroperoxides and, more generally, to oxidized species (Debellefontaine and Foussard 2000). The organic hydroperoxides formed are relatively unstable and decompose to form subsequent intermediates with lower carbon numbers. These reactions continue rapidly until the formation of carbon dioxide and low molecular weight acids, which require more severe conditions to be oxidised (Collyer 1995). Moreover, the general form of the main developed reactions during wet oxidation is collected in Equations 7-11 (Kolaczkowski et al. 1999).

$$\text{Organic matter} + \mathrm{O_2} \rightarrow \mathrm{CO_2} + \mathrm{H_2O} + \mathrm{RCOOH} \tag{7}$$

$$\text{Sulphur compounds} + \mathrm{O_2} \rightarrow \mathrm{SO_4}^{2-} \tag{8}$$

$$\text{Halogens compounds} + \mathrm{O_2} \rightarrow \mathrm{Cl^-} \text{ or } \mathrm{F^-} \text{ or } \mathrm{Br^-} + \mathrm{CO_2} + \mathrm{RCOOH} \tag{9}$$

$$\text{Phosphorous compounds} + \mathrm{O_2} \rightarrow \mathrm{PO_4}^{3-} \tag{10}$$

$$\text{Nitrogenuos compounds} + \mathrm{O_2} \rightarrow \mathrm{CO_2} + \mathrm{H_2O} + \mathrm{NH_3}\ (\text{and/or }\ \mathrm{N_2}, \mathrm{NO_3}^{2-}) \tag{11}$$

3. MATERIALS AND METHODS

3.1. SLUDGE SAMPLES

The thermal hydrolysis and wet oxidation experiments were performed with thickened activated sludge obtained from a municipal WWTP of the region (Asturias-Spain). The sludge was extracted of a unit of thickening by flotation and stored at 4 ºC until its subsequent use. As a rule, the sludge was replaced every two weeks in order to avoid any effect in the results by deterioration of this. The general characteristics of the sludge samples used in the experiments are summarized in Table 3.1.

Table 3.1. General characteristics of the activated sludge samples used in the experiments.

Parameter	Range
TSS (g/L)	21.5-32
VSS (g/L)	15.5-27
TCOD (mg O_2/L)	26000-37000
SCOD (mg O_2/L)	200-1000
Soluble TOC (mg C/L)	400
SVI (mL/g)	31-47
CST (s)	205
pH	6.5-6.9

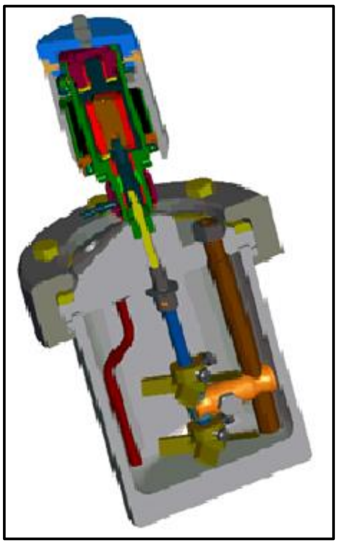
TSS (total suspended solids), VSS (volatile SS), TCOD (total chemical oxygen demand), SCOD (soluble COD), TOC (total organic carbon), SVI (sludge volume index) and CST (capillary suction time).

3.2. EXPERIMENTAL SETUP

The experimental setup for thermal hydrolysis and wet oxidation experiments was equipped by a stirred cylindrical reactor of 1 litre of capacity. A constant flow of nitrogen (thermal hydrolysis) or oxygen (wet oxidation), previously conditioned in a humidifier until a near level to the saturation point, was fed since the beginning of the experiment. The main components of the equipment are:

3.2.1. Reactor *(PARR series 4520)*

It is a cylindrical tank built in stainless steel (SS316) and of 1 litre of capacity, which is equipped with a two six-blade agitators. The geometric characteristics of the reactor are specified in the Figure 3.1.

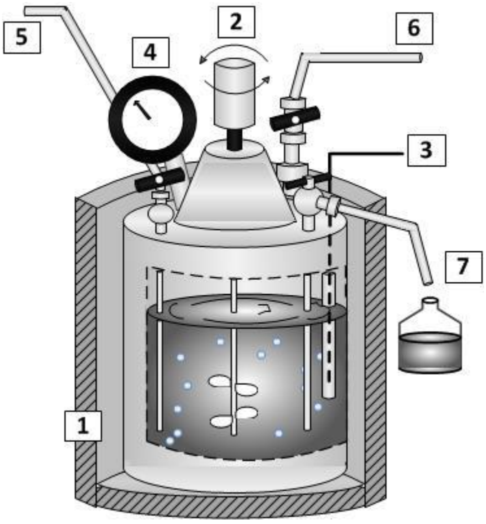


Reactor PARR series 4520

- Diameter of the tank: 0.10 m
- Height of the tank: 0.14 m
- Bubbling orifice surface: $7 \cdot 10^{-7}$ m^2
- Diameter of the stirrer: 0.06 m
- Width of the blades: 0.01 m
- Length of the blades: 0.02 m
- Distance between the propellers: 0.05 m

Figure 3.1. Dimensions of the Reactor PARR used in the experiments.

The reactor is wrapped in a heating jacket in order to carry out the reaction at the temperature established. In the Figure 3.2 is shown a scheme of the reactor as well as its main components.



(1) Heating jacket.
(2) Mechanical agitation by means of a magnetic rotor equipped with two propellers (each having six blades).
(3) Temperature control through a thermocouple connected to a PID controller.
(4) Pressure gauge.
(5) Oxygen inlet.
(6) Gas outlet.
(7) Sampling device.

Figure 3.2. Schematic diagram of the reactor and its components.

3.2.2. Humidifier

It is formed by a tank of 2 litres of capacity. Oxygen or nitrogen was bubbled inside to ensure that those were with moisture content close to the saturation point before being introduced into the reactor. Temperatures and pressures conditions in both humidifier and reactor were set to carry out the reaction. In order to avoid leak of water from the humidifier to the reactor, the former was filled up to 70% of its capacity approximately, and its temperature was established 5 ºC lower than that of the reactor. In addition, a non-return valve is disposed between the humidifier and the reactor.

3.2.3. Control system

It consists of a P.I.D. controller through which the temperature of both the humidifier and the reactor, as well as the flow rate of oxygen or nitrogen fed in the reactor was controlled. The flow rate controller acts directly on an electrovalve (BROOKS mass flow controller 5850), which can measure flow rates from 2 mL/min to 2000 mL/min.

The pressure in the reactor was regulated by means of a pressure gauge installed in it, as well as a back pressure valve located in the gases outlet. The stirring speed was established through the magnetic stirrer installed in the reactor. It was handled from the P.I.D. controller.

The experimental device is equipped with two rupture discs (*PARR Inconel rupture disc*). A disc is installed in the reactor and the other in the humidifier, both with rupture pressures of 115 atm at 473 K. The maximum temperature supported by the equipment is 623 K.

3.3. EXPERIMENTAL PROCEDURE

Initially, the operating conditions to carry out the reaction were established (pressure, temperature, oxygen or nitrogen flow rate and stirring speed). The amount of raw sludge or sludge fraction to be fed in the reactor was chosen in basis to the specifications of the operating manual. Basically, the volume of fed sample at the used working conditions was between 500-700 mL.

Once the reactor has been charged with the sample, the temperature and the oxygen (wet oxidation) or nitrogen (thermal hydrolysis) flow rate were set through the P.I.D. controller. The pressure was adjusted manually by means of the backpressure valve and controlled in basis to the value registered in the pressure gauge. The stirring speed and the gas flow rate were maintained at 500 rpm and 1.2 L/min, respectively, for all the experiments.

Eight samples were taken at different times of reaction. The first sample was taken when the temperature reached 100 ºC, in order to determine whether any change occurred during the heating period. The following samples were withdrawn during the next 2 h, after reaching the operating conditions. The volume of extracted sample was around 30-35 mL, which once collected was cooled by cold water and subjected to analysis.

During the sludge fractions experiments, the first tests were performed with the raw sludge and the naked cells to avoid any change in their structure that might take place during storage, since this could affect the results. In the Figure 3.3, it is showed a image of the experimental setup used. A scheme of the experimental setup can also be seen in the Figure 1 of the articles II and VI located on the sections 4.2 and 4.4, respectively.

Figure 3.3. Experimental setup used in the thermal hydrolysis and wet oxidation experiments.

3.4. EXTRACTION METHOD OF EPS

The extraction method applied is mainly based on the one proposed by Li and Yang, 2007. A volume of 450 mL of sludge was collected in a centrifuge bottle of 500 mL and subjected to centrifugation at 4000 g (4 ºC, 5 min), employing a Kubota 6500 centrifuge. The supernatant obtained, corresponding to the SMP fraction, was removed and stored, whilst the pellet deposited at the bottom of the bottle was re-suspended with distilled water until its initial volume. Then, the sample was centrifuged again (6000 g, 4 ºC and 15 min) and the resulting supernatant, which corresponds to LB-EPS, was also extracted and stored. Finally, the pellet was again re-suspended to its original volume with distilled water at 65 ºC and placed in a thermostatically controlled water bath at the same temperature, for 30 min. Afterwards, the sample was centrifuged for the third time (8000 g, 4 ºC and 15 min).

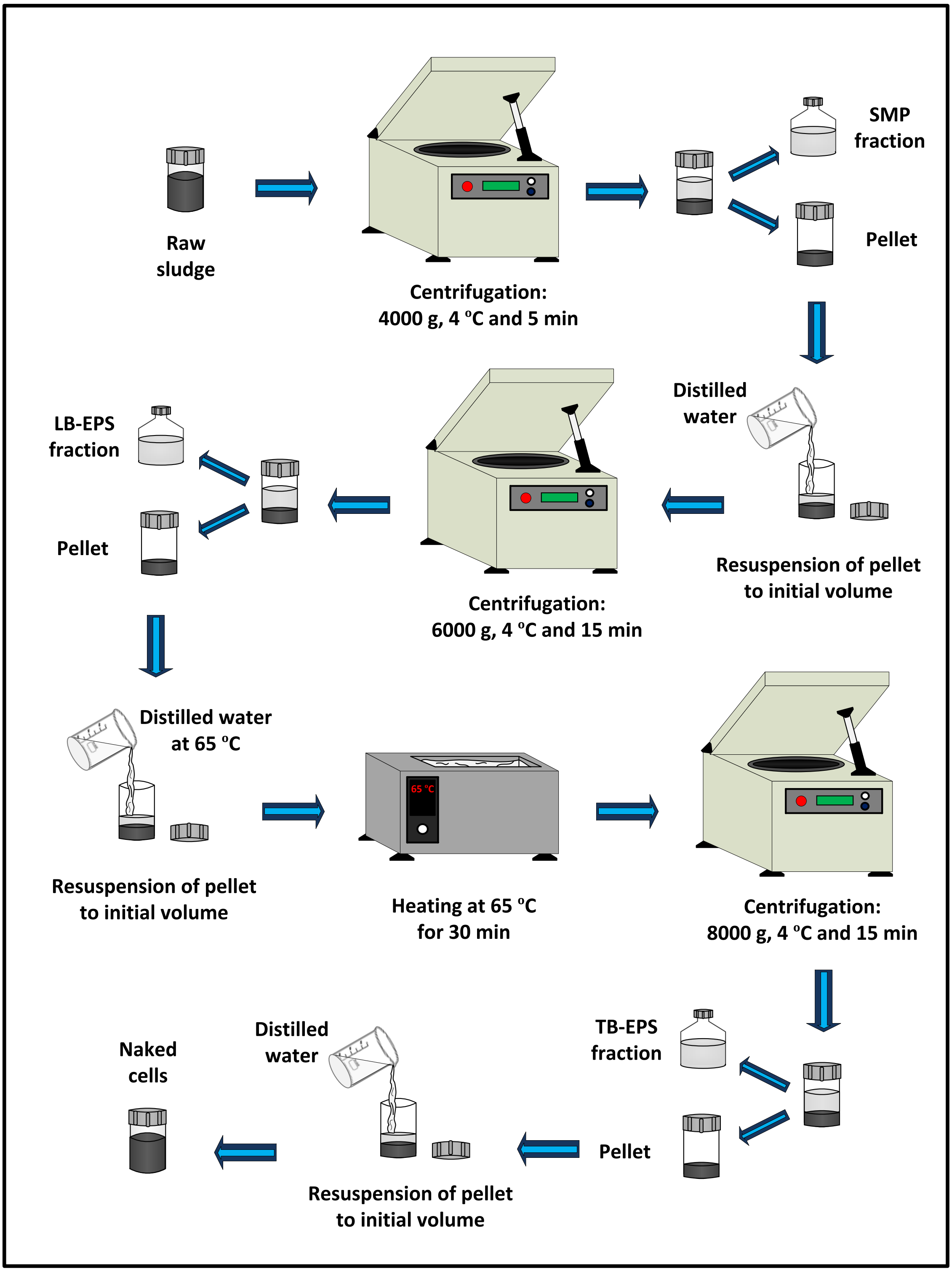


Figure 3.4. Procedure used for EPS extractions from activated sludge.

The supernatant, corresponding to TB-EPS, was removed and stored, while the residue, that is to say, the “naked cells”, was also re-suspended and stored at 4 ºC. A flow chart of this method is shown in the Figure 3.4. In order to remove any material in suspension, SMP, LB-EPS and TB-EPS were filtered through a gravimetric filter paper after extraction, and then centrifuged at 12000 g, 4 ºC and 15 min. Finally, these samples were stored at -20 ºC until being used in WO experiments.

3.5. ANALYTICAL METHODS

The analytical methods used to characterize both initial and treated samples by either thermal hydrolysis or wet oxidation are summarized in Table 3.2 As a general procedure, the samples once collected were analysed during the following 24 hours, these being conserved at 4 ºC during that period.

Table 3.2. Analytical methods used in the experiments.

Parameter	Analytical method/Equipment
Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)	Varian 670-IR spectrometer
Capillary suction time (CST)	OFITE Capillary Suction Timer (294-50)
Carbohydrates	Dubois method, spectrophotometric (at 492 nm), glucose as standard. Helios γ spectrophotometer (Thermo scientific)
Chemical oxygen demand: total (TCOD), soluble (SCOD) and solid (COD_S)	Spectrophotometric (at 600 nm), dichromate method (APHA, 1998). DR2500 spectrophotometer (Hach Company, USA)
Colour number (CN)	Spectrophotometric (at 436, 525 and 620 nm) ISO 7887:2011. T80 UV/VIS spectrophotometer (PG Instruments Ltd)
DNA	Diphenylamine method, spectrophotometric (at 600 nm), DNA calf thymus as standard. Helios γ spectrophotometer (Thermo scientific)
pH	Selective electrode (JENWAY 3510 pH Meter)
Polymers size distribution	High performance liquid chromatography (Agilent 1200, Agilent Technologies Inc., California, USA)

Parameter	Analytical method/Equipment
Proteins and humic acids	Modified Lowry method, spectrophotometric (at 580 nm), BSA and humic acids as standard, respectively. Helios γ spectrophotometer (Thermo scientific)
Readily biodegradable COD (RBCOD)	Respirometric method, oxygen uptake rate
Rheological tests	Rotational rheometer Haake MARS II
Sludge volume index (SVI)	APHA, 1998
Soluble total organic carbon (TOC)	TOC analyzer (Shimadzu TOC-V_{CSH})
Suspended solids: total (TSS), volatile VSS) and fixed (FSS)	Gravimetric (APHA, 1998)
Uronic acids	Blumenkrantz and Asboe-Hansen method, spectrophotometric (at 520 nm), glucuronic acid as standard. Helios γ spectrophotometer (Thermo scientific)
UV-VIS scans	Spectrophotometric (from 190 to 900 nm). T80 UV/VIS spectrophotometer (PG Instruments Ltd)

3.5.1. Carbohydrates *(Dubois method)*

Carbohydrates were measured according to the following procedure. To 1 mL of sample was firstly added 0.5 mL of a phenol solution (5%) and then, 2.5 mL of concentrated H_2SO_4. The mixture was shaken in a vortex mixer and left to rest for 30 min. Finally, the absorbance was measured at a wavelength of 492 nm. To perform the curve of calibration, a stock solution of glucose at a concentration of 0.1 g/L was prepared. From this, standard solutions at a concentration among 0-90 µg/L were made. The standard solutions were analysed in the same way as the samples.

3.5.2. Proteins and humic acids *(Modified Lowry method)*

Proteins and humic acids were determined by measuring the absorbance in presence (total abs) and absence (blind abs) of $CuSO_4$. The solutions used to carry out the analysis were prepared as follow. Solution A was made by dissolving 40 g of Na_2CO_3 and 8 g of NaOH in 2 litres of distilled water. Solution B1 was prepared by adding 1 g of $CuSO_4 * 5\ H_2O$ in 100 mL of distilled water. Solution B2 was made by dissolving 2.68 g of potassium sodium tartrate tetrahydrate in 100 ml of distilled water. The solutions were conserved at 4 ºC to avoid any deterioration.

In the moment of analysing the samples, the following solutions were prepared from A, B1 and B2 solutions. Solution C1 was made by mixing A, B1 and B2 in proportions of 50:0.5:0.5 (volume). Solution C2 was prepared as C1 but instead of adding B1 was used distilled water. Each sample must be analysed with both, C1 and C2 solutions. Afterwards, to 1 mL of sample was added 5 mL of C1 (total abs) or C2 (blind abs) and left in darkness for 15 min. Then, 0.3 mL of Folin-ciocalteu's phenol reagent 2N diluted at 50% was added and once more the mixture was left in darkness for 30 min. Eventually, the absorbance at 580 nm was measured. The interferences between proteins and humic acids in the samples were corrected by using Equations 12-13. The curves of calibration for proteins (solution C1) and humic acids (solution C2) were performed with BSA and humic acid standards, respectively, preparing standard solutions at concentrations among 0-450 µg/L.

$$\text{Proteins abs} = 1.25\,(\text{total abs} - \text{blind abs}) \qquad (12)$$

$$\text{Humic acids abs} = \text{blind abs} - 0.2\ \text{proteins abs} \qquad (13)$$

3.5.3. Uronic acids *(Blumenkrantz and Asboe-Hansen method)*

Uronic acids are widely determined as representative of mucopolysaccharide acids in biological substances. To perform the analysis, a 0.15% solution of meta-hydroxydiphenyl in 0.5% NaOH, as well as a 0.0125 M solution of tetraborate in concentrated sulfuric acid, were prepared. To 0.4 mL of sample containing from 1 to 40 µg uronic acids, 2.4 mL of sulfuric acid/tetraborate solution was added. The tubes were refrigerated in crushed ice. The mixture was shaken in a vortex mixer and the tubes heated in a water bath at 100 ºC for 5 min. After cooling in a water-ice bath, 40 µL of the m-hydroxydiphenyl solution was added. Subsequently, the tubes were shaken and sonicated in order to eliminate any bubble. After 5 min, the absorbance at 520 nm was measured. As carbohydrates produce a pinkish chromogen with sulfuric acid/tetraborate at 100 ºC, a blank sample was run without addition of the reagent, which was replaced by 40 µL of 0.5% NaOH. A stock solution of glucuronic acid at a concentration 0.1 µg/µL was prepared to perform a curve of calibration. From the stock solution, standard solutions were made at concentrations among 0-0.1 µg/µl.

3.5.4. DNA *(Diphenylamine method)*

Firstly, a solution of acetaldehyde at a concentration of 16 mg/mL was prepared and stored at 4 ºC. Subsequently, diphenylamine reagent was prepared by dissolving 1.5 g of diphenylamine in 100 mL of acetic acid and adding 1.5 mL of concentrated H_2SO_4. The reagent was stored at 4 ºC in the darkness to avoid any deterioration. At the time of the analysis, a new solution was made by mixing 0.1 mL of aqueous acetaldehyde (16 mg/mL) for each 20 mL of diphenylamine reagent.

The analysis was performed with the samples containing DNA, which were diluted with 0.5 N $HClO_4$ up to reach a final concentration between 0.02 and 0.25 µg/mL. Subsequently, 1 mL of the diluted samples was mixed with 2 mL of the diphenylamine solution containing acetaldehyde. The colour was developed by heating the mixture at 30 ºC during 16-20 hours. Once this period has elapsed, the absorbance was measured at a wavelength of 600 nm.

In order to prepare a curve of calibration, a stock solution was made by dissolving DNA (Calf-thymus) at about 0.4 mg/mL in 5 mM NaOH. From this, working standards at concentrations between 0-0.25 µg/mL were prepared (every 3 weeks) by mixing the stock solution with 0.5 N $HClO_4$ and heating at 70 ºC for 15 min. Both, stock solution and working standards were stored at 4 ºC.

3.5.5. Total Organic Carbon *(TOC)*

TOC in liquid samples was measured by using a TOC-V_{CSH} from Shimadzu (Figure 3.5). TOC is determined as the difference between total carbon (TC) and inorganic carbon (IC). During TC analysis, the sample is burned in a reactor filled with a catalyst of Pt supported on alumina spheres at 953 K. The TC of the sample is oxidised up to form CO_2 and analysed by a non-dispersive infrared detector (NDIR). This latter provides a peak whose area is proportional to the amount of carbon present in the sample.

On the other hand, air is bubbled through the sample in presence of orthophosphoric acid (25% w/v) during IC analysis. Carbonates and bicarbonates (IC) contained in the samples are decomposed up to form CO_2, this latter being also analysed by the NDIR. Calibration curves were performed from standard solutions prepared with acid potassium phthalate and a mixture of carbonates and bicarbonates, which were dissolved in ultrapure water (resistivity 18.2 MΩ·cm at 298 K). The samples before being injected were filtered through a PTFE syringe filter of 0.45 mm of pore size. The standard deviation of the equipment was around 1%.

Figure 3.5. TOC analyzer Shimadzu TOC-VCSH used in the TOC determination.

3.5.6. Readily biodegradable chemical oxygen demand *(RBCOD)*

The method is based on determining the effect of introducing a liquid sample on the oxygen uptake rate (OUR) of an activated sludge. The analysis of RBCOD was carried out in a 250 mL jacketed vessel, which was maintained at 20 ºC by means of a thermostatically controlled water bath. The experimental setup used in the analysis is showed in Figure 3.6. Below it is described step by step the procedure followed to perform the analysis.

Figure 3.6. Experimental setup used to analyse RBCOD of samples.

1. The ratio of initial substrate concentration to initial biomass concentration (So/Xo) was established in 0.05-0.10 g COD g VSS^{-1}. Low values of So/Xo are suitable to avoid long time of analysis.
2. An activated sludge with a concentration of volatile suspended solids among 3.5 and 5.5 g/L was aerated until to achieve endogenous conditions. Then, 170 mL of this was charged in the reactor and 5 drops of nitrification inhibitor were added. The system was stirred to 250 rpm and the temperature was adjusted at 20 ºC.
3. The aeration was carried out in feeding cycles (1 min feeding and 6 min turned off), which were controlled by using a Cebek I-111 cyclic timer. An YSI Professional Optical Dissolved Oxygen instrument was employed to measure and to store the data of the variation in the oxygen concentration with the time. This was located on an opposite position to the air diffuser in order to avoid direct contact with air bubbles.
4. The sample was centrifuged (10000g, 10 min) and filtered (0.45 µm PVDF filters) in order to remove any colloidal organic material present. Then, its pH was adjusted to a value between 6.5 and 7.5. Afterwards, 50 mL of the sample, which was previously diluted until to achieve the ratio So/Xo established, was injected in the reactor.
5. The OUR measurements were maintained until the sludge reached the endogenous conditions again. A final sample of the reactor was extracted to be analysed its soluble COD. The final sample must be centrifuged and filtered before being analysed as it was done with the initial sample.
6. The OUR was estimated by calculating the slope of the oxygen drop for each measurement cycle. Finally, the RBCOD of the sample was estimated from Equation 14 (Orhon and Çokgör 1997), being necessary to subtract the endogenous cumulative oxygen uptake of the total OUR.

$$\mathrm{RBCOD} = \frac{1}{(1-\mathrm{Y_H})}\int_{t_0}^{t_1} \mathrm{OUR}\ \partial t \qquad (14)$$

Where YH is the heterotrophic yield coefficient

As YH depends of the sludge characteristics, it was necessary to carry out a previous test using sodium acetate as an organic standard. The value of YH was calculated from Equation 15, obtaining a value of 0.74 mg COD mg COD^{-1}.

$$Y_H = 1 - \frac{\int \mathrm{OUR}}{\mathrm{COD\ degradation}} \qquad (15)$$

3.5.7. Size exclusion chromatographic analysis

The size distribution of both EPS and sludge biopolymers solubilised by hydrothermal treatments were determined by high performance liquid chromatography analysis (Agilent 1200, Agilent Technologies Inc., California, USA). A Yarra SEC-2000 column 300 x 7.8 mm, with a total column volume of 11.8 mL (estimated with a $NaNO_3$ solution), was used. The detection of soluble biopolymers was carried out with a diode array UV detector at 210, 260 and 280 nm, and at room temperature. 20 µl of sample previously filtered through 0.45 µm PVDF filters (Millipore) were injected, with a mobile phase flow of 1 mL/min. The mobile phase consisted of a buffer solution of 9 mM NaCl, 0.9 mM Na_2HPO_4 and 0.005% NaN_3, with an ionic strength of 0.02. The pH of this solution was adjusted at 7.0 ± 0.1 with phosphoric acid. The column was calibrated with a Protein Standard Mix 15-600 kDa supplied by Sigma-Aldrich (69385), which was composed by four proteins: Ribonuclease A (13.7 kDa), Albumin (44.3 kDa), γ-Globulin (150 kDa) and Thyroglobulin (670 kDa), as well as by a low molecular weight marker, ρ-aminobenzoic acid (0.14 kDa). The coefficient of determination obtained for the calibration curve was $R^2=0.93$. The samples with a high concentration of soluble biopolymers were diluted with the mobile phase before being injected. The presence of hydrophobic interactions between the column packing material and sludge biopolymers were determined by using a mobile phase of 5% (v/v) of methanol, 9 mM NaCl, 0.9 mM Na_2HPO_4 and 0.005% NaN_3, with a pH adjusted at 7.0 ± 0.1.

4. RESULTS AND DISCUSSION

In this chapter, the results obtained during the development of this thesis are presented through of five sections. Seven research articles already published were distributed in the first four sections, corresponding to the hydrothermal treatments of sludge. The fifth section collects the study of the effect of wet oxidation on physicochemical characteristics and composition of different landfill leachates. The identification of the organic compounds present in the leachates was performed during a research stay in a foreign group. This last work is also presented following the structure of a research article. A brief description of the content of each section as well as the article references that compose it is presented below.

4.1. Study of the formation of valuable compounds from sludge by means of hydrothermal treatments.

A bibliographic analysis of the works carried out employing hydrothermal techniques to treat sewage sludge is presented, this being focused on the data collection concerning the formation of compounds of interest.

I. *Suárez-Iglesias, O., Urrea, J.L., Oulego, P., Collado, S. and Díaz, M. (2017) Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review. Science of the Total Environment 584–585, 921-934.*

4.2. Effect of operating conditions of hydrothermal treatments on sludge properties.

The effect of the operating variables, temperature and pressure, during thermal hydrolysis and wet oxidation of sludge was studied. The evolution in the sludge characteristics such as, settleability, solubilisation and mineralisation, as well as the changes in the viscosity and the flow behaviour were analysed. Some equations to describe the influence of TSS of the sludge on the rheological parameters of the Herschel-Bulkley model used during thermal hydrolysis were established. In addition, a reaction mechanism was proposed for the wet oxidation of sludge and its kinetics constants were calculated.

II. *Urrea, J.L., Collado, S., Laca, A. and Díaz, M. (2015) Rheological behaviour of activated sludge treated by thermal hydrolysis. Journal of Water Process Engineering 5(0), 153-159.*

III. *Urrea, J.L., Collado, S., Laca, A. and Díaz, M. (2014) Wet oxidation of activated sludge: Transformations and mechanisms. Journal of Environmental Management 146(0), 251-259.*

4.3. Comparison between thermal hydrolysis and wet oxidation of sludge.

The effect of thermal hydrolysis and wet oxidation on COD, TOC, SVI, colour and pH parameters of sludge was compared and analysed. Likewise, the differences on the composition and the size distribution of the solubilised biopolymers were determined.

IV. *Urrea, J.L., García, M., Collado, S., Oulego, P. and Díaz, M. (2018) Sludge hydrothermal treatments. Oxidising atmosphere effects on biopolymers and physical properties. Journal of Environmental Management 206, 284-290.*

4.4. Effect of wet oxidation on structural fractions from sludge.

The sludge was separated into its structural components corresponding to: SMP, LB-EPS, TB-EPS and naked cells, and each fraction was subjected separately to treatment by wet oxidation. A wide variety of properties were analysed, such as solubilisation, oxidability, mineralisation degree, mean oxidation number of organic carbon, composition and size distribution de biopolymers, colour, UV-Vis spectrum and biodegradability. Then, the results were compared with those obtained from the raw sludge. Furthermore, the effect of solubilised biopolymers on settleability and dewaterability of sludge during wet oxidation was explained. Eventually, a kinetic model that includes the effects of oxygen concentration and temperature of the process, as well as the rates of formation and reaction of the main solubilised biopolymers was proposed and validated.

V. *Urrea, J.L., Collado, S., Oulego, P. and Díaz, M. (2017b) Wet oxidation of the structural sludge fractions. Journal of Cleaner Production 168(Supplement C), 1163-1170.*

VI. *Urrea, J.L., Collado, S., Oulego, P. and Díaz, M. (2016) Effect of wet oxidation on the fingerprints of polymeric substances from an activated sludge. Water Research 105, 282-290.*

VII. *Urrea, J.L., Collado, S., Oulego, P. and Díaz, M. (2017a) Formation and Degradation of Soluble Biopolymers during Wet Oxidation of Sludge. ACS Sustainable Chemistry & Engineering 5(4), 3011-3018.*

4.5. Effect of wet oxidation on physicochemical characteristics and composition of different landfill leachates.

Physicochemical characteristics and composition of three samples of landfill leachates (young, old and biologically stabilised ones), as well as the changes occurred during the wet oxidation of these, were analysed and compared. Liquid Chromatography mass spectrometry (LC-MS) was used to identify organic compounds present in the leachates, whilst physicochemical characteristics of those were analysed by COD, TOC, mean oxidation number of organic carbon, colour, specified ultraviolet absorption, pH and elemental analysis.

VIII. *Wet oxidation effects on LC-MS fingerprints for different landfill leachates.*

4.1. STUDY OF THE FORMATION OF VALUABLE COMPOUNDS FROM SLUDGE BY MEANS OF HYDROTHERMAL TREATMENTS

I. Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review.

Science of the Total Environment 584–585 (2017) 921–934

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Review

Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review

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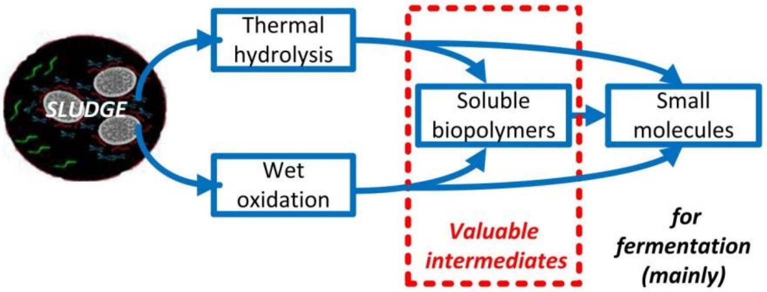
HIGHLIGHTS

- Thermal hydrolysis solubilizes proteins, carbohydrates, lipids and phosphorus
- Wet oxidation mainly produces soluble volatile fatty acids.
- Temperature, reaction time and type of sludge determined the obtained products.
- There is a lack of product characterization and absence of economic evaluations.

GRAPHICAL ABSTRACT



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ABSTRACT

Sewage sludge is considered a costly waste, whose benefit has received a lot of attention for decades. In this sense, a variety of promising technologies, such as thermal hydrolysis and wet oxidation, are currently employed. Thermal hydrolysis is used as a pretreatment step ahead of anaerobic digestion processes and wet oxidation is intended for the solubilization and partial oxidation of the sludge. Such processes could be utilized for solubilizing polysaccharides, lipids, fragments of them and phosphorus (thermal hydrolysis) or for generating carboxylic acids (wet oxidation). This article compiles the available information on the production of valuable chemicals by these techniques and comments on their main features. Temperature, reaction duration times and sludge characteristics influence the experimental results significantly, but only the first two variables have been thoroughly studied. For thermal hydrolysis, a rise of temperature led to an increase in the solubilized biomolecules, but also to a greater decomposition of proteins and undesirable reactions of carbohydrates with themselves or with proteins. At constant temperature, the amounts of substances that can be recovered tend to become time independent after several minutes. Diluted and activated sludges seem to be more readily hydrolyzable than the thickened and primary ones. For wet oxidation, the dependence of the production of carboxylic acids with

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Abbreviations: Carb, carbohydrates; col, colloidal; DKN, dissolved Kjeldahl nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; HTC, hydrothermal carbonization; HTG, hydrothermal gasification; HTL, hydrothermal liquefaction; Lip, lipids; PCOD, particulate chemical oxygen demand; Prot, proteins; TH, thermal hydrolysis; SBOD, soluble biochemical oxygen demand; sCarb, soluble carbohydrates; SCOD, soluble chemical oxygen demand; SCWO, supercritical wet oxidation; sLip, soluble lipids; sProt, soluble proteins; SubCWO, subcritical wet oxidation; TBOD, total biochemical oxygen demand; TCarb, total carbohydrates; TCOD, total chemical oxygen demand; TDS, total dissolved solids; TKN, total Kjeldahl nitrogen; TLip, total lipids; TOC, total organic carbon; TON, total organic nitrogen; TP, total phosphorus; TProt, total proteins; TS, total solids; TSS, total suspended solids; TVS, total volatile solids; VDS, volatile dissolved solids; VFA, volatile fatty acids; VSS, volatile suspended solids; WO, wet oxidation; WWTP, wastewater treatment plant.

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temperature and time is not simple: their concentration can increase, decrease or go through a maximum. At high temperatures, acetic acid is the main carboxylic acid obtained. Concentrated, fermented and secondary sludge seem to be more suitable for yielding higher amounts of acid than diluted, undigested and primary ones.

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Contents

1. Introduction

Sewage sludge is a broad term that refers to several aqueous suspensions of solids generated during the decontamination of the influents to Wastewater Treatment Plants (WWTP). Sewage sludge is called "primary" if it comes from the primary treatment (mechanical processes, such as gravitational settling and air flotation, which separate heavy solids and oils from the raw wastewater) and "secondary" if it is mainly comprised of microorganisms from the biological treatment that follows the primary one (where microbial activity removes suspended and dissolved organic matter, but also causes an increase in the amount of biomass, which has to be purged). Table 1 sums up some characteristics of both suspensions (Li and Noike, 1992; Metcalf and Eddy Inc. et al., 2003; Bougrier et al., 2008). Additionally, a "tertiary sludge" can be originated, if further purification of the effluent from the secondary treatment is required (e.g., elimination of specific nutrients as phosphorus or nitrogen). Due to the growing environmental concerns over the last decades, new WWTPs have been built and part of the existing ones have been upgraded (Ødegaard, 2004; Rulkens, 2004), leading to a significant rise of the global sewage sludge production.

Although the operating conditions of the biological treatment can be adapted to accumulate polyhydroxyalkanoates in the microorganisms (Chua et al., 2003) or to produce aerobic granular sludge, which can be utilized as a source of biodegradable plastics or as adsorbent (Zhang et al., 2016), the WWTPs are reluctant to modify these conditions, if the overall performance of the plant is good. In this sense, it is more desirable: i) to deposit the residual sludge in landfills, ii) to use it as fertilizer/soil conditioner, or, instead of giving it a final destination, iii) to use it for the production of energy or valuable compounds (Ødegaard et al., 2002). This last alternative refers to a variety of processes, such as anaerobic digestion (Appels et al., 2008), pyrolysis and gasification (Manara and Zabaniotou, 2012), mono-incineration and co-combustion (Werther and Ogada, 1999) and hydrothermal methods (Hii et al., 2014). Anaerobic digestion produces biogas, pyrolysis and gasification transform the sludge into fuel, incineration and combustion allow for the recovery of energy by means of heat exchangers, and hydrothermal methods generate energy, chemicals and gases (Rulkens, 2008; Tyagi and Lo, 2013). Unfortunately, the decreasing capacities of landfills, the scarcity of appropriate regions to construct new sites, the legal limitations applied to landfilling, the necessity of stabilizing the sludge (due to its poor physical nature) and the presence of heavy metals and other toxic organics in it, make options i) and ii) troublesome (Werther and Ogada, 1999). Anaerobic fermentation is widely used, but it is a slow process, which requires large reactors and can be easily inhibited by organics present in the sludge or generated during the digestion (Anjum et al., 2016). Besides, pyrolysis, gasification, incineration and combustion require extensive drying of the material, with the subsequent high energy costs (Libra et al., 2011; Xue et al., 2015a).

Table 1
General characteristics of primary and secondary sludge.

Parameter	Primary sludge	Secondary sludge
Total solids (% TS)	2.0–8.0	0.83–3.3
Organic solids (% TS)	60–80	59–88
Grease, fats and lipids (% TS)	13–65	2–12
Protein (% TS)	20–30	32–41
Nitrogen (N, % TS)	1.5–4.0	2.4–5.0
Phosphorus (P, % TS)	0.17–0.6	0.6–2.3
Cellulose (% TS)	8.0–15.0	n.a.[a]
Carbohydrates (% TS)	n.a.[a]	6.1–9.8
pH	5.0–8.0	6.5–8.0
Organic acids (mg/L as acetic acid)	200–2000	16–1700

[a] n.a. not available.

Hydrothermal technologies can be defined as those performed in dense water at elevated temperatures and pressures, namely, above 100 °C and 0.101 MPa (Byrappa and Yoshimura, 2013), and are categorized into oxidative and non-oxidative techniques (Hii et al., 2014). The first ones are carried out in oxidative atmospheres (air, oxygen or hydrogen peroxide), and are generically called wet oxidations (WO): subcritical wet oxidation (SubCWO) is performed below the supercritical point of water, whereas supercritical wet oxidation (SCWO) if performed above it. The second ones are implemented in inert atmospheres and comprise thermal hydrolysis (TH), hydrothermal carbonization (HTC), hydrothermal liquefaction (HTL) and hydrothermal gasification (HTG). HTC, HTL and HTG lead to the production of solid, liquid and gaseous fuels, like pyrolysis and gasification, but are advantageous in that there is no need to dry the resulting sludge before processing it. TH usually ranges from 100 to 180 °C at the saturation pressures, and is commonly employed as pretreatment for the anaerobic treatment of activated sludge (Foladori et al., 2010). Since most of the organic substances in this sludge are in the particulate form and only a small percentage of them are dissolved, they are not readily available for digestion. TH breaks particles and microbial cells, releasing either their content, which allows a better fermentation, or the bond water, which improves the sludge dewaterability. It also decomposes lipids (Lip), proteins (Prot) and carbohydrates (Carb) into lighter molecules, as schematically represented in Fig. 1. HTC is operated at 180–220 °C (saturation pressures between 1.0 and 2.32 MPa, respectively), and mainly generates a coal called hydrochar, together with minor amounts of liquid biocrude and gas (Ekpo et al., 2016; Matsumura, 2016; Wagner and Pruss, 2002). HTL works at higher temperatures (up to 370 °C,

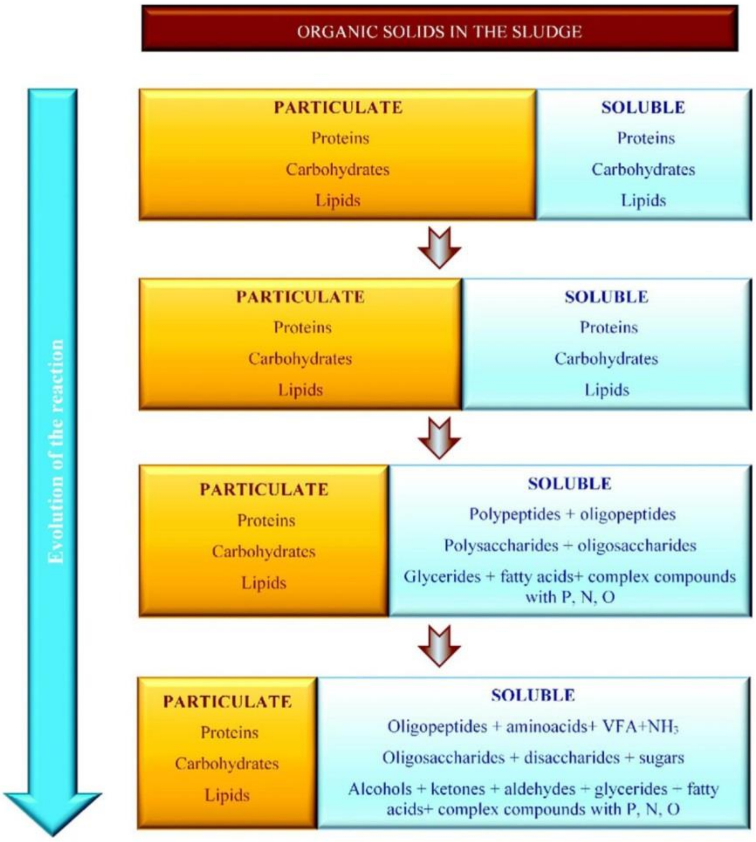


Fig. 1. Idealized evolution with time and/or temperature of some organic compounds during thermal hydrolysis of sewage sludge.

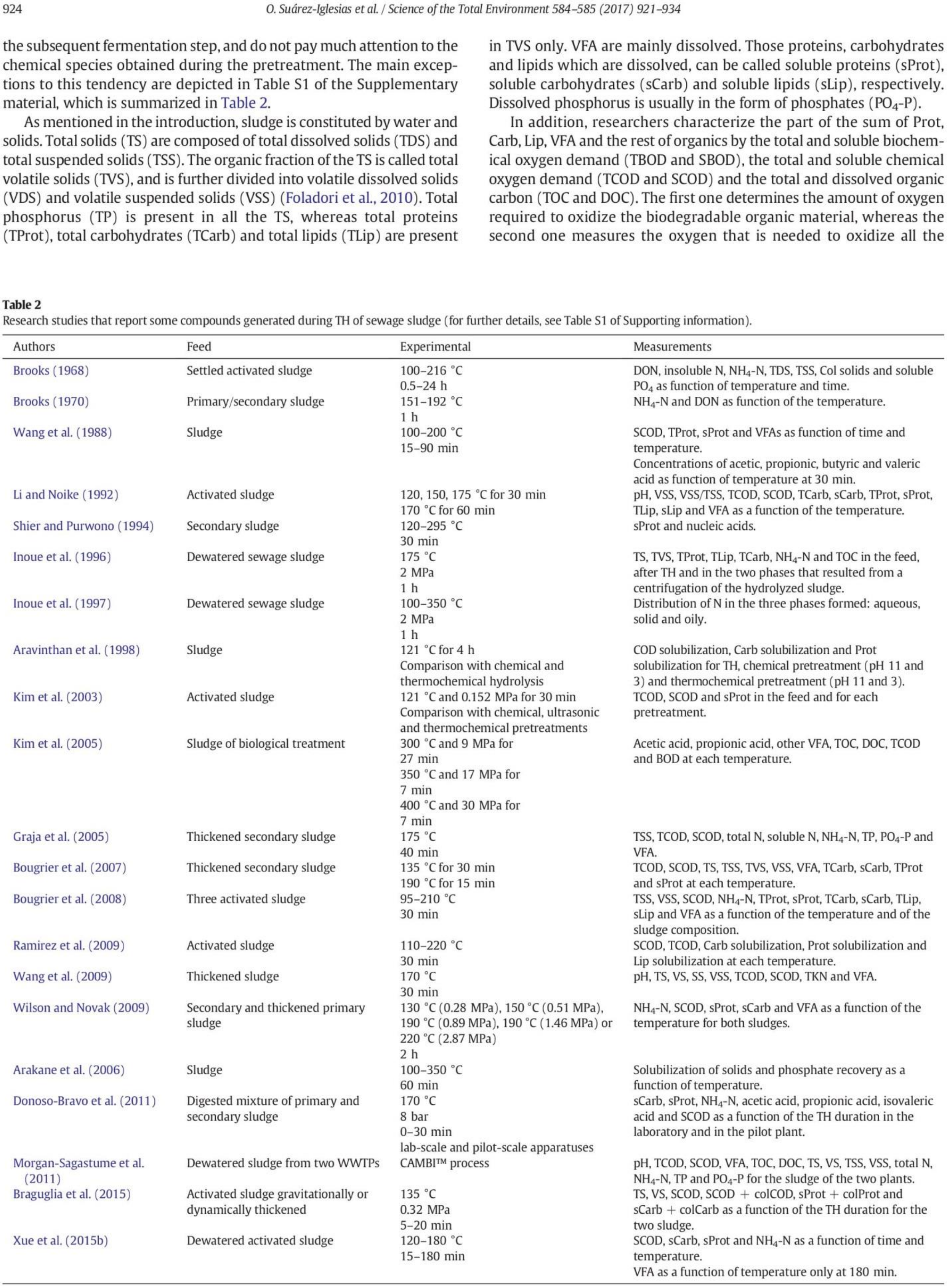
corresponding to a saturation pressure of 21.05 MPa), where the liquid oil becomes predominant; and HTG is suitable at conditions above the critical point of water (373.94 °C and 22.06 MPa), where the fuel gas is the main product (Dote et al., 1993; He et al., 2014; Wagner and Pruss, 2002). Beyond the critical temperature, there is no liquid water or steam, but a new phase called "supercritical", with properties such as density, viscosity, diffusivity and specific heat, very different from those of water in its ordinary states. Additionally, supercritical water has low polarity, and is able to dissolve fully organic compounds and gases such as oxygen, nitrogen and argon, whereas inorganic salts become almost insoluble in it (Matsumura, 2016; Qian et al., 2016).

On the other hand, SubCWO typically takes place at temperatures between 120 and 370 °C (Rulkens and Bien, 2004): the heat allows for the hydrolyzation of complex organic compounds into simpler molecules (as in TH), but the presence of an oxidant avoids that some of these fragments, which are unstable and reactive, repolymerize into carbonaceous or oily compounds. Therefore, the main products of this SubCWO are molecular volatile fatty acids (VFA), non-aromatic alcohols, ketones, ammonia, carbon dioxide and water. As temperature increases, the proportion of organic material mineralized become greater, and for SCWO, light organic compounds are completely oxidized.

There are various generic reviews on hydrothermal technologies; however, very few are focused on their applicability to sewage sludge for the production of resources. In fact, and to the best of our knowledge, there are only two papers dealing with this matter: those by He et al. (2014) and Qian et al. (2016). Nevertheless, these studies analyze the publications on fuel gases generation by means of HTC and SCWO, and neither of them evaluates the production of valuable substances. The aim of the present work is to compile and discuss on the papers that deal with the production of Prot, Carb, Lip, VFA and phosphorus, among others, by means of TH, SubCWO and SCWO. All of these compounds have interesting industrial applications. For instance, Prot can be used as animal food (Hwang et al., 2008. Prot and Carb can also serve as emulsifiers (García-Becerra et al., 2010a, 2010b. Lipids are converted into biodiesel (Siddiquee and Rohani, 2011) and VFAs produced biodegradable plastics, generate bioenergy and can also be employed as carbon sources for the removal of biological nutrients (Lee et al., 2014). Phosphorus is a non-renewable resource, chiefly obtained from phosphate rock, whose reserves are estimated to be insufficient to meet the demand within 50–400 years (van Dijk et al., 2016). Therefore, its recovery from wastes (not only sludge, but wastewater and sludge ashes, too) and subsequent recycling is an interesting and technologically feasible option (Egle et al., 2016), although there is a lack of active support for P-recycling in the states regulations (Hukari et al., 2016) HTC and HTL are beyond the scope of this study, due to the complexity of hydrochars and biocrudes.

2. Thermal hydrolysis

TH, together with other techniques, such as biological hydrolysis (also called hydrolysis at low temperatures), advanced oxidation below 100 °C, ultrasounds, centrifugation, grinding and alkali or acid addition at low/high temperatures, are commonly used as pretreatments to increase biogas production in the anaerobic digestion process (Anjum et al., 2016; Appels et al., 2008; Carrère et al., 2010; Müller, 2001; Neyens and Baeyens, 2003; Pérez-Elvira et al., 2006). Nonetheless, after extensive literature research, it has been found that most of the papers which deal with thermal hydrolysis are mainly focused on



the subsequent fermentation step, and do not pay much attention to the chemical species obtained during the pretreatment. The main exceptions to this tendency are depicted in Table S1 of the Supplementary material, which is summarized in Table 2.

As mentioned in the introduction, sludge is constituted by water and solids. Total solids (TS) are composed of total dissolved solids (TDS) and total suspended solids (TSS). The organic fraction of the TS is called total volatile solids (TVS), and is further divided into volatile dissolved solids (VDS) and volatile suspended solids (VSS) (Foladori et al., 2010). Total phosphorus (TP) is present in all the TS, whereas total proteins (TProt), total carbohydrates (TCarb) and total lipids (TLip) are present in TVS only. VFA are mainly dissolved. Those proteins, carbohydrates and lipids which are dissolved, can be called soluble proteins (sProt), soluble carbohydrates (sCarb) and soluble lipids (sLip), respectively. Dissolved phosphorus is usually in the form of phosphates (PO$_4$-P).

In addition, researchers characterize the part of the sum of Prot, Carb, Lip, VFA and the rest of organics by the total and soluble biochemical oxygen demand (TBOD and SBOD), the total and soluble chemical oxygen demand (TCOD and SCOD) and the total and dissolved organic carbon (TOC and DOC). The first one determines the amount of oxygen required to oxidize the biodegradable organic material, whereas the second one measures the oxygen that is needed to oxidize all the

Table 2
Research studies that report some compounds generated during TH of sewage sludge (for further details, see Table S1 of Supporting information).

Authors	Feed	Experimental	Measurements
Brooks (1968)	Settled activated sludge	100–216 °C 0.5–24 h	DON, insoluble N, NH$_4$-N, TDS, TSS, Col solids and soluble PO$_4$ as function of temperature and time.
Brooks (1970)	Primary/secondary sludge	151–192 °C 1 h	NH$_4$-N and DON as function of the temperature.
Wang et al. (1988)	Sludge	100–200 °C 15–90 min	SCOD, TProt, sProt and VFAs as function of time and temperature. Concentrations of acetic, propionic, butyric and valeric acid as function of temperature at 30 min.
Li and Noike (1992)	Activated sludge	120, 150, 175 °C for 30 min 170 °C for 60 min	pH, VSS, VSS/TSS, TCOD, SCOD, TCarb, sCarb, TProt, sProt, TLip, sLip and VFA as a function of the temperature.
Shier and Purwono (1994)	Secondary sludge	120–295 °C 30 min	sProt and nucleic acids.
Inoue et al. (1996)	Dewatered sewage sludge	175 °C 2 MPa 1 h	TS, TVS, TProt, TLip, TCarb, NH$_4$-N and TOC in the feed, after TH and in the two phases that resulted from a centrifugation of the hydrolyzed sludge.
Inoue et al. (1997)	Dewatered sewage sludge	100–350 °C 2 MPa 1 h	Distribution of N in the three phases formed: aqueous, solid and oily.
Aravinthan et al. (1998)	Sludge	121 °C for 4 h Comparison with chemical and thermochemical hydrolysis	COD solubilization, Carb solubilization and Prot solubilization for TH, chemical pretreatment (pH 11 and 3) and thermochemical pretreatment (pH 11 and 3).
Kim et al. (2003)	Activated sludge	121 °C and 0.152 MPa for 30 min Comparison with chemical, ultrasonic and thermochemical pretreatments	TCOD, SCOD and sProt in the feed and for each pretreatment.
Kim et al. (2005)	Sludge of biological treatment	300 °C and 9 MPa for 27 min 350 °C and 17 MPa for 7 min 400 °C and 30 MPa for 7 min	Acetic acid, propionic acid, other VFA, TOC, DOC, TCOD and BOD at each temperature.
Graja et al. (2005)	Thickened secondary sludge	175 °C 40 min	TSS, TCOD, SCOD, total N, soluble N, NH$_4$-N, TP, PO$_4$-P and VFA.
Bougrier et al. (2007)	Thickened secondary sludge	135 °C for 30 min 190 °C for 15 min	TCOD, SCOD, TS, TSS, TVS, VSS, VFA, TCarb, sCarb, TProt and sProt at each temperature.
Bougrier et al. (2008)	Three activated sludge	95–210 °C 30 min	TSS, VSS, SCOD, NH$_4$-N, TProt, sProt, TCarb, sCarb, TLip, sLip and VFA as a function of the temperature and of the sludge composition.
Ramirez et al. (2009)	Activated sludge	110–220 °C 30 min	SCOD, TCOD, Carb solubilization, Prot solubilization and Lip solubilization at each temperature.
Wang et al. (2009)	Thickened sludge	170 °C 30 min	pH, TS, VS, SS, VSS, TCOD, SCOD, TKN and VFA.
Wilson and Novak (2009)	Secondary and thickened primary sludge	130 °C (0.28 MPa), 150 °C (0.51 MPa), 190 °C (0.89 MPa), 190 °C (1.46 MPa) or 220 °C (2.87 MPa) 2 h	NH$_4$-N, SCOD, sProt, sCarb and VFA as a function of the temperature for both sludges.
Arakane et al. (2006)	Sludge	100–350 °C 60 min	Solubilization of solids and phosphate recovery as a function of temperature.
Donoso-Bravo et al. (2011)	Digested mixture of primary and secondary sludge	170 °C 8 bar 0–30 min lab-scale and pilot-scale apparatuses	sCarb, sProt, NH$_4$-N, acetic acid, propionic acid, isovaleric acid and SCOD as a function of the TH duration in the laboratory and in the pilot plant.
Morgan-Sagastume et al. (2011)	Dewatered sludge from two WWTPs	CAMBI™ process	pH, TCOD, SCOD, VFA, TOC, DOC, TS, VS, TSS, VSS, total N, NH$_4$-N, TP and PO$_4$-P for the sludge of the two plants.
Braguglia et al. (2015)	Activated sludge gravitationally or dynamically thickened	135 °C 0.32 MPa 5–20 min	TS, VS, SCOD, SCOD + colCOD, sProt + colProt and sCarb + colCarb as a function of the TH duration for the two sludge.
Xue et al. (2015b)	Dewatered activated sludge	120–180 °C 15–180 min	SCOD, sCarb, sProt and NH$_4$-N as a function of time and temperature. VFA as a function of temperature only at 180 min.

organic matter by means of a strong chemical oxidant. TOC quantifies the amount of carbon dioxide generated after subjecting the sample to strong oxidation (Metcalf and Eddy Inc. et al., 2003).

Occasionally, some authors expressed the concentration of the interesting compounds in terms of COD (Donoso-Bravo et al., 2011; Kim et al., 2005; Li and Noike, 1992; Morgan-Sagastume et al., 2011; Wang et al., 2009).

Since valuable substances are mainly in the particulate form (i.e. in the suspended particles), it is interesting to increase their solubilization in order to improve the production of resources. Most of the papers listed in Tables 2 and S1 deal with the influence of heating time and/or heating temperature on the amount of SCOD, DOC, sProt, sCarb, sLip, PO_4-P and dissolved organic nitrogen (DON) produced. On the other hand, the rise in the VFA concentration is not due to solubilization, but to decomposition of part of the organic matter. There is an article that compares a primary sludge with a secondary one (Wilson and Novak, 2009), and another one focused on the scale-up of TH from laboratory to pilot plant (Donoso-Bravo et al., 2011). There are also two publications that compare TH with other pretreatments (Aravinthan et al., 1998; Kim et al., 2003) and one paper that studies the effect of the different organic loading rates (Braguglia et al., 2015). For the sake of clarity, it has to be said that in this last publication, organic matter is not only classified as suspended and soluble, but also as colloidal matter (col), which corresponds to particles of 0.45–1.2 μm. The work of Shier and Purwono (1994) has also been included in Tables 2 and S1 because, in spite of being carried out in presence of a certain concentration of air, they repeated their experiments after purging with nitrogen and no change in their results was observed.

The degree of solubilization of COD, Prot, Lip, Carb and phosphorus is usually defined by Eq. (1) (Aravinthan et al., 1998; Bougrier et al., 2008; Braguglia et al., 2015; Donoso-Bravo et al., 2011; Graja et al., 2005; Ramirez et al., 2009).

$$\left(\begin{matrix}\text{Solubilization}\\ \text{degree}\end{matrix}\right) = \frac{\text{soluble concentration in the treated effluent} - \text{soluble concentration in the feed}}{\text{total concentration in the feed} - \text{soluble concentration in the feed}} \quad (1)$$

Nevertheless, other authors, such as Li and Noike (1992) and Kim et al. (2005, 2003) calculated it according to Eq. (2):

$$\left(\begin{matrix}\text{Solubilization}\\ \text{degree}\end{matrix}\right) = \frac{\text{soluble concentration in the treated effluent}}{\text{total concentration in the treated effluent}} \quad (2)$$

Note that both expressions become the same if the soluble concentration of the considered species in the feed is negligible and the total concentration remains constant during the TH. In this sense, and within an experimental error of 5–10%, the flat concentration (or a slight decrease) of TS, TVS and/or TCOD indicated that most of the organic matter is solubilized and not mineralized (Bougrier et al., 2007; Braguglia et al., 2015; Graja et al., 2005; Li and Noike, 1992; Ramirez et al., 2009). In the works of Morgan-Sagastume et al. (2011) and of Kim et al. (2005), the sharp decrease in some of these parameters reveals a notable degree of mineralization which is better explained in the publication by Kim et al. (2005), since the operating temperatures were well above the typical range for TH. Unfortunately, many researchers measured the total concentration of the organics of interest in the feed and not in the thermally hydrolyzed effluents, which makes it difficult to know the true fate of the suspended matter. On the other hand, it is stated that at constant temperature, most of the solubilization (and decomposition) occurred in the first 10–30 min (Braguglia et al., 2015; Wang et al., 1988). So, the pre-heating time, or the time required to raise the temperature from ambient to the operating one, has significant influence on that solubilization (Donoso-Bravo et al., 2011; Xue et al., 2015b). A notable exception is the work of Brooks (1968), where some parameters exhibit a considerable dependence on time.

For a given time, SCOD, sProt, sCarb and sLip increase with increasing temperatures. Bougrier et al. (2008) and Li and Noike (1992) pointed out that carbohydrates were more hydrolyzable than proteins, and proteins more hydrolyzable than lipids, but in the paper by Ramirez et al. (2009), proteins were released from the VSS fraction more easily than carbohydrates. It has been reported that, at temperatures of 130–190 °C, (Bougrier et al., 2008) or around 220 °C (Ramirez et al., 2009), sCarb decreases because of the reactions of such carbohydrates with themselves or with soluble proteins, yielding other compounds. Nonetheless, this does not seem to be a general feature of TH, but a characteristic of some sludges, since Wilson and Novak (2009) observed a continuous increase in sCarb at temperatures from 130 to 220 °C for both the primary and the secondary sludges treated by them. Furthermore, hydrolyzed sludge at temperatures above 175 °C usually contains also some toxic components (Stuckey and McCarty, 1984), which can be a major drawback in the subsequent processing of this stream.

In the case of proteins, the rise in sProt with temperature can be accompanied by an increase in the ammonia-nitrogen concentration (NH_4-N), as shown in Fig. 2. This implies that part of TProt is being degraded (Xue et al., 2015b). Occasionally, sProt also suffers degradation, and then, the increase in NH_4-N corresponds to a decrease of the DON. Brooks (1970, 1968) and Inoue et al. (1997) established the degradation temperature between 150 and 200 °C, and Shier and Purwono (1994) at 150–155 °C. Moreover, a noticeable increase in the VFA concentrations with time or with temperature is not followed by a rise of the NH_4-N concentration, revealing that VFA are produced by lipid degradation instead of protein decomposition (Bougrier et al., 2008; Donoso-Bravo et al., 2011). Lipids are not easily measurable, and experimental errors are common: e.g. Bougrier et al. (2008) observed that the higher the temperature, the higher the TLip amount, which makes no physical sense.

Protein concentration can be measured by the Lowry method and its variants, which uses bovine serum albumin as standard (Bougrier et al., 2008, 2007; Braguglia et al., 2015; Li and Noike, 1992; Ramirez et al., 2009; Shier and Purwono, 1994; Wilson and Novak, 2009; Xue et al., 2015b) or by quantifying the organic nitrogen and assuming that proteins contain 16.0 wt% of nitrogen (Donoso-Bravo et al., 2011; Inoue et al., 1996; Wang et al., 1988). Total or dissolved organic nitrogen (TON or DON) is estimated by deducting the NH_4-N to the total or dissolved Kjeldahl nitrogen (TKN or DKN), respectively. However, Morgan-Sagastume et al. (2011) compared both techniques, and observed differences of several orders of magnitude (0.04% of VS with Lowry versus 61% of VS with organic N). Such differences can be attributed to the fact that the Lowry method quantifies the actual peptide-bonds of long-chain proteins (with the interference of tryptophan and tyrosine), but the organic nitrogen method also estimates oligopeptides and amino acids, which are produced by TH of proteins. Differences of orders of magnitude indicate that proteins are not only solubilized, but also broken down into very small fragments.

VFA concentration seems to increase with both temperature (Bougrier et al., 2008; Li and Noike, 1992; Wilson and Novak, 2009; Xue et al., 2015b) and time (Donoso-Bravo et al., 2011), although the scattering of the experimental data suggests the presence of maxima in some cases (Wang et al., 1988). Above 300 °C, the higher the temperature, the lower the total VFA concentration (Kim et al., 2005). Acetic acid is the most produced acid in the TH of Donoso-Bravo et al. (2011), Wang et al. (1988) and Xue et al. (2015b)), although, in the first case, the high amount of propionic acid in the feed masks its production. In the high-temperature TH experiment by Kim et al. (2005), it is not possible to elucidate which is the most produced acid, since the initial amount of each one was not determined.

COD solubilization in secondary sludge is more easily accomplished than in primary sludge, whereas for protein solubilization, the opposite is true (Wilson and Novak, 2009). However, keeping in mind that the rise of the percentages of NH_4-N and VFA in the secondary sludge is

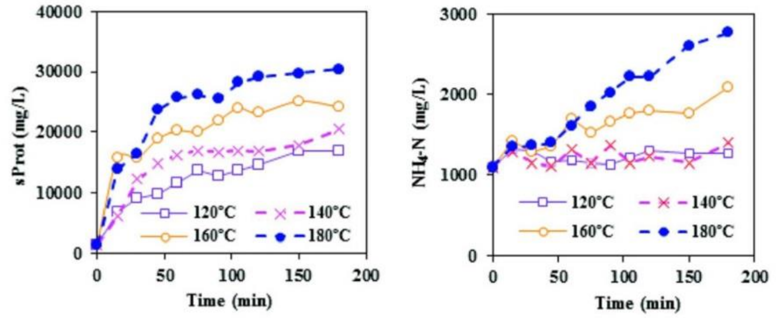


Fig. 2. Concentrations of a) sProt and b) NH_4-N at different treatment temperatures in a dewatered secondary sludge (figures adapted from Xue et al., 2015b). Arrows indicate the preheating time for each temperature.

considerably higher than those attained in the primary one, it is almost sure that protein decomposition is reducing the amount of TProt available for solubilization. The increase in the percentage of sCarb is also higher in the activated sludge, which confirms that the sludge from the secondary treatment is more readily solubilized than that from the primary treatment.

When TS and COD of the same sludge are doubled by thickening, COD solubilization decreases noticeably and perhaps, so does the release of both proteins and carbohydrates, as well, which can be explained by the high solids content dissipating the heat and thereby reducing the possibility of floc disruption (Braguglia et al., 2015). Regarding the influence of the scale at which the experiments are performed, that is, lab or pilot plant scale, Donoso-Bravo et al. (2011) concluded that solubilization of COD was similar in both systems, but protein solubilization was lower at pilot plant scale, whereas carbohydrate solubilization was greater in the lab tests. They attributed it all to a bad temperature control in the small-scale experimental setup. Moreover, the NH_4-N tended to be slightly higher in the lab-experiments. In the pilot plant ones, acetic acid concentration increases with time, propionic acid remains constant and isovaleric acid goes through a maximum at 10 min, whereas in the small-scale experiments, the trends were less clear, and the three acids seemed to go through a maximum at 10 min.

Alkaline hydrolysis at room temperature can perform better (Kim et al., 2003) or worse (Aravinthan et al., 1998) than thermal hydrolysis, depending on the experimental conditions. Acid hydrolysis gives lower solubilizations of COD and proteins than the alkaline one, but improves the release of carbohydrates. The combination of chemicals with heat seems to be more beneficial than heat alone (Aravinthan et al., 1998; Kim et al., 2003; Shier and Purwono, 1994).

Phosphorus solubilization was only reported by three authors (Brooks, 1968; Graja et al., 2005; Morgan-Sagastume et al., 2011), whereas phosphorus recovery (by precipitation as magnesium ammonium phosphate) was mentioned by a fourth one (Arakane et al., 2006), but no systematic study of the features was conducted. Some comments on the temperature dependence of the solubilization/recovery were made by Brooks (1968) and by Arakane et al. (2006), whereas Graja et al. (2005) and Morgan-Sagastume et al. (2011) simply highlighted that the reduction of TP could be higher than the rise of soluble phosphate. The temperature dependence disclosed by Arakane et al. (2006) for phosphorus recovery after precipitation (53% at 130 °C, 95% at 216 °C, 75% at 350 °C) contradicted the trend established by Brooks (1968) for solubilization (58% at 130 °C and 13% at 216 °C), despite of having a close value at 130 °C. The decrease of TP observed in the experiments of both Graja et al. (2005) and Morgan-Sagastume et al. (2011) was attributed by Graja et al. (2005) to the precipitation of calcium and magnesium phosphates in the reactor. High solubilizations can be achieved by acid hydrolysis at pH 1–2 and temperatures around 140–150 °C, which is the base of the KREPRO process (Cassidy, 1998; Hansen et al., 2000), but, strictly speaking, this is “thermochemical hydrolysis” rather than “thermal hydrolysis”, and this is the reason why it has not been included in Tables 2 and S1.

Finally, it should be indicated that there is a lack of in-depth characterization of the products generated during the thermal treatment. For instance, with the sole exception of Shier and Purwono (1994), no author studied the molecular weight distribution of the resulting sProt, sCarb and sLip, and none of them compared it with the distribution before the TH. The determination of functional properties (such as foaming, complexing, antioxidant and surfactant capacity) of the obtained biomolecules and the occurrence and fate of certain microcontaminants (persistent organic pollutants, pharmaceuticals, personal care products, etc.), are not studied either. Obviously, this step is essential in deciding if the hydrolyzed sewage sludge could be suitable for the recovery of valuable biomolecules. It is also worthy to note that there is no information about methods of downstream processing of the hydrolyzed sewage sludge for the separation and purification of interesting biopolymers, nor economical estimations. Fig. 3 summarizes these comments.

3. Wet oxidation

With the exception of some papers, such as that by Kang et al. (2001), which reported on the production of amino acids from waste fish entrails, the majority of the works are focused on VFA, which are the main substances generated by WO of biomass (He et al., 2008; Hii et al., 2014), and especially, on acetic acid, whose concentrations usually exceed those of any other acids. Among these studies, it is particularly interesting the research done by Jin et al. (2010, 2007, 2006, 2005, 2002), who produces the deicer Ca/Mg acetate from food wastes and proposed a two-step method for improving the yield of acetic acid from rice hull, potato starch and cellulose. Unfortunately, sewage sludge has not been studied as thoroughly as these wastes, and many of the papers compiled in Table 3, which is an extract of Table S2 in the Supplementary material, report on the concentrations of VFA and other chemicals without the specific aim of resource production.

Hurwitz and Dundas (1960), indicated in their earlier works that the concentration of the feed did not influence importantly the performance of SubCWO, but these authors did not keep rigorous control of variables such as temperature, residence time and oxidant amount, which affect the oxidation process as well. In fact, WO products depend on the sludge characteristics (Lendormi et al., 2001; Shanableh and Jomaa, 2006, 2005). Gapes et al. (2013) stated that the SubCWO of fermented sludge instead of raw one could be advantageous in improving the yield or the purity of the generated acids, but unfortunately, no additional studies dealing with this subject have been published.

Fig. 4 displays the time and temperature dependence of the acetic acid concentration of the sludge used in the study by Shanableh and Gloyna (1991), Shanableh and Jomaa (2001) and Yin et al. (2015). The

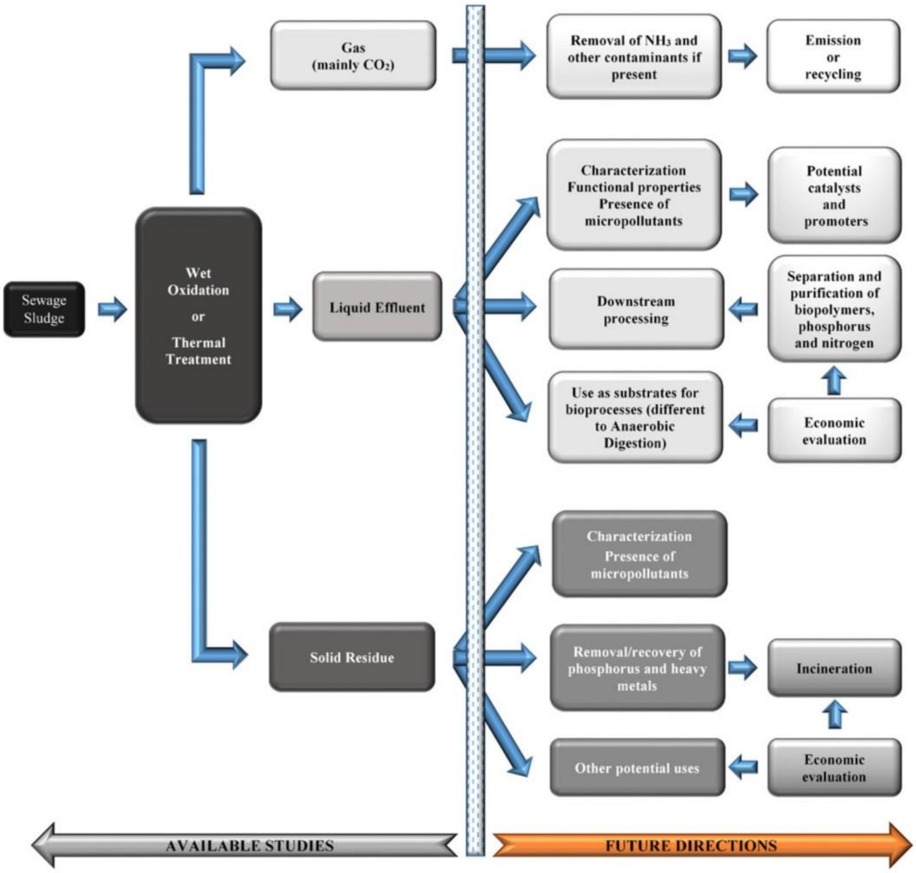


Fig. 3. Summary of the available studies and the future directions on the identified knowledge gaps related to the production of valuable compounds from sewage sludge.

first one was a secondary sludge fed with industrial wastewater and oxidized with O_2, the second one was a mixture of primary and secondary sludge from a WWTP under the action of H_2O_2 and the third one was a sludge of unknown origin treated with an unknown oxidant. In the SubCWO of Yin et al. (2015) and within the experimental error, the amount of acid augments monotonically with both temperature and residence time, as in the SubCWOs of Baroutian et al. (2016) and Prince-Pike et al. (2015). Moreover, at a constant time of 30–90 min, the increase in acid concentration in the range 220–260 °C is greater than the rises observed in the ranges 180–220 °C and 260–300 °C. This is in agreement with the findings of Friedman et al. (1988), who saw a similar abrupt rising between 250 and 270 °C for a digested sludge oxidized with air for 1 h. Nevertheless, the performance of the experiments by Shanableh and Gloyna (1991) and of Shanableh and Jomaa (2001) is very different. There is only concordance at 150 °C with Yin et al. (2015), where the higher the time, the higher the acetic concentration. From 200 to 350 °C, both Shanableh and Gloyna (1991) and Shanableh and Jomaa (2001) observed maxima (Fig. 4a and b). Beyond the critical temperature, Shanableh and Jomaa (2001) continued seeing maxima, whereas Shanableh and Gloyna (1991) reported a monotonic fall of the acetic acid concentration with increasing times, which implies that the variation from subcritical to supercritical water does not always mean a sudden change of the oxidation processes. The trend of the measurements by Shanableh (2000) and Shanableh and Shimizu (2000), carried out with secondary sludge in a continuous flow reactor for 15 and 30 min, is the same than those of the mixed sludge of Shanableh and Jomaa (2001) in a batch reactor. Additionally, it is interesting to point out that researchers as Teletzke et al. (1967) and Wu et al. (1987) saw maxima on the temperature dependence of their acid generation at 225 °C for a fixed reaction time. So, the qualitative form of the concentration-temperature-time plot is likely to be a combination of Fig. 4b and c, this being equal to Fig. 4a at low temperatures and low residence times, while presenting maxima at higher temperatures. As the temperature increases, the maxima are lower and appear at lower times; and at supercritical conditions (but not necessarily in the close vicinity of the supercritical temperature, perhaps far beyond), maxima vanish.

The effect of other variables in the generation of acetic acid, as raw sludge concentration and oxidant to TCOD ratio, has been less studied. In the case of an anaerobically digested sludge, Wu et al. (1987) reported that, for 1.1–10% TS, the higher the solids in the feed, the higher the acid generation, in agreement with Khan et al. (1999), who oxidized a secondary sludge with 0.12–3.8% TVS. These results are rather predictable, since the higher the solids, the higher the organic load, and therefore, more compounds can be degraded to acetic acid. The influence of the oxygen content at three different temperatures was analyzed by Prince-Pike et al. (2015) and by Baroutian et al. (2015), who found inconsistent trends, as can be read in Table S2. The latter attributed these trends to the fact that reaction kinetics, solubility and gas–liquid mass transfer depend on the interaction of temperature and oxidant amount, and not on each parameter individually.

With regard to the other VFA, it seems that, if there are considerable amounts present in the feed, their concentrations fall as the reaction progresses at a given temperature (Andrews et al., 2015; Baroutian et al., 2016; Prince-Pike et al., 2015), although in some of the experiments by Shanableh and Jomaa (2001) this trend is not so clear. If they are not

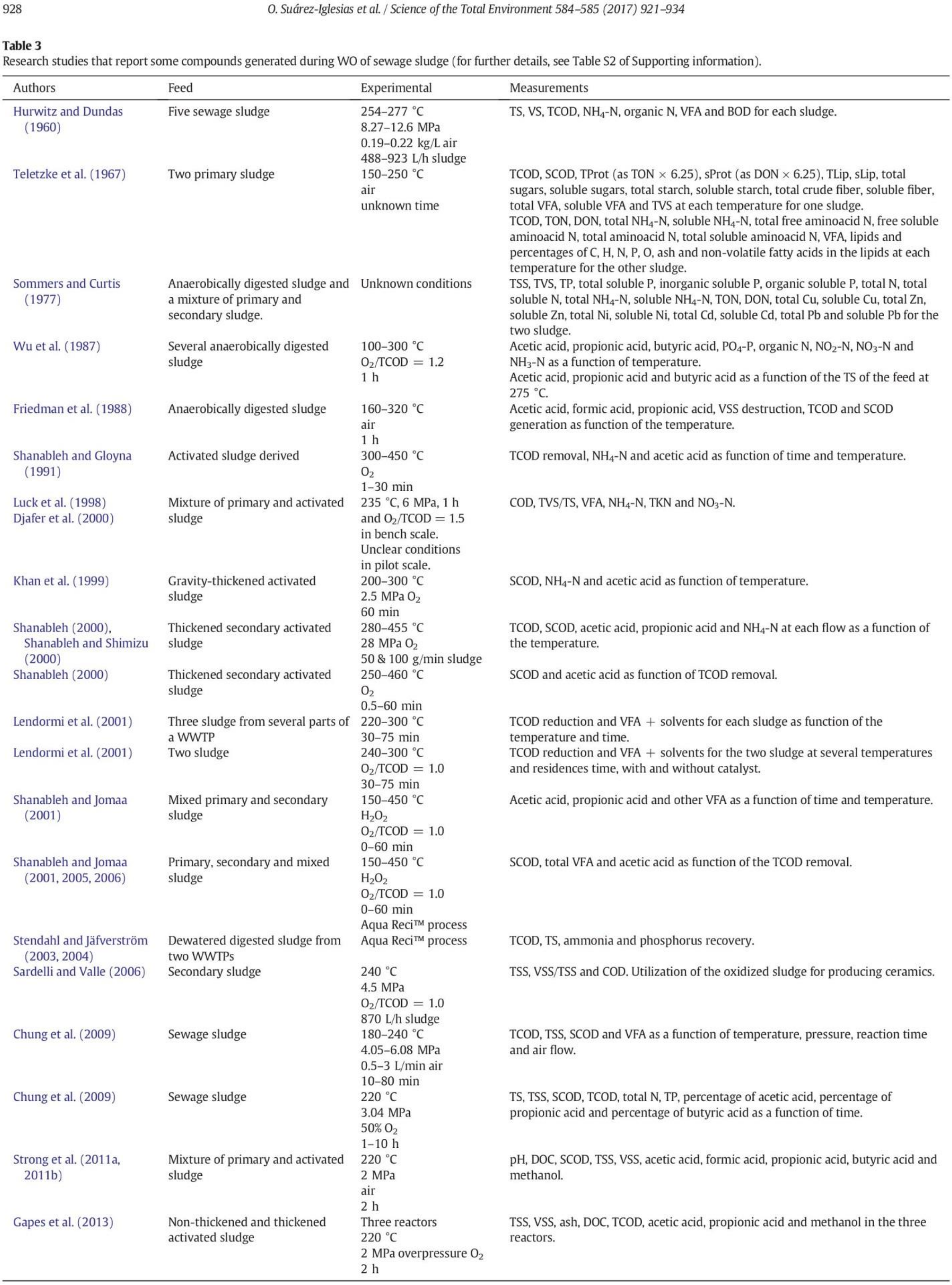


Table 3
Research studies that report some compounds generated during WO of sewage sludge (for further details, see Table S2 of Supporting information).

Authors	Feed	Experimental	Measurements
Hurwitz and Dundas (1960)	Five sewage sludge	254–277 °C 8.27–12.6 MPa 0.19–0.22 kg/L air 488–923 L/h sludge	TS, VS, TCOD, NH_4-N, organic N, VFA and BOD for each sludge.
Teletzke et al. (1967)	Two primary sludge	150–250 °C air unknown time	TCOD, SCOD, TProt (as TON × 6.25), sProt (as DON × 6.25), TLip, sLip, total sugars, soluble sugars, total starch, soluble starch, total crude fiber, soluble fiber, total VFA, soluble VFA and TVS at each temperature for one sludge. TCOD, TON, DON, total NH_4-N, soluble NH_4-N, total free aminoacid N, free soluble aminoacid N, total aminoacid N, total soluble aminoacid N, VFA, lipids and percentages of C, H, N, P, O, ash and non-volatile fatty acids in the lipids at each temperature for the other sludge.
Sommers and Curtis (1977)	Anaerobically digested sludge and a mixture of primary and secondary sludge.	Unknown conditions	TSS, TVS, TP, total soluble P, inorganic soluble P, organic soluble P, total N, total soluble N, total NH_4-N, soluble NH_4-N, TON, DON, total Cu, soluble Cu, total Zn, soluble Zn, total Ni, soluble Ni, total Cd, soluble Cd, total Pb and soluble Pb for the two sludge.
Wu et al. (1987)	Several anaerobically digested sludge	100–300 °C O_2/TCOD = 1.2 1 h	Acetic acid, propionic acid, butyric acid, PO_4-P, organic N, NO_2-N, NO_3-N and NH_3-N as a function of temperature. Acetic acid, propionic acid and butyric acid as a function of the TS of the feed at 275 °C.
Friedman et al. (1988)	Anaerobically digested sludge	160–320 °C air 1 h	Acetic acid, formic acid, propionic acid, VSS destruction, TCOD and SCOD generation as function of the temperature.
Shanableh and Gloyna (1991)	Activated sludge derived	300–450 °C O_2 1–30 min	TCOD removal, NH_4-N and acetic acid as function of time and temperature.
Luck et al. (1998) Djafer et al. (2000)	Mixture of primary and activated sludge	235 °C, 6 MPa, 1 h and O_2/TCOD = 1.5 in bench scale. Unclear conditions in pilot scale.	COD, TVS/TS, VFA, NH_4-N, TKN and NO_3-N.
Khan et al. (1999)	Gravity-thickened activated sludge	200–300 °C 2.5 MPa O_2 60 min	SCOD, NH_4-N and acetic acid as function of temperature.
Shanableh (2000), Shanableh and Shimizu (2000)	Thickened secondary activated sludge	280–455 °C 28 MPa O_2 50 & 100 g/min sludge	TCOD, SCOD, acetic acid, propionic acid and NH_4-N at each flow as a function of the temperature.
Shanableh (2000)	Thickened secondary activated sludge	250–460 °C O_2 0.5–60 min	SCOD and acetic acid as function of TCOD removal.
Lendormi et al. (2001)	Three sludge from several parts of a WWTP	220–300 °C 30–75 min	TCOD reduction and VFA + solvents for each sludge as function of the temperature and time.
Lendormi et al. (2001)	Two sludge	240–300 °C O_2/TCOD = 1.0 30–75 min	TCOD reduction and VFA + solvents for the two sludge at several temperatures and residences time, with and without catalyst.
Shanableh and Jomaa (2001)	Mixed primary and secondary sludge	150–450 °C H_2O_2 O_2/TCOD = 1.0 0–60 min	Acetic acid, propionic acid and other VFA as a function of time and temperature.
Shanableh and Jomaa (2001, 2005, 2006)	Primary, secondary and mixed sludge	150–450 °C H_2O_2 O_2/TCOD = 1.0 0–60 min Aqua Reci™ process	SCOD, total VFA and acetic acid as function of the TCOD removal.
Stendahl and Jäfverström (2003, 2004)	Dewatered digested sludge from two WWTPs	Aqua Reci™ process	TCOD, TS, ammonia and phosphorus recovery.
Sardelli and Valle (2006)	Secondary sludge	240 °C 4.5 MPa O_2/TCOD = 1.0 870 L/h sludge	TSS, VSS/TSS and COD. Utilization of the oxidized sludge for producing ceramics.
Chung et al. (2009)	Sewage sludge	180–240 °C 4.05–6.08 MPa 0.5–3 L/min air 10–80 min	TCOD, TSS, SCOD and VFA as a function of temperature, pressure, reaction time and air flow.
Chung et al. (2009)	Sewage sludge	220 °C 3.04 MPa 50% O_2 1–10 h	TS, TSS, SCOD, TCOD, total N, TP, percentage of acetic acid, percentage of propionic acid and percentage of butyric acid as a function of time.
Strong et al. (2011a, 2011b)	Mixture of primary and activated sludge	220 °C 2 MPa air 2 h	pH, DOC, SCOD, TSS, VSS, acetic acid, formic acid, propionic acid, butyric acid and methanol.
Gapes et al. (2013)	Non-thickened and thickened activated sludge	Three reactors 220 °C 2 MPa overpressure O_2 2 h	TSS, VSS, ash, DOC, TCOD, acetic acid, propionic acid and methanol in the three reactors.

Table 3 (*continued*)

Authors	Feed	Experimental	Measurements
Gapes et al. (2013)	Raw and fermented sludge	220 °C 20 bar overpressure O_2 (4–5 MPa total pressure 4 h	VFA yield, VFA purity, acetic acid yield and acetic acid purity for both sludge.
Andrews et al. (2015)	Mixture of anaerobically-digested primary and secondary sludge	220 °C 4.3 MPa total pressure O_2 1–5 h	VSS, acetic acid, propionic acid and other VFA as function of time.
Baroutian et al. (2015)	Mixture of anaerobically-digested primary and secondary sludge	220–240 °C O_2 to biomass ratio of 1:1–2:1 (2 to 4 MPa O_2) 60 min unspecified stirring speed	TSS, VSS, TCOD, DOC, acetic acid, propionic acid, butyric acid, isobutyric acid, NH_4-N and DNK as function of temperature and of the oxygen to biomass ratio.
Prince-Pike et al. (2015)	Mixture of anaerobically-digested primary and secondary sludge	The same than Baroutian et al. (2015) 300–500 rpm 0–60 min	PCOD, TCOD, SCOD, acetic acid and other VFA as a function of time, stirring speed and pressure.
Prince-Pike et al. (2015)	Mixture of anaerobically-digested primary and secondary sludge	Unspecified temperature 20% oxidant 0–260 min	PCOD, sCOD, TCOD, acetic acid and other VFA as a function of time.
Yin et al. (2015)	Dewatered sewage sludge	180–300 °C unknown oxidant 0–90 min	DOC, NH_4-N, sCarb, sProt and acetic acid as a function of time and temperature.
Baroutian et al. (2016)	Mixture of anaerobically-digested primary and secondary sludge.	220–240 °C 0–60 min oxygen to biomass ratio of 1:1 (2 MPa O_2)	Acetic, propionic, butyric, isobutyric and pentanoic acids and methanol as a function of time and temperature.
Urrea et al. (2016)	Activated sludge	190 °C 65 bar Oxygen 0–210 min 500 rpm	Molecular weight distribution of the products generated as a function of reaction time.

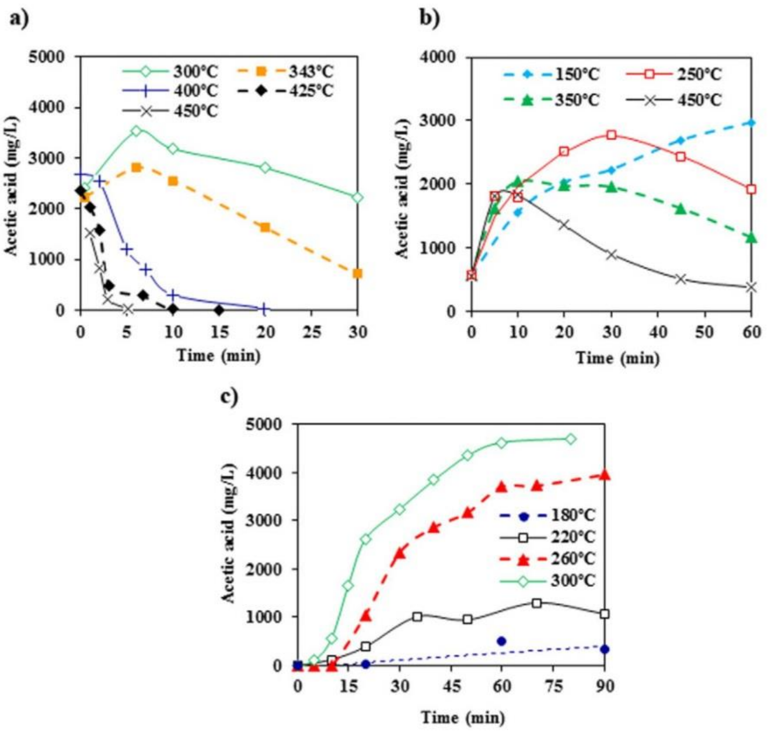


Fig. 4. Temperature and reaction time dependence of the acetic acid concentration for a) Shanableh and Gloyna (1991); b) Shanableh and Jomaa (2001) and c) Yin et al. (2015). Figure has been adapted from these three references.

present in the raw sludge or their concentrations are low, they are produced (Gapes et al., 2013; Strong et al., 2011a) and can remain or disappear (Shanableh and Jomaa, 2001). At a constant reaction time, the concentrations of these VFA decrease with increasing temperature (Baroutian et al., 2016; Shanableh, 2000; Wu et al., 1987) or go through a maximum (Friedman et al., 1988). Chung et al. (2009) reported that in their sewage sludge, propionic acid concentration rises from 0 mg/L at 180 °C to 13.5 mg/L at 240 °C, but this is probably because the propionic acid concentration goes through a maximum at higher temperatures. The effect of oxygen amount at 60 min and 220 °C, 230 °C and 240 °C is as complex as in the case of acetic acid (Baroutian et al., 2015). At constant temperature and time, Wu et al. (1987) observed that propionic and butyric acid concentrations did not become higher when increasing the TS of the feed, but go through maxima at around 6.4% TS. Occasionally, other substances than VFA are formed during the reaction, as formaldehyde, alcohols and/or ketones (Baroutian et al., 2016; Gapes et al., 2013; Khan et al., 1999; Lendormi et al., 2001; Strong et al., 2011a).

In Table S2, TCOD decreases with both temperature and oxidation time (Chung et al., 2009; Lendormi et al., 2001; Shanableh, 2000; Shanableh and Gloyna, 1991). Between 220 and 300 °C, Lendormi et al. (2001) pointed out that the higher the TCOD reduction, the higher the fraction of this TCOD that was due to VFA (acetic acid plus others). These authors, at 240 °C, observed that the use of a copper based catalyst allowed for a greater reduction of TCOD but had no effect on the fraction due to VFA. Nonetheless, this last finding could be a coincidence, since Luck et al. (1998) also tested the utilization of copper at 235 and 285 °C, and only found a significant reduction of the organic matter at 235 °C. In this regard, they insinuate that this poor performance of the catalyst at high temperatures was caused by the increase in the TCOD fraction due to VFA, and especially, to acetic acid.

As mentioned in Section 2, VFA are mainly dissolved, and therefore, their contribution to the TCOD is also a contribution to the SCOD. Thus, the SCOD and DOC evolution with time and temperature for the sewage sludge of Chung et al. (2009) and of Yin et al. (2015) were plotted in Fig. 5a and b, respectively. Qualitatively speaking, they are very similar: at low temperatures the soluble organic matter rises with time and at high temperatures, it goes through a maximum. Prince-Pike et al. (2015) also saw this maximum in the SCOD concentration in their experiments with a fermented mixture of primary and secondary sludge. The increase is due to solubilization (as in TH) and the fall is caused by oxidization of the dissolved compounds in the liquid phase, the first stage being faster than the second one. Furthermore, in the work by Chung et al. (2009), the solubilization step is quicker than in the work by Yin et al. (2015), and this fact can explain why the temperature dependence in Fig. 5a at 10 min and 20 min resembles that in Fig. 5b at 55 min and 80 min. For a fixed time of 60 min, Baroutian et al. (2015) observed maxima in the DOC vs. temperature plot, as well.

The shape of the figure curves at 180–220 °C suggests that it could be a narrow range of temperatures where, after passing through the maximum with time, SCOD would quickly reach a plateau. Urrea et al. (2016) have demonstrated this behaviour in a recent paper. Additionally, it was determined, for the first time ever, the molecular weight distribution of the solubilized matter during a SubCWO treatment by size exclusion chromatography (Fig. 6, adapted from Urrea et al. (2016)), although the solute-column interactions made that part of the molecules appeared at elution volumes higher than the total volume of the column.

Besides, Yin et al. (2015) also studied the time and temperature dependence of sProt, NH4-N and sCarb, and found that at 220–300 °C, sProt and sCarb went through a maximum with the reaction time, and concluded that, the higher the temperature, the lower the time at which the maximum appeared. NH_4-N rose monotonically with time and temperature, being indicative of considerable decomposition of TProt. At 180 °C, sProt reached a maximum, but it was not clear if sCarb reached it as well or increased monotonically with time.

At constant temperature, NH_4-N falls with increasing time in the publication by Shanableh (2000), and, at a constant reaction time, it augments with temperature in the experiments by Teletzke et al. (1967) and by Wu et al. (1987). Nonetheless, in the WO of Sommers and Curtis (1977), the NH_4-N in the oxidized effluent is lower than in the feed, and in the paper of Baroutian et al. (2015), NH_4-N decreases with increasing temperature after 60 min reaction (except at oxygen to biomass ratio of 2:1, where the ammonia nitrogen goes through a maximum at 230 °C). The data of these last two groups could be in accordance with the results of Shanableh and Gloyna (1991), who observed maxima in the plots of NH_4-N vs. time, and that the higher the temperature, the lower the time at which the maximum appeared. In principle, it could be thought that NH_3 was oxidized to N_2, but Baroutian et al. (2015) stated that there was no evidence of molecular nitrogen formation.

At fixed temperature, oxygen pressure and reaction time, the higher the TVS in feed, the higher the production of ammonia (Khan et al., 1999).

Teletzke et al. (1967) measured the nitrogen due to amino acids, and compared it with the non-ammonia nitrogen, finding that the first one was always considerably lower than the second one, and proving again that the use of DON × 6.25 or of TON × 6.25 as a method for the estimation of protein concentration was not precise. At a constant oxidation time, it was observed that the higher the temperature, the lower the TCOD, the TVS, and total organic compounds, except for VFA. SCOD, sProt, sLip went through a maximum, as in the work by Yin et al. (2015) at low reaction times. Most of the amino acids in the oxidized sludge, both free and forming polypeptides, were soluble, and the higher the temperature, the higher the fraction of free amino acids. The lipids of raw sludge and sludge oxidized at 175 °C were composed of around 50% of total non-volatile fatty acids (fatty acid esters + free acids) and in all the oxidized sludges, nearly all of these acids were in the free state. Sugars were not present in the feed, and the small

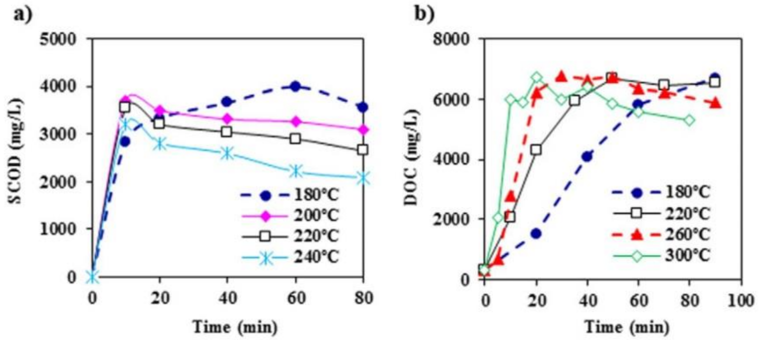


Fig. 5. Time and temperature dependence of a) SCOD in the sludge of Chung et al. (2009); b) DOC in the sludge of Yin et al. (2015). Figures have been adapted from these two references.

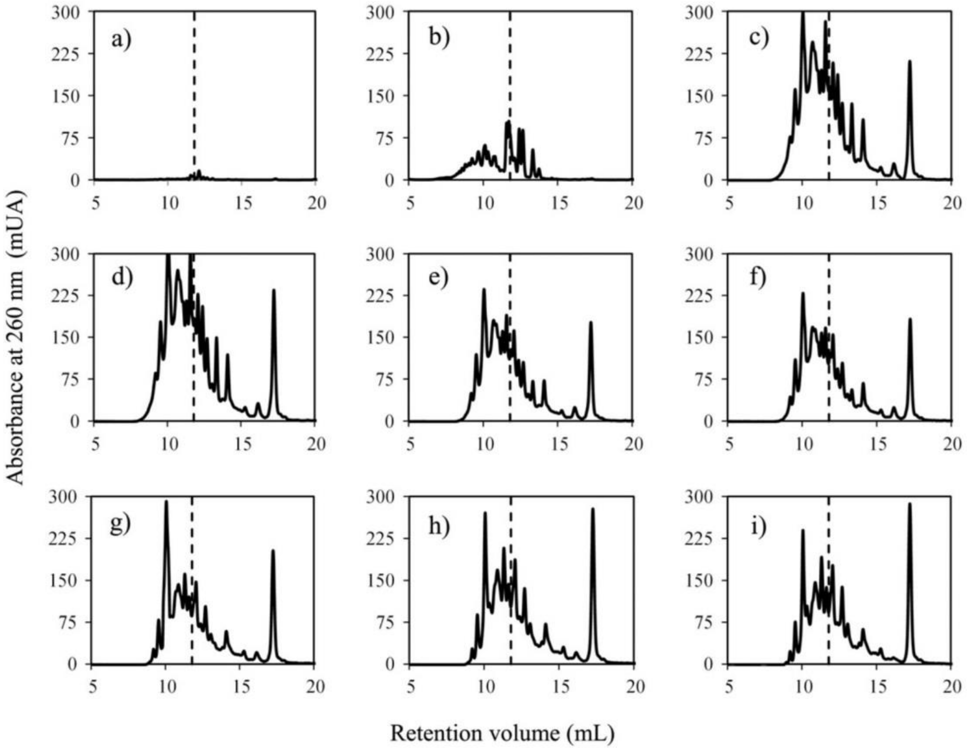


Fig. 6. Evolution of Spanish activated sludge fingerprints during WO at 65 bar, according to Urrea et al. (2016): a) Feed; b) 40 min; c) 90 min; d) 100 min; e) 110 min; f) 120 min; g) 140 min; h) 170 min; i) 210 min. Temperature: 100 °C at 40 min, and 190 °C at 90 min and from then on. Flow rate of the mobile phase: 1 mL/min. Total volume of the column (indicated with a dashed line): 11.8 mL. Calibration curve: V(mL) = 15.36–3.532 log [M(kDa)].

amounts found in the sludge treated at 150 and 175 °C were attributed to hydrolysis of carbohydrates.

Both Teletzke et al. (1967) and Wu et al. (1987) alleged that soluble phosphorus decreases because it is precipitated as calcium and magnesium salts. Sommers and Curtis (1977) pointed out that WO augments the percentage of particulate phosphorus and reduces the percentage of the soluble one with respect to the feed. These high quantities of this valuable element in the particles, led Stendahl and Jäfverström (2003, 2004) to propose its recovery by leaching after filtering, and also led Sardelli and Valle (2006) to suggest the use of the dried solids in devising ceramic products. Additionally, the presence of phosphates in the solid, together with hydroxides and carbonates, can allow for the immobilization/recovery of most of the heavy metals present in the non-oxidized sludge (Djafer et al., 2000; Friedman et al., 1988), although there are cases in which the WO facilitates the solubilization of several metals (Sommers and Curtis, 1977).

In conclusion, the available literature is mainly focused on maximizing the production of acetic acid in order to improve the subsequent process of anaerobic digestion. For this reason, the effect of the operating conditions of wet oxidation on the formation and selectivity of other products, including alcohols, ketones and more complex VFA, should be studied in depth. Likewise, the use of different catalysts or oxidation promoters, which can enhance either the functional properties or the amount of the biomolecules generated, is another topic, which needs further investigation. The use of the effluent treated by wet oxidation as a substrate for other bioprocesses different than the anaerobic digestion could potentially become a future research line. Eventually, most of the literature deals with the characterization of the liquid effluent after wet oxidation, whereas very few papers describe the properties and potential uses of the solid residue (non-solubilized matter). Besides, none of them discusses the presence of micropollutants, the reduction or formation of these contaminants during the oxidation (Collado et al., 2013) and the quality of the produced products. Part of these comments are summarized in Fig. 3.

4. Comparison of thermal hydrolysis and wet oxidation

Table 4 sums up the findings of the few works that compare the performance of TH and SubCWO for the same sludge (no SCWO data available). These findings are explained in detail in Table S3 of the Supplementary material. Some of the studies on TH are performed at temperatures higher than 180 °C, where the formation of hydrochars and bio oils can become important, but nothing is said about these products in the cited texts. The biodegradability and the toxicity of the hydrolyzed material in these conditions was not addressed, either. As expected, when SubCWO is performed at temperature equal to or higher than that of TH (but always above 120 °C), oxidation is more effective than heating alone in the solubilization of nutrients, producing VFA and reducing solids and total organics (Brooks, 1968; Marshall and Gillespie, 1974; Quitain et al., 2002; Strong et al., 2011b; Yang et al., 2010).

When SubCWO is carried out at a temperature lower than that of TH, the results can be the opposite (Yang et al., 2010). Quitain et al. (2002) compared the amount and type of VFA produced at 250, 300 and 350 °C and observed that formic acid concentration fell when temperature was increased from 250 °C to 350 °C, and acetic acid content rose, whereas the production of lactic acid decreased with temperature for SubCWO and went through a tiny maximum for TH.

Aggrey et al. (2011) compared TH at 140 °C, SubCWO at 220 °C and the two-stage process that Jin et al. (2007, 2006, 2005) developed to increase the production of acetic acid from vegetable wastes. This two-stage process consisted of an initial TH at 140 °C to generate furans

Table 4
Research studies that compare the compounds generated during the application of TH and WO to the same sludge in the same experimental device (for further details, see Table S3 of Supporting information).

Authors	Feed	Experimental	Measurements
Brooks (1968)	Activated sludge	100–197 °C air or argon 1 h	SCOD, DON, insoluble N, total N, NH_4-N, SS, TS, Col solids, VFA and soluble PO_4 as a function of temperature for TH and WO.
Marshall and Gillespie (1974)	Activated sludge	TH at 149–250 °C for 20–45 min WO at 149–315 °C with O_2 for 30–45 min 0.7–20.7 MPa 1750 rpm	TS, nitrogen and phosphate.
Quitain et al. (2002)	Dewatered sewage sludge	250–350 °C at saturation H_2O_2 unspecified time	Succinic acid, lactic acid, propionic acid, acetic acid and formic acid as a function of temperature for TH and WO.
Yang et al. (2010)	Waste activated sludge	WAO at 100–300 °C TH at 200 °C 1 MPa 30 min	TCOD, SCOD/TCOD, BOD/TCOD, VFA and acetic acid for the three WAO and the TH.
Strong et al. (2011a, 2011b)	A mixture of belt-pressed activated sludge and primary one	TH at 140 & 165 °C under N_2 WO at 220 °C under O_2 20 bar 2 h 300 rpm	TSS, VSS, carbon, nitrogen, pH, DOC, SCOD, VFA, methanol and acetic, formic, propionic, butyric acid for the two TH and the WO.
Aggrey et al. (2011)	Mixed primary and secondary sludge	TH (140 °C, 1 MPa, N_2, 120 min) WO (220 °C,1 MPa, O_2, 120 min) a two-stage process (TH + WO) 355 rpm	SCOD/TCOD, SCOD/DOC, unidentified DOC, TCOD removal, Carb (galactosamine, glucosamine, arabinose, galactose, glucose, xylose and mannose), acids (formic, acetic, propionic, butyric and pentanoic) and yield and purity of the acetic acid produced in the TH, in the WO and in the 2-stage process.

and lactic acid, followed by a SubCWO at 220 °C for converting these chemicals into the desired acid. However, in opposition to the results of vegetable wastes, SubCWO alone gives the best yield and purity of acetic acid for sewage sludge, proving that the information obtained from vegetables cannot be extrapolated to that obtained with sludge. Furthermore, it was found that TH alone generates more soluble carbohydrates than SubCWO or TH + SubCWO, and that the two-stage method produces more soluble glucose, unidentified DOC, formic, propionic, butyric and pentanoic acids than SubCWO or TH.

Table S4 of the Supplementary material compiles the effect of varying the oxygen to TCOD ratio from 0 to 1, 2 or 4, and Table 5 is a short version of it. Additionally, Shanableh and Jomaa (2001) compared the performance of a primary sludge with that of a secondary one in subcritical conditions. On the contrary, the publications in Tables 4 and S3, Goto et al. (1998) and Qian et al. (2015), reported on the presence of hydrochar, and Qian et al. (2015) analyzed the composition of the synthesis gas formed.

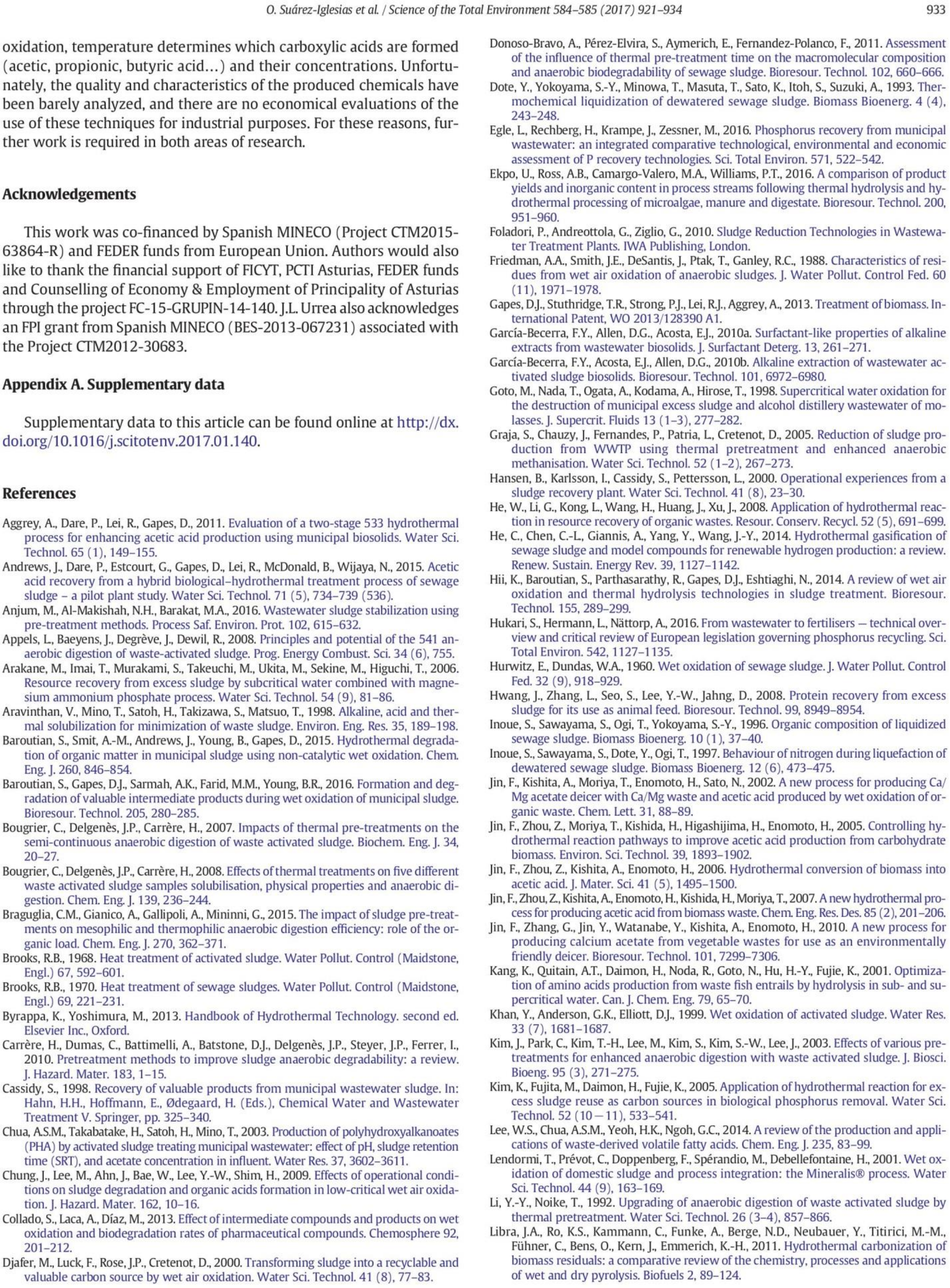
When increasing the amount of oxidant, VFA concentrations go through a maximum or decline continuously, achieving a value of zero when O_2/TCOD = 1.0 at 385 °C and when the ratio was 0.5 at 450 °C. At subcritical conditions, the zero VFA content is not achieved even at a ratio of 2.0. Acetic acid is the most abundant VFA, followed by propionic acid (Goto et al., 1998; Shanableh and Jomaa, 2001). Secondary sludge generates more acetic acid than the primary one.

5. Conclusions

Sewage sludge contains high amounts of water, and therefore, thermal hydrolysis and wet oxidation can be used to obtain chemical compounds from it. Thermal hydrolysis is a non-oxidative technology, which solubilizes proteins, carbohydrates, lipids or cut them into small fragments, whereas wet oxidation tends to degrade these substances into volatile fatty acids, mainly acetic acid. Thermal hydrolysis is usually employed as pretreatment for the methanogenic fermentation, whereas wet oxidation is commonly utilized for the final destruction of the sludge. The literature concerning the generation of valuable products by both methods is relatively scarce. However, interesting conclusions can be achieved: i) secondary sludge seems to be more suitable than the primary one for both processes. ii) reaction time is more important for wet oxidation than for thermal hydrolysis. iii) In thermal hydrolysis processes, temperature enhances the solubilization of biomolecules but also their decomposition and unwanted reactions, whereas in wet

Table 5
Research studies focused on the effect of the oxygen level fed into the reactor on the products obtained during hydrothermal treatment of sewage sludge (for further details, see Table S4 of the Supplementary material).

Authors	Feed	Experimental	Measurements
Goto et al. (1998)	Sludge	385 °C 30 MPa H_2O_2 O_2/TCOD: 0–1 30 min	TOC, NH_4-N and formic, acetic, propionic, butyric, isovaleric and succinic acid as a function of the oxidant to TCOD ratio.
Shanableh and Jomaa (2001)	Primary and secondary sludge	H_2O_2 300 °C O_2/TCOD: 0–2 30 min	Acetic, propionic and other acids for the two sludge as a function of the oxidant to TCOD ratio.
Qian et al. (2015)	Sewage sludge	450 or 550 °C 25 MPa H_2O_2 O_2/TCOD = 0–4 20 min	TOC, NH_4-N, total average relative molecular weight, percentage of remaining TS, percentage of acids, hydrocarbons and esters, percentage of amides, percentage of phenol, cresols and aromatic acids, percentage of pyridine, pyrazine and related compounds and percentage of cyclic ketonic compounds for 450 °C (O_2/TCOD = 0.0 and 0.5) and 550 °C (O_2/TCOD = 4.0).



oxidation, temperature determines which carboxylic acids are formed (acetic, propionic, butyric acid…) and their concentrations. Unfortunately, the quality and characteristics of the produced chemicals have been barely analyzed, and there are no economical evaluations of the use of these techniques for industrial purposes. For these reasons, further work is required in both areas of research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.01.140.

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4.2. EFFECT OF OPERATING CONDITIONS OF HYDROTHERMAL TREATMENTS ON SLUDGE PROPERTIES

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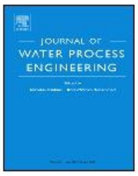

Rheological behaviour of activated sludge treated by thermal hydrolysis

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ABSTRACT

The effects that thermal hydrolysis produces on the rheological properties of activated sludge of a sewage treatment plant were analyzed. Tests were carried out using thermal hydrolysis at different temperatures (160–200 °C) and pressures (40–80 bar), to determine their effect on the characteristics of the waste and the evolution of the transformations that the sludge undergoes during this treatment. A decrease in the concentration of solids has a positive influence on the rheological parameters of the effluent. Empirical equations are proposed for modelling these changes. Thermal hydrolysis causes a considerable reduction in the apparent viscosity of up to two orders of magnitude, improving the fluidity of the effluent and its fluid behaviour is transformed from that of a Bingham plastic for the initial sludge, to a Newtonian fluid at the end of the treatment. The increase in temperature meant an improvement in the rates of transformations of the material, while the changes in pressure had only a minimal effect.

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1. Introduction

The use of activated sludge systems in urban wastewater treatment plants (WWTPs) is accompanied by the inevitable inconvenience of the generation of an excess of sludge, which usually possesses difficult dehydration characteristics. These are associated with the presence of extracellular polymers (ECP), which are present in variable amounts and produced as a highly hydrated capsule surrounding the bacterial cell wall [1]. These ECP also significantly affect flocculation, settling, dewatering, heavy metal absorption and biodegradability [2]. The achievement of sludge with a lower moisture content requires pre-treatment processes, in particular the modification of the structure of the flocs of which it is composed, causing the bacterial cells to rupture and release their content [3,4]. Several pre-treatments can be considered to accelerate the hydrolysis of sludge, such as thermal, alkaline, Fenton, ozone, ultrasonic or mechanical treatments, together with various treatments employing a combination of techniques [5].

Thermal hydrolysis is a process that has the purpose of increasing the solubilization of organic matter through partial or total destruction of the cell structures of the bacteria generated by the heating of the sludge. It is usually employed as a pre-treatment of sludge, prior to its being introduced into an anaerobic digester, improving the rate of degradation of the components, which are more easily assimilated by the microorganisms and also increasing the production of biogas [6–8]. The good results achieved with thermal hydrolysis in the fractionation of macromolecules have allowed its application in the treatment of other residues [9].

The use of thermal hydrolysis leads to increased speed, stability and performance during anaerobic digestion, in addition to reducing the viscosity of the sludge, thus making it easier to handle. The best results are obtained when operating with secondary sewage sludge, although, any type of sludge can be treated by this process, with good results [10]. The conditions of operation usually include temperatures in the range of 160–180 °C, pressures below 13 bar and residence times of 30 min. The temperature is considered to be the most important factor because it seems to determine the extent to which the disintegration of the sludge occurs [11]. The use of higher temperature leads to the formation of dissolved organic compounds of a biorefractary nature [12,13], known as melanoidins [14,15].

The reactions involved in the thermal hydrolysis of sludge and their kinetics are not well documented. Burger and Parker [16] tested a model for activated sludge subjected to thermal hydrolysis, based on results of respirometry and using a phased approach to estimate the best adjustment parameters of the model. Imbierowicz and Chacuk [17] propose a lumped kinetic model, assuming that

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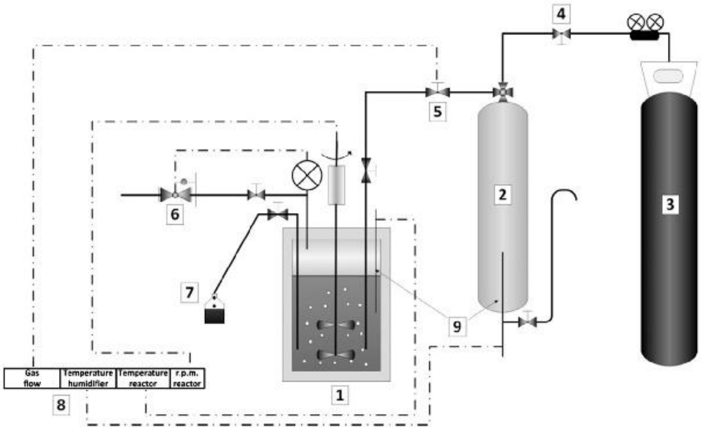

Fig. 1. Scheme of the experimental setup. (1) Reactor, (2) humidifier, (3) gas tank, (4) flow controller, (5) check valve, (6) pressure control, (7) sampling, (8) controller PID, (9) thermocouple.

during the heating, two reactions in parallel are produced. In the first reaction, organic particles are solubilized, which increases the concentration of dissolved organic carbon in the liquid phase, while for the second reaction an insoluble solid phase that decomposes into carbon dioxide is obtained.

The rheological behaviour of the sludge is an important property that needs to be studied for the different pre-treatments, since the viscosity affects operations such as pumping of sludge, the hydrodynamics of bioreactors and the transfer of oxygen. An understanding of the behaviour of the material, through the study of its deformation and flow caused by the application of a shear, is considered crucial for improving the management of wastewater treatment plants, for example for the calculation of pressure losses during transport in pipes, the selection of pumps and for the design of aeration systems [18,19].

The main rheological models used to characterize steady state laminar flow for suspensions of microorganisms are the Bingham model and the Herschel–Bulkley model, in particular when the characterization is performed starting from rest [20].

The flow characteristics of the sludge are highly dependent on the concentration of solids [21–23]. It has been suggested that the concentration of suspended solids has an exponential relationship with the viscosity of the sludge [24]. In another study by Baroutian et al. [25], using the Herschel–Bulkley model for a mixture of primary and secondary sewage sludge, it was determined that the concentration of solids and the temperature have a significant impact on the yield stress and the parameters of the model.

Although several studies related to the rheological characterization of sewage sludge have been carried out [26,27], there is little information available on the changes in the properties of the sludge when treated by thermal hydrolysis.

The aim of this study is to analyze the changes in the chemical and rheological characteristics of sewage sludge when subjected to treatment by thermal hydrolysis, including the effect of temperature and pressure conditions.

2. Materials and methods

Thickened activated sludge from a municipal WWTP (Asturias, Spain) was used for the experiments. This sludge had a concentration of total suspended solids (TSS) = 23.3 g/l, total chemical oxygen demand (TCOD) = 27,550 mgO_2/l, soluble chemical oxygen demand (SCOD) = 650 mgO_2/l and pH = 6.69. To maintain the initial conditions, the sludge was stored at a temperature of 4 °C.

A one-litre PARR reactor (series 4520) was used to carry out the hydrolysis of the sludge. It was equipped with a stirrer with two propellers to enable continuous mixing of the solid and liquid phase. A 2 l steel humidifier tank was installed, through which the gas was passed before being introduced into the reactor. A proportional integral differential controller was used to regulate the temperature of the reactor and the humidifier and also the gas flow rate. As a safety measure, both the reactor and the humidifier were filled to only 70% capacity. A diagram of the system is shown in Fig. 1.

Once fed into the reactor, the sludge was preheated to 70 °C, then the temperature was set at the defined value for the experiment and the pressure was adjusted by a feedback control valve located at the gas output. To increase the pressure inside the reactor, pure nitrogen was injected at a fixed flow rate of 1200 ml/min. The stirrer speed was maintained at 500 rpm during the experiment. An experiment was repeated twice under the same conditions, showing that the procedure has good reproducibility, error values lower than 15% of the average value being obtained.

A series of analyses were performed to characterize the initial sludge and the samples taken at different times. Analytical parameters of total (TCOD) and soluble chemical oxygen demand (SCOD), total (TSS) and volatile (VSS) suspended solids, sludge volume index (SVI) and pH were determined. All these analyses were carried out in accordance with the Standard Methods [28].

Rheological tests were carried out using a rotational rheometer Haake MARS II with a Peltier unit to control the temperature. A plate/plate measuring system (PP60Ti) was used, with a gap of 1 mm. Flow curves were carried out in CR mode from 0.1 to 100 s^{-1} in 180 s at 25 °C and temperature ramps were developed at a constant shear rate of 10 s^{-1} from 5 to 100 °C in 600 s. Before starting any measurement, the samples rested for at least 5 min, allowing induced stresses to relax. All tests were carried out at least in duplicate.

3. Results and discussion

3.1. Characterization of the transformations of the sludge

3.1.1. Thermal hydrolysis at different temperatures

The results showed a slight improvement in the rate at which the transformations of the sludge occurred when the temperature increased from 160 to 200 °C (Fig. 2). It can be observed that the

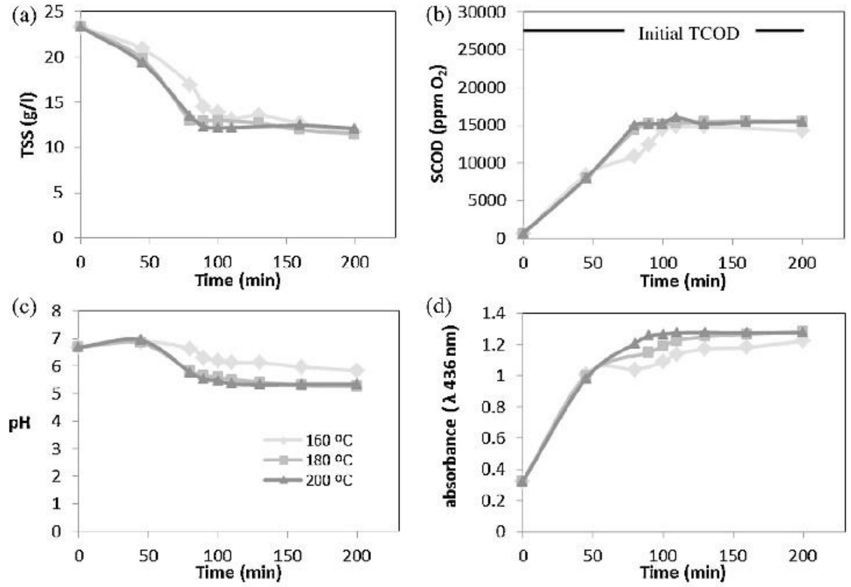

Fig. 2. Influence of temperature on the thermal hydrolysis (a) TSS, (b) SCOD, (c) pH, (d) absorbance. In all cases $P = 60$ bar.

transformations, in particular the solubilization, reach a limiting value after 80 min when working at the highest temperature, while 30 min more is needed when operating at 160 °C.

The reduction of the TSS obtained when the reaction temperature is reached (around 80 min) was 27% at 160 °C. This value was significantly lower compared with those obtained at 180 and 200 °C (44 and 42%). For the three temperatures used, the final reduction of TSS after more than 120 min reaches 50%. This phenomenon can also be observed in the evolution of SCOD. When the reaction temperature was attained, the SCOD reached 39% of the TCOD at 160 °C, while at 180 and 200 °C it reached 52 and 54% respectively. The final SCOD reduction reaches 56%.

The changes in pH were caused mainly by the formation of ammonia and volatile fatty acids produced by the hydrolysis of proteins and lipids [11]. The use of higher temperature means a greater acidification of the medium, suggesting an increased production of short chain fatty acids. The final pH at 160, 180 and 200 °C was 5.84, 5.28 and 5.34 respectively. During the course of the treatment, the supernatant acquired a dark colour, which became more intense with time, but once the solubilization reactions came to a halt, the colour of the reaction mixture did not change. The absorbance intensity measured at a wavelength of 436 nm gave an initial value of 0.32, while the values for the final effluent corresponded to 1.22, 1.28 and 1.27 for 160, 180 and 200 °C respectively.

3.1.2. Thermal hydrolysis at different pressures

The results obtained from experiments at different pressures (40, 60 and 80 bar) did not show significant improvement in the transformations of the sludge during the process. Few differences in the measured parameters of TSS, SCOD, absorbance and pH were observed between the different experiments (Fig. 3). As was seen before, the reactions showed a greater activity up to the time of reaching the defined reaction temperature (80 min), and from the moment the parameters reached stable values in the analysis, no important change was observed by prolonging the time of treatment. Under the before-mentioned conditions, changes in pressure had only a minimal effect on the observed transformations.

3.1.3. Sludge settling

The sedimentation capacity of the sludge is considered to be an important feature for determining how difficult it is to manage this type of waste. The SVI (volume occupied by a gram of TSS after 30 min of sedimentation) was used as an approximate measure to predict the settling capacity of the sludge after thermal hydrolysis. This treatment generated an adverse effect on the SVI during start-up, which increased due to the decrease in the concentration of TSS. However, once a stable value in the solids concentration is reached, the SVI decreased. These changes occurred sooner when the temperature was higher, which led to an improvement in settleability which was achieved more rapidly (Fig. 4a). For experiments conducted at different pressures, the values of SVI were similar (Fig. 4b). The results show that prolonging the treatment beyond the point when the solubilization reactions come to an end leads to a subsequent higher compaction of solid material, possibly due to the reduction in the particle size which allows better agglomeration.

3.2. Rheology

Rheology tests were conducted on samples taken at different times. In this way it was possible to measure the changes in fluid behaviour during treatment, and in particular those in the parameters involved in the Herschel–Bulkley model ($\tau = \tau_0 + K\dot{\gamma}^n$), used for its characterization. The rheological behaviour of the sludge mainly depended on the concentration of the solids present, which in turn depended heavily on the treatment temperature.

3.2.1. Model parameters

A comparison was made of the evolution of the sludge parameters τ_0 (yield stress), K (consistency) and n (flow behaviour index) after thermal hydrolysis. With respect to the τ_0 value, the initial sludge gave a value of 3.7 Pa, this then being promptly reduced by the hydrolysis. Hydrolysis at a temperature of 160 °C was sufficient to drive its value to zero. The values of K and n present a reverse tendency, when one increases the other decreases. For the initial sludge, values of $K = 0.075$ Pa s^n and $n = 1$ were obtained. Therefore,

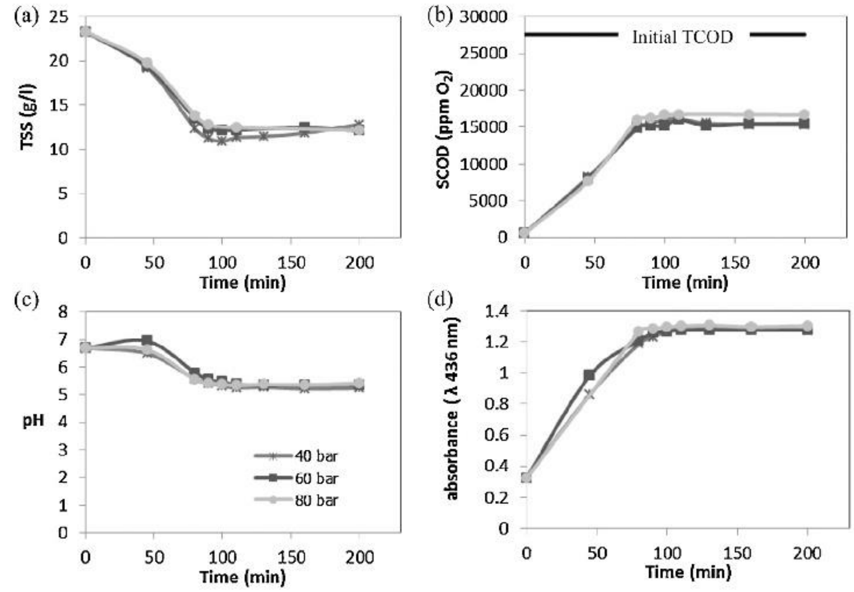

Fig. 3. Influence of pressure on the thermal hydrolysis (a) TSS, (b) SCOD, (c) pH, (d) absorbance. In all cases $T = 200\,^{\circ}C$.

behaviour corresponding to a Bingham plastic was identified for the initial sludge, since it was necessary to exceed a stress threshold level ($\tau_0 = 3.7$ Pa) to begin its flow, which was linear, in agreement with the value of n. During the first warm-up phase ($T = 100\,^{\circ}C$), the value of K increased while n decreased. Later, when higher temperatures were reached, K decreased to values close to zero, while n increased to values near to one. Mori et al. [20] found similarly that an increase in TSS increases the value of both τ_0 and K, while the value of n is reduced. These contrasting changes in K and n are perfectly compatible and, in fact, are to be expected, because during thermal hydrolysis the solids concentration is gradually reduced, rather than increasing. The increase in the temperature caused an acceleration in the changes of the magnitudes of these parameters, while a variation in the pressure did not affect the values of these (Fig. 5).

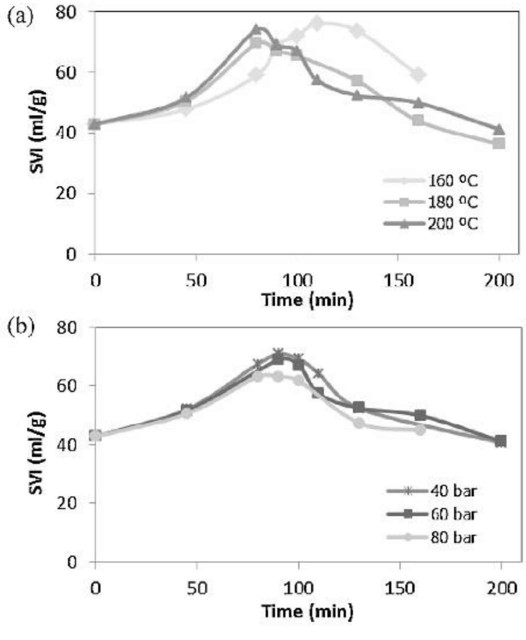

Fig. 4. Evolution of the SVI (a) for different temperatures and (b) for different pressures.

3.2.2. *Changes in the type of flow*

Fig. 6 shows the rheograms obtained for the initial sludge and final samples from all experiments. As can be observed in Fig. 6, the changes experienced in the type of flow were the same regardless of the conditions of hydrolysis and, as indicated previously, at higher temperature these changes occurred faster. In the initial sludge, the flow corresponded to a Bingham plastic ($\tau_0 > 0$, $n = 1$). In this case, the forces of cohesion that exist between the flocs cause a strong interaction between the particles, producing behaviour similar to that of a single solid mass. To overcome the resistance to movement it is necessary to exceed a level of initial stress, resulting in deformation of the flocs that are rearranged to finally flow linearly. Subsequently, when hydrolysis began to take place, the values of τ_0 decreased quickly until they were close to zero due to the destruction of the flocs. At this moment, pseudoplastic behaviour of the fluid ($\tau_0 = 0$, $n < 1$) was observed, probably due to the presence in the medium of polymers released by the bacteria after lysis. In this type of flow, the shear stress deforms and organizes these polymers in the direction of flow, and therefore the sludge flows better. At the end of treatment an effluent with a Newtonian flow was obtained ($\tau_0 = 0$, $n = 1$). This easier flow was due to the reduction in the amount and size of the particles and polymers, causing a decrease in the interaction between them, the sludge therefore behaving more like a homogeneous liquid. It is evident that the use of thermal hydrolysis improves the fluidity of the sludge, facilitating its subsequent handling in operations of pumping and transport. In Fig. 6, from the slope of the flow, it can be observed that treatment at $160\,^{\circ}C$ (0.0029 Pa s) produced a final sludge with a viscosity two times higher than that seen under the other conditions used in thermal hydrolysis. The difference between operating temperatures of

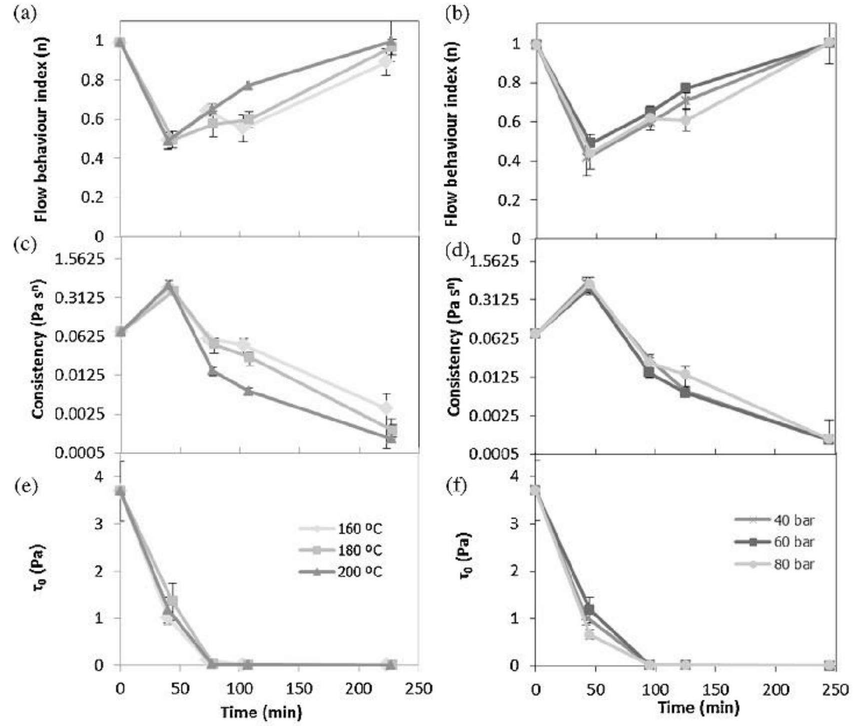

Fig. 5. Changes in the rheological parameters of the sludge with the treatment: figures (a), (c) and (e) for different temperatures (P = 60 bar); figures (b), (d) and (f) for different pressures (T = 200 °C).

180 °C (0.0015 Pa s) and 200 °C (0.0013 Pa s) was much smaller. For the pressure range of 40–80 bar, the viscosity was similar, reaching values from 0.0010 to 0.0013 Pa s.

3.2.3. Influence of TSS on the rheological properties

An analysis of the influence of the TSS on the parameters of τ_0, K and n was carried out, and empirical equations for modelling the changes in the rheology of the sludge during thermal treatment were determined. To carry out the analysis, the data from the experiments at different temperatures were selected. These were chosen because they showed greater difference in TSS concentration between the samples taken at the same times.

From Fig. 7, it can be seen that the value of τ_0 behaves exponentially with the increase in TSS (Eq. (1)). It is also observed that when the concentration of 15 g/l is exceeded, the value of τ_0 is greater than zero, the sludge thus showing plastic behaviour. Moreover, K and n were fitted to a polynomial function of third and second order respectively for the range between 10 and 23 g/l of TSS (Eqs. (2) and (3)). For concentrations outside this range, the expected value for n is 1 and for K close to 0. Fig. 7 shows that during the decline in the TSS from 23 g/l, the sludge begins a transition in the type of flow, changing to a fluid that possesses a plastic character together with pseudoplastic behaviour ($\tau_0 > 0$, $n < 1$). During this period, n increased and K decreased. When the TSS reach values lower than 15 g/l, the sludge loses its plastic characteristic and maintains the pseudoplastic behaviour ($\tau_0 = 0$, $n < 1$). From this point, a further decline in the TSS leads to a further transition stage of the flow towards a Newtonian fluid, which is seen at TSS concentrations below 10 g/l. During this last transition stage, an increase in n and a decline in K were noted.

$$\tau_0 = 0.00001\exp(0.5569 \times \text{TSS}) \quad R^2 = 0.9785 \tag{1}$$

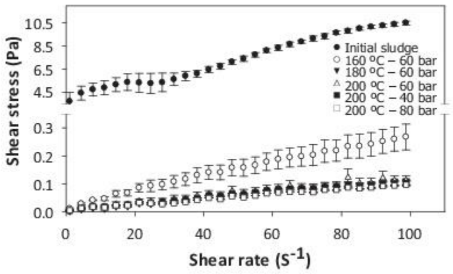

Fig. 6. Rheogram of the initial sludge and of final samples for all conditions of thermal hydrolysis.

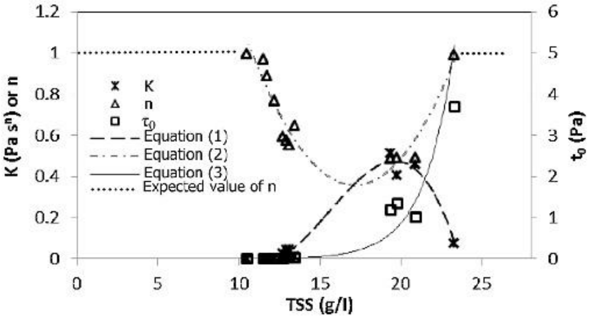

Fig. 7. Evolution de τ_0, K and n in function of the TSS concentration.

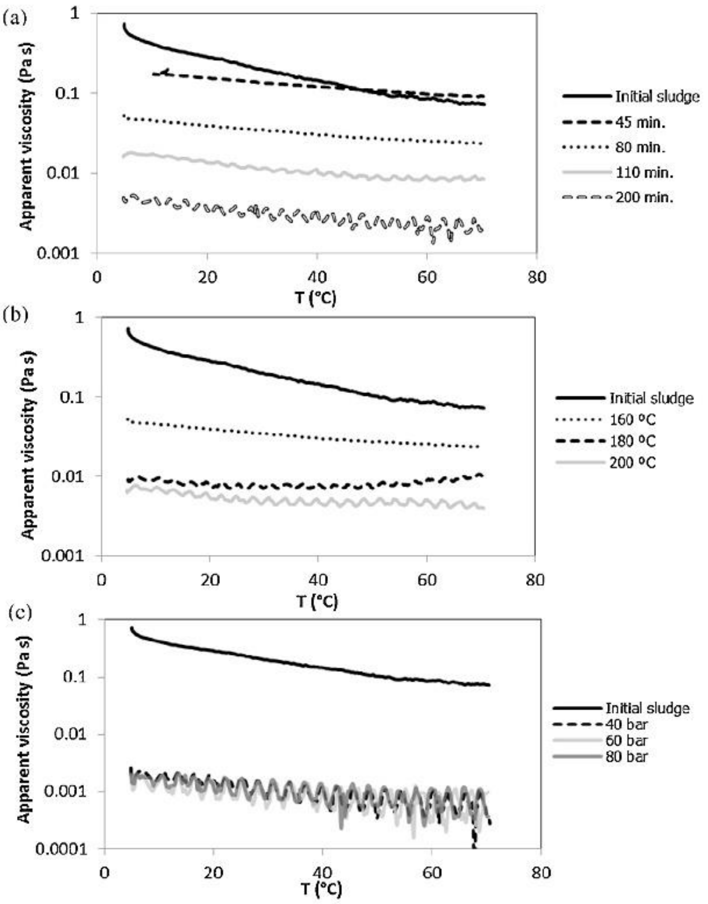

Fig. 8. Apparent viscosity for the thermal hydrolysis treatment (a) evolution at 160 °C and 60 bar, (b) results after of 80 min reaction at different temperatures, (c) final effluent (after 200 min) at different pressures (T = 200 °C).

$$K = -0.0018 \times \text{TSS}^3 + 0.086 \times \text{TSS}^2 - 1.2496 \times \text{TSS} + 5.7881 \quad R^2 = 0.9773 \tag{2}$$

$$n = 0.016 \times \text{TSS}^2 - 0.54491 \times \text{TSS} + 5.0631 \quad R^2 = 0.8871 \tag{3}$$

3.2.4. Apparent viscosity

The treatment of sludge by thermal hydrolysis achieved a significant decrease in its apparent viscosity. The results presented in Fig. 8a show that before reaching temperatures of 100 °C (45 min) the fluid still had values similar to those of the initial sludge, so that the problems of handling would still persist. At the time of reaching 160 °C (around 80 min), a reduction in the viscosity was more evident, becoming up to 15 times less than its initial value. With a stronger treatment, and after longer reaction times, the apparent viscosity decreases with the decreasing concentration of solids.

Moreover, the increase in temperature also had a favourable effect on the rate of apparent viscosity reduction (Fig. 8b). The percentage of VSS solubilized at 80 min was 29.1, 44.7 and 47.2%, for 160, 180 and 200 °C respectively. The same tendency was found when comparing the final effluent results.

From the results obtained working at different pressures, it was observed that the changes during the course of treatment were similar for samples taken at the same times. In Fig. 8c the results obtained from the final effluent are shown, and it is observed that they reach the same value for all conditions.

The effect of temperature on the viscosity of the hydrolysed sludge (T_1) was analyzed, also taking into account the influence of the temperature selected for the thermal hydrolysis (T_2). To carry out the study, the viscosity data at different values of T_1 (5–75 °C) for the final samples obtained at different T_2, were used. The data were fitted to the Arrhenius equation (Eq. (4)), used for Newtonian fluids. Where μ is the viscosity, A and B are constants that are dependent on the fluid and T is the absolute temperature (K). For each sample a value of A and B was determined. Later, the results of A and B are plotted against T_2, showing a linear correspondence of each term with T_2 (Eqs. (5) and (6)), where an increase in T_2 caused a decrease in A and an increase in B. Finally, by replacing A and B in Eq. (4) with Eqs. (5) and (6), Eq. (7) was obtained, which allows the calculation of the viscosity as a function of T_1 and T_2, for samples with Newtonian behaviour. This equation was tested with the final treatment samples, showing a good fit with the experimental data (error values lower than 15%). Additionally, it was observed that the higher the value of T_2, the lower is the effect of T_1 on the viscosity.

$$\ln \mu = A + \frac{B}{T_1} \tag{4}$$

$$A = -0.054 \times T_2 + 13.265 \quad R^2 = 0.9458 \tag{5}$$

$$B = 6.9325 \times T_2 + 1697.4 \quad R^2 = 0.9279 \tag{6}$$

$$\mu = \exp\left(-0.54 \times T_2 + 13.265 + \frac{6.9325 \times T_2 + 1697.4}{T_1}\right) \tag{7}$$

4. Conclusions

Thermal hydrolysis treatment of sludge represents a favourable alternative for improving the management of this type of waste, as it reduces the solid fraction, generating an effluent with characteristics of best dewaterability and easier handling. The temperature has proved to be the most influential parameter for this technology, while the changes in pressure did not produce significant changes in the characteristics of the effluent.

The increase in temperature improves the rate of solubilization reactions, requiring less reaction time. The decrease in the concentration of TSS by heat treatment affected the interaction between the particles of the sludge, which generated a positive effect on the rheological characteristics of the effluent.

A reduction in the apparent viscosity of up to two orders of magnitude was achieved, as well as the transformation of the fluid from a Bingham plastic, in the case of the initial sludge, to one with Newtonian flow at the end of the treatment. It was also demonstrated that the reduction in the solids content due to thermal hydrolysis reduces the yield stress value τ_0. However, for K and n the evolution is more complex. Initially, K increased and n decreased until that the flow changed to pseudoplastic but after that, K decreased and n increased with reaction time until the treated sludge achieved a Newtonian behaviour. The modelling of the rheological parameters showed an exponential growth of τ_0 with an increase in TSS, while K and n were fitted to a polynomial function of third and second order respectively.

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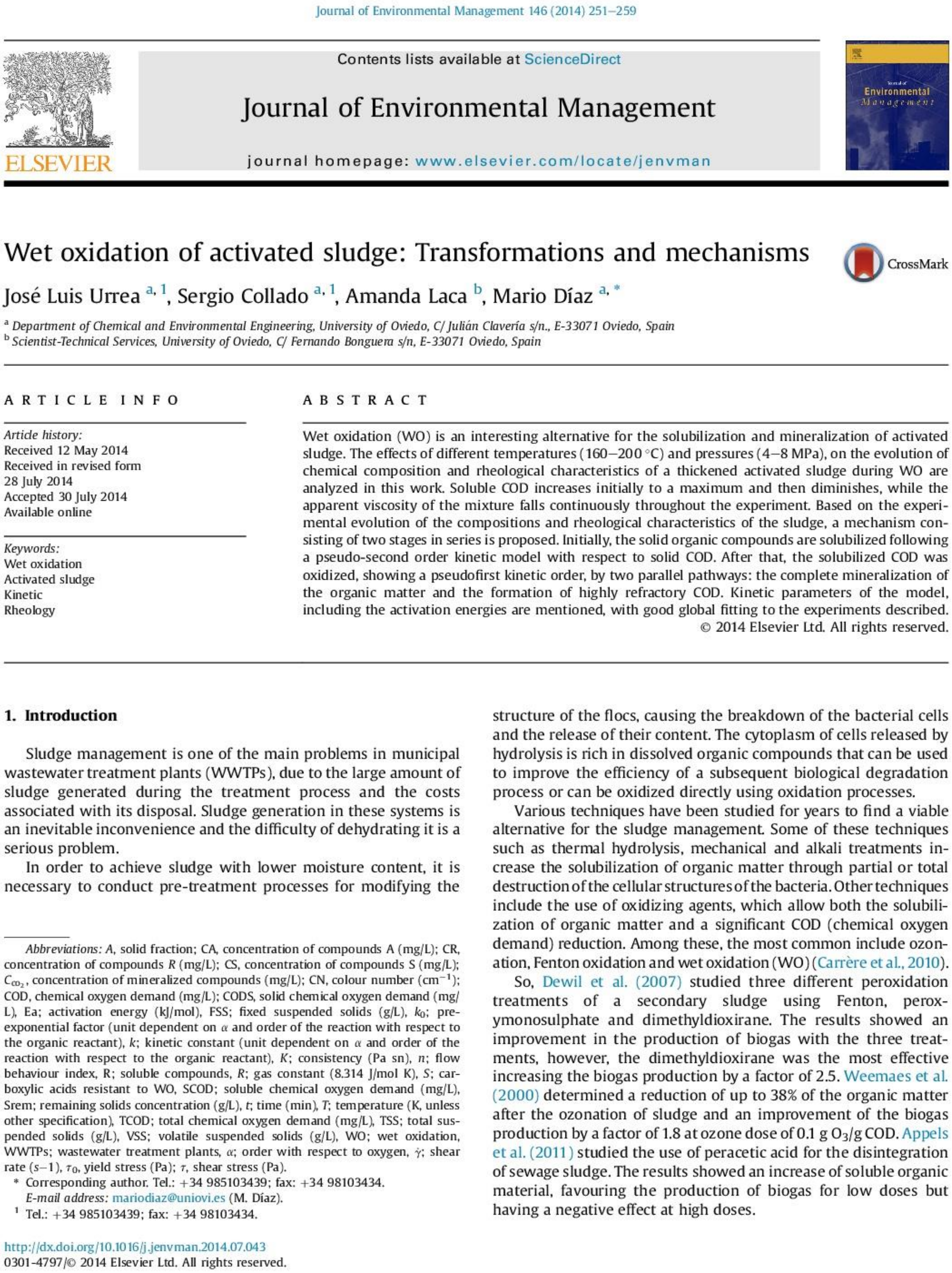

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Wet oxidation of activated sludge: Transformations and mechanisms

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ABSTRACT

Wet oxidation (WO) is an interesting alternative for the solubilization and mineralization of activated sludge. The effects of different temperatures (160–200 °C) and pressures (4–8 MPa), on the evolution of chemical composition and rheological characteristics of a thickened activated sludge during WO are analyzed in this work. Soluble COD increases initially to a maximum and then diminishes, while the apparent viscosity of the mixture falls continuously throughout the experiment. Based on the experimental evolution of the compositions and rheological characteristics of the sludge, a mechanism consisting of two stages in series is proposed. Initially, the solid organic compounds are solubilized following a pseudo-second order kinetic model with respect to solid COD. After that, the solubilized COD was oxidized, showing a pseudofirst kinetic order, by two parallel pathways: the complete mineralization of the organic matter and the formation of highly refractory COD. Kinetic parameters of the model, including the activation energies are mentioned, with good global fitting to the experiments described.

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1. Introduction

Sludge management is one of the main problems in municipal wastewater treatment plants (WWTPs), due to the large amount of sludge generated during the treatment process and the costs associated with its disposal. Sludge generation in these systems is an inevitable inconvenience and the difficulty of dehydrating it is a serious problem.

In order to achieve sludge with lower moisture content, it is necessary to conduct pre-treatment processes for modifying the structure of the flocs, causing the breakdown of the bacterial cells and the release of their content. The cytoplasm of cells released by hydrolysis is rich in dissolved organic compounds that can be used to improve the efficiency of a subsequent biological degradation process or can be oxidized directly using oxidation processes.

Various techniques have been studied for years to find a viable alternative for the sludge management. Some of these techniques such as thermal hydrolysis, mechanical and alkali treatments increase the solubilization of organic matter through partial or total destruction of the cellular structures of the bacteria. Other techniques include the use of oxidizing agents, which allow both the solubilization of organic matter and a significant COD (chemical oxygen demand) reduction. Among these, the most common include ozonation, Fenton oxidation and wet oxidation (WO) (Carrère et al., 2010).

So, Dewil et al. (2007) studied three different peroxidation treatments of a secondary sludge using Fenton, peroxymonosulphate and dimethyldioxirane. The results showed an improvement in the production of biogas with the three treatments, however, the dimethyldioxirane was the most effective increasing the biogas production by a factor of 2.5. Weemaes et al. (2000) determined a reduction of up to 38% of the organic matter after the ozonation of sludge and an improvement of the biogas production by a factor of 1.8 at ozone dose of 0.1 g O_3/g COD. Appels et al. (2011) studied the use of peracetic acid for the disintegration of sewage sludge. The results showed an increase of soluble organic material, favouring the production of biogas for low doses but having a negative effect at high doses.

Abbreviations: A, solid fraction; CA, concentration of compounds A (mg/L); CR, concentration of compounds R (mg/L); CS, concentration of compounds S (mg/L); C_{CO_2}, concentration of mineralized compounds (mg/L); CN, colour number (cm^{-1}); COD, chemical oxygen demand (mg/L); CODS, solid chemical oxygen demand (mg/L), Ea; activation energy (kJ/mol), FSS; fixed suspended solids (g/L), k_0; pre-exponential factor (unit dependent on α and order of the reaction with respect to the organic reactant), k; kinetic constant (unit dependent on α and order of the reaction with respect to the organic reactant), K; consistency (Pa sn), n; flow behaviour index, R; soluble compounds, R; gas constant (8.314 J/mol K), S; carboxylic acids resistant to WO, SCOD; soluble chemical oxygen demand (mg/L), Srem; remaining solids concentration (g/L), t; time (min), T; temperature (K, unless other specification), TCOD; total chemical oxygen demand (mg/L), TSS; total suspended solids (g/L), VSS; volatile suspended solids (g/L), WO; wet oxidation, WWTPs; wastewater treatment plants, α; order with respect to oxygen, $\dot{\gamma}$; shear rate (s−1), τ_0, yield stress (Pa); τ, shear stress (Pa).

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Moreover, the WO process has been adapted for the treatment of sewage sludge as an alternative to incineration (Lin et al., 1996). Organic solids present in the sludge are oxidized in aqueous phase by injecting air or oxygen in a reactor designed to operate at elevated temperatures and pressures. Operating conditions usually include temperatures between 160 and 300 °C and pressures between 2 and 20 MPa. The products obtained are inorganic salts, carbon dioxide and water when the oxidation is complete or simple forms of biodegradable compounds when it is partial (Abelleira et al., 2012; Collado et al., 2012; Chung et al., 2009). Carboxylic acids of low molecular weight (mainly acetic acid) are formed as intermediate products during the WO of organic compounds (Inamura, 1999; Genç et al., 2002). These are known to have a high resistance to oxidation by WO, more severe conditions being necessary to achieve complete oxidation (Shende and Mahajani, 1997). The degree of oxidation is mainly a function of temperature, oxygen partial pressure, residence time and pollutant oxidisability (He et al., 2008; Padoley et al., 2012). The individual and interactive effect of the reaction temperature, oxygen to biomass ratio and mixing intensity during the WO of sludge have been examined by Baroutian et al. (2013).

Two kinds of reactions occur during the WO process, a solubilization effect caused by thermal hydrolysis and oxidation, which takes place in series or in parallel, depending on the oxygen supply (Mucha and Zarzycki, 2008). The use of this technology achieves a sludge that settles and can be filtered more easily (Khan et al., 1999), and also reduces the organic load.

Generally, WO is used as a pre-treatment step in which the sludge is solubilized and the soluble fraction is partially oxidized, yielding biodegradable products and the COD being reduced. Therefore, a subsequent biological treatment is necessary in order to complete the mineralization of the effluent (Mishra et al., 1995).

In order to improve the degradation rate, the addition of catalysts during the sludge WO has also been studied by several authors (Bernardi et al., 2010). The use of catalyst causes that refractory compounds are more susceptible to oxidation and allows the selection of milder operating conditions (Hii et al., 2014). Nevertheless, the need for a subsequent operation for the recovery of the catalyst must be considered, in order to avoid a negative impact on the environment (Collyer, 1995).

Most of the studies have reported that the chemical reaction of WO chiefly proceeds via free radical reactions (Luan et al., in press). However, different types of chemical reactions that can cause/lead to oxidation of organic compounds under WO treatment include auto-oxidation (free radical reactions involving oxygen), heterolytic/homolytic cleavage (oxidative or non-oxidative thermal degradation), hydrolysis, decarboxylation, alkoxide formation followed by subsequent oxidation (alkaline solution), and carbanion formation followed by subsequent oxidation (alkaline solution) (Bhargava et al., 2006).

The reaction kinetics for sludge subjected to WO has been addressed by some researchers. Li et al. (1991) proposed a generalized kinetic model for WO of organic compounds based on three groups of organic substances. This model includes all initial and intermediate organic compounds (group A), that can be directly oxidized to the end products (group C) or form refractory intermediates represented by acetic acid (group B) that are also oxidized to form the end products (group C). This model was successfully applied in the WO of an activated sludge, providing a good fit with the experimental data. Nevertheless, it has been mentioned that this model includes an excess of adjustable parameters and this may be the reason for its good fit when it is applied to the WO of different types of compounds (Zhang and Chuang, 1999).

Mucha and Zarzycki (2008) studied the kinetics for a sludge that had previously been thermally hydrolyzed and separated into two fractions (solid and liquid), which were subsequently submitted separately to WO. They proposed a model for each oxidized fraction. For the liquid phase, two reaction pathways were suggested in which a gas and a liquid, this latter formed by compounds resistant to oxidation, were obtained as final products. In the case of the solid phase, they propose an initial decomposition that gives a gas product and an intermediate liquid product, which in turn is decomposed to more gas product and a final liquid product. In the oxidation of the intermediate liquid, the kinetic constants obtained from the WO of the liquid phase are used, assuming that the kinetics in this phase are the same.

The rheological behaviour of the sludge is an important property to consider, since its viscosity affects operations such as pumping, the hydrodynamics of bioreactors and the transfer of oxygen. For this reason rheology studies are considered to be important for improving management in wastewater treatment plants, for example for the calculation of pressure losses during transport in the pipes, the selection of pumps and for the design of aeration systems (Ratkovich et al., 2013; Seyssiecq et al., 2003).

It has been suggested by several authors that the concentration of suspended solids has an exponential relationship with the viscosity of the sludge (Mori et al., 2006; Tixier et al., 2003). At high concentrations of solids, the space between the structural units of suspension smaller, leading to stronger inter-particle interactions and hence the higher apparent viscosity of sludge (Eshtiaghi et al., 2013). The use of pre-treatment techniques, which allow a reduction in the solids content, generated a positive effect on the viscosity of the sludge. In a previous work (Urrea et al., 2014) the effect of thermal hydrolysis on rheological properties of an activated sludge was investigated, concluding that the reduction in the amount and size of the particles and polymers caused a decrease in the interaction between them. This, in turn, favoured the flow and caused a decrease in the apparent viscosity.

The aim of this work is to analyse the effects of temperature and pressure on the wet oxidation of an activated sludge, following the evolution of the chemical and rheological characteristics of the sludge. After discussion of the results, a model is presented to describe the kinetics of the reactions.

2. Material and methods

2.1. Experimental setup

The tests were performed with thickened activated sludge from a municipal WWTP in Asturias, Spain. This sludge had the following characteristics (mean values): total suspended solids (TSS) 32.25 g/L, total chemical oxygen demand (TCOD) 34,018 mg/L, soluble chemical oxygen demand (SCOD) 1797 mg/L and pH of 6.95.

The experimental equipment used was composed of a PARR series 4520 reactor of one litre, equipped with a stirrer with two propellers to maintain continuous mixing of the solid phase and liquid. A gas humidifier, formed by a steel tank of two litres, through which the gas was passed before it was introduced into the reactor, was used. A proportional integral differential controller handled the reactor and humidifier temperatures and the gas flow. As a safety condition, both the reactor and the humidifier were filled to 70% of their capacities.

Initially, the reactor was charged with sludge and preheated to 70 °C. Once this value was reached, the desired operating values of temperature and pressure were fixed. The pressure was adjusted through a backpressure controller located at the end of the gas line.

The stirrer speed was adjusted to 500 rpm for all the experiments and the feed gas was pure oxygen, which was introduced from the start of the preheating period, maintaining a fixed flow rate of 1200 mL/min for all experiments.

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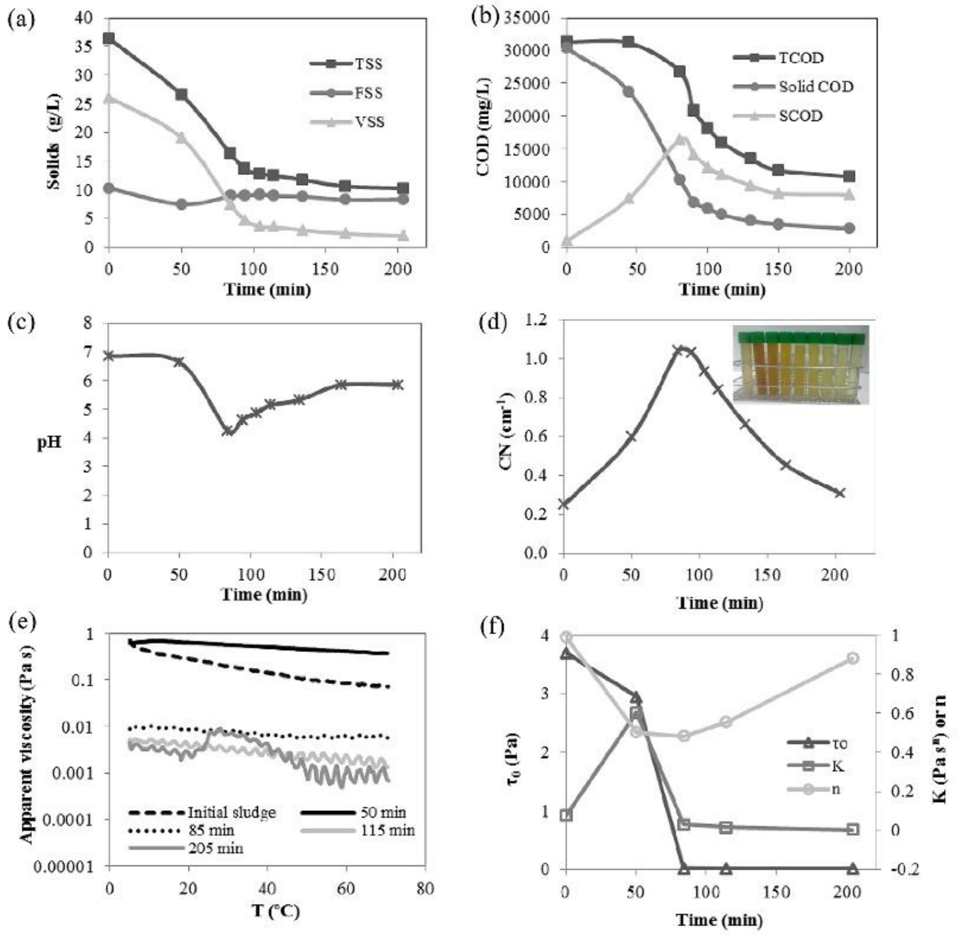


Fig. 1. Evolution of the characteristics of an activated sludge treated by wet oxidation (200 °C and 6 MPa oxygen). (a) solids, (b) COD, (c) pH, (d) CN, (e) apparent viscosity, (f) τ_0, K and n (Herschel-Bulkley model).

2.2. Methods analysis

The analyses performed to characterize the initial sludge and the samples taken at different reaction times were TCOD, SCOD, TSS, VSS (volatile suspended solids), FSS (fixed suspended solids) and pH (measured when the samples have cooled to room temperature). All these analyses were performed according to Standard Methods (APHA, 1998). To evaluate the evolution of colour during the treatment the spectral absorption coefficient (SAC) of the samples at three different wavelengths (436, 525 and 620 nm) was measured and the results were expressed as the colour number (CN) as defined in Equation (1) (Tizaoui et al., 2007).

$$\mathrm{CN} = \frac{\mathrm{SAC}_{436}^2 + \mathrm{SAC}_{525}^2 + \mathrm{SAC}_{620}^2}{\mathrm{SAC}_{436} + \mathrm{SAC}_{525} + \mathrm{SAC}_{620}} \tag{1}$$

The rheological analyses were carried out with a MARS II rotational rheometer (Haake). Before starting any measurement, the sample rested for at least 5 min, allowing the stresses induced during sample load to relax. All tests were conducted in CR mode, employing a plate/plate measuring system (PP60Ti) with a gap of 1 mm. Temperature ramps were carried out from 5 to 100 °C at a heating rate of 9.5 °C/min and a constant shear stress of 10 s^{-1}, whereas flow curves were determined at room temperature (25 °C) from 0.1 to 100 s^{-1} in 3 min. Temperature was controlled by means of a Peltier unit. All measurements were conducted at least in duplicate.

3. Results and discussion

3.1. Characteristics of the transformations of the sludge subjected to WO (Model experiment. 200 °C and 6 MPa oxygen)

3.1.1. Solids

Initial WO results for the sludge at 200 °C and 6 MPa showed that a high degree of solubilization (defined as the percentage reduction in suspended solids for a given time) (Equation (2)) can be achieved using this technique. The TSS showed a fast reduction, obtaining a 55% reduction of the initial TSS when the reaction temperature was achieved (85 min) (see Fig. 1a). After that, TSS concentration continued decreasing, a 72% reduction being observed when the reaction ended. Moreover, it can be also seen in Fig. 1a that when only considering VSS, the degree of solubilization was around 93% at the end of the experiment, whereas FSS concentration remained constant during the reaction period.

$$\text{Solubilization degree}_{(TSS)} = \frac{TSS_{t=0} - TSS_t}{TSS_{t=0}} * 100 \quad (2)$$

3.1.2. COD

The treatment of sludge by WO caused a rapid decrease in the solid phase COD (COD_S), which was transformed to SCOD (soluble COD). In Fig. 1b it is observed that the SCOD evolution showed a maximum (19651 mg/L) when the reaction temperature was achieved (85 min) and after that it began to decrease. This behaviour can be explained if it is borne in mind that a hydrolysis reaction prevailed during the first minutes of reaction, when TSS was high; oxidation reactions being more important at the end of the reaction period, when SCOD was high. Thus, hydrolysis reactions led to the initial increase in SCOD and the reduction in TSS. When the oxidation reactions prevailed, due to the higher SCOD, a decline in the TCOD caused by mineralization is observed. This also indicates that oxidation of the organic matter during WO of sludge required a previous solubilization of the sludge. Finally, it may also be noted that the TCOD tended to stabilise at an approximately constant value at high reaction times, which suggests the formation of refractory products, mainly carboxylic acids of low molecular weight. According to Fig. 1b, this COD that is refractory to oxidation represented around one third of the initial TCOD.

3.1.3. pH

Fig. 1c shows an initial decrease in the pH during the first 85 min and then, a short recovery of the pH values, with a final pH of 6 at the end of the reactions. The initial fast acidification of the medium was attributed to the release during the lysis cell of compounds, some of them having acidic character (humic acids, DNA, fatty acids and uronic acids). These compounds undergo transformations during the subsequent oxidation reactions, until that finally are decomposed to form either carbon dioxide and water or organic acids. As hydrolysis reactions decline, the contribution of these compounds to the reaction medium (liquid phase) decreases, thus the oxidation reactions become most predominant leading to a slow re-alkalinization of the medium as it is shown in Fig. 1c.

3.1.4. Colour

The treatment also caused a change in the colour of the supernatant (Fig. 1d). During the first 85 min, a dark brown liquid was observed, probably due to the solubilization of compounds. The presence of this colour is presumably caused by the formation of biorefractory molecules called melanoidins due to Maillard reactions (Dwyer et al., 2008b).

However, after 85 min, the colour was progressively reduced until a clarified effluent was obtained. It can be concluded that oxidation reactions also reduce the colour of the supernatant, which mainly appears during the initial hydrolysis of the TSS. This colour reduction can be associated with the degradation of melanoidins in accordance with Dwyer et al. (2008a). These authors found that the brown colour associated with melanoidins is caused by conjugated carbon–carbon double bonds, which are broken by the attack of the hydroxyl radicals generated by the advanced oxidation processes.

3.1.5. Rheology

The evolution of the apparent viscosity of the sludge during the WO process is shown in Fig. 1e. It can be seen that a reduction of two orders of magnitude is observed at the end of the treatment, probably due to the decrease in the TSS (Ratkovich et al., 2013). When a temperature of 100 °C is reached (50 min), the apparent viscosity does not show a reduction with respect to that of the initial sludge. It was also observed in this sample, once extracted from the reactor, that an increase in temperature during the rheology analysis did not affect its apparent viscosity, contrary to what occurred with the initial sludge. It can be seen that when the temperature in the WO reached 100 °C, the apparent viscosity of the sludge become more stable, and during the rheology analysis it showed even poorer viscosity characteristics than those of the untreated sludge. To achieve a reduction in the apparent viscosity it was necessary to reach higher temperatures, as seen in the sample taken when the reaction temperature was reached (85 min). When comparing the samples corresponding to treatment times of 115 and 205 min, only a small difference is observed between the two. This is probably the result of fast solubilization of solids at the beginning of the reaction period, which, after a treatment time of 115 min, had already reached 87% of the total that was achieved at the end of the experiment.

The measurement of the flow curves for the initial sludge and samples taken at different times of treatment were adjusted to the Herschel-Bulkley model ($\tau = \tau_0 + K\dot{\gamma}^n$). The results obtained for the consistency (K), the flow behaviour index (n) and yield stress (τ_0) are shown in Fig. 1f.

The flow behaviour for the initial sludge corresponded to a Bingham plastic, ($\tau_0 > 0$, $n = 1$) in which it is necessary to overcome an initial stress level before the start of linear flow. During the treatment, the solubilization of the solids caused the values of τ_0 and n to decrease, so when the operating temperature was reached, a value close to zero for τ_0 was obtained. In this way a modification in the flow was detected, the sludge changing in its behaviour from a Bingham plastic to a pseudoplastic fluid ($\tau_0 = 0$, $n < 1$). For higher treatment times, the flow behaviour index increases, reaching a final value of around 1. In this case, the rheological behaviour of the sludge corresponds to a Newtonian fluid. This new change of the sludge from pseudoplastic to Newtonian fluid can be attributed to the breakdown of the polymeric substances, which were initially released in the medium during the lysis of the cells, and which are one of the causes of the pseudoplastic behaviour of a fluid.

3.2. Effect of the operating conditions

3.2.1. Temperature

To analyse the effect of temperature, three experiments were carried out at a constant pressure of 6 MPa and at operating temperatures of 160, 180 and 200 °C (see Fig. 2). The results showed an improvement in the evolution of the characteristics of the sludge with temperature. To review the results in greater detail, it can be seen that a more marked decrease in the solids concentration was observed when temperature was increased. VSS reductions of 84.4, 87.6 and 92.6% were obtained at the end of the experiment for temperatures of 160, 180 and 200 °C respectively (Fig. 2a). Similar behaviour was observed for the TCOD. The higher the temperature, the higher the reduction in the TCOD reached at the end of the treatment, with values of 43, 51 and 66% for 160, 180 and 200 °C respectively (Fig. 2b). Fig. 2c shows that the pH reached the minimum value (close to 4) faster when the temperature was higher. However, a higher temperature also caused a higher increase in pH after the minimum pH was reached. pH values at the end of the reaction time of 4.35, 4.97 and 5.85 were obtained for 160, 180 and 200 °C respectively. This behaviour indicates that the solubilization of compounds that acidifies the medium during the hydrolysis and its subsequent oxidation are faster at higher temperatures. This was also corroborated by the colour evolution. The colour also showed a maximum, which was more marked at higher temperatures, indicating a faster solubilization. Moreover, the increase in the temperature also allowed a more clarified effluent at the end of the experiment, the SCOD mineralization being faster (Fig. 2d).

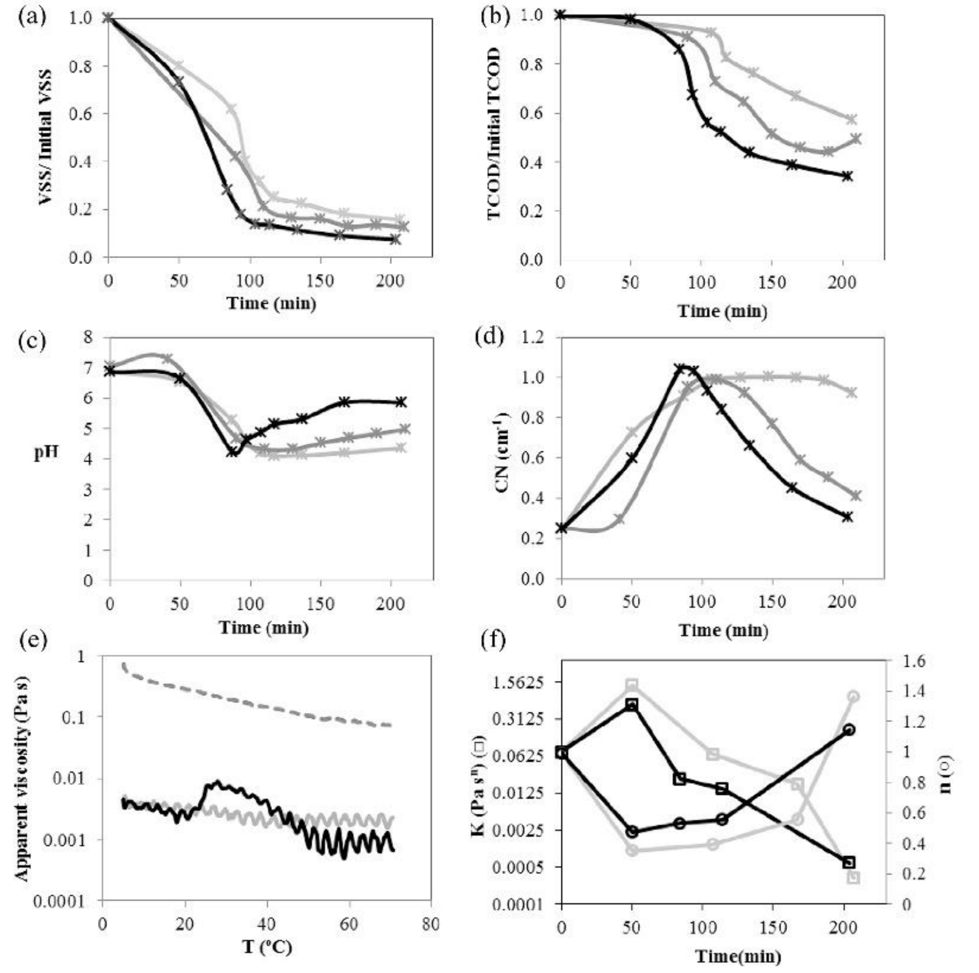


Fig. 2. Effect of temperature on the wet oxidation of sludge (a) VSS, (b) TCOD, (c) pH, (d) CN, (e) apparent viscosity (after 200 min), (f) K and n. — — initial sludge, —— 160 °C, —— 180 °C, —— 200 °C. In all cases P = 6 MPa, initial VSS = 26 g/L, initial TCOD = 35660 mg/L.

Fig. 2e shows rheology tests for the experiments carried out to 160 and 200 °C. A reduction in viscosity of two orders of magnitude can be seen from this figure, regardless of the selected temperature. The effect of temperature on k and n was not very marked (Fig. 2f). For both temperatures, Newtonian flow was observed at the end of the experiment. These results reveal that the use of WO, even at moderate temperatures (160 °C), helps to solve the problems of the viscosity of the sludge by favouring its fluidity. From an industrial point of view, this reduces the operating costs of the pumping equipment.

3.2.2. Pressure

In order to investigate the effect of pressure, experiments at 4, 6 and 8 MPa were carried out whilst maintaining a fixed temperature of 200 °C. Results showed that the increase in pressure had only a slight effect on the WO of the sludge during the initial stage of hydrolysis, similar degrees of solubilization being reached with each of the selected pressures (91 ± 2% COD solubilized).

As shown in Fig. 3a, the impact of pressure on VSS reduction was not very significant, a reduction of 90% being obtained at all the pressures. An increase in the pressure involved a higher dissolved oxygen concentration in the medium. However, cellular lysis is mainly due to a hydrolytic process, on which the oxygen concentration has little effect. It can also be noted that the VSS reduction occurred during the first 100 min, after which a constant value was reached. However, the increase in pressure had a more marked effect during oxidation reactions, leading to a fast reduction in the TCOD due to higher levels of dissolved oxygen (Fig. 3b). Similar changes in pH were seen for the three pressures (Fig. 3c). The colour initially progressed from yellow to brownish, then reached a maximum intensity, after which it became weaker, a pale yellow final effluent being obtained for the three pressures used (Fig. 3d). Nevertheless, the lower the pressure, the more intense was the colour that was seen during the first minutes. This behaviour can be explained bearing in mind that the products of hydrolysis intensify the colour whereas their oxidation causes a decolouration of the medium. As the pressure has little impact on the hydrolysis mechanism, similar colour evolution would be expected for the three experiments. However, an increase in pressure favours the oxidation of these coloured compounds, causing a faster reduction of the intensity. Finally, regarding the rheology, analyses also indicated a very slight effect on the apparent viscosity (Fig. 3e).

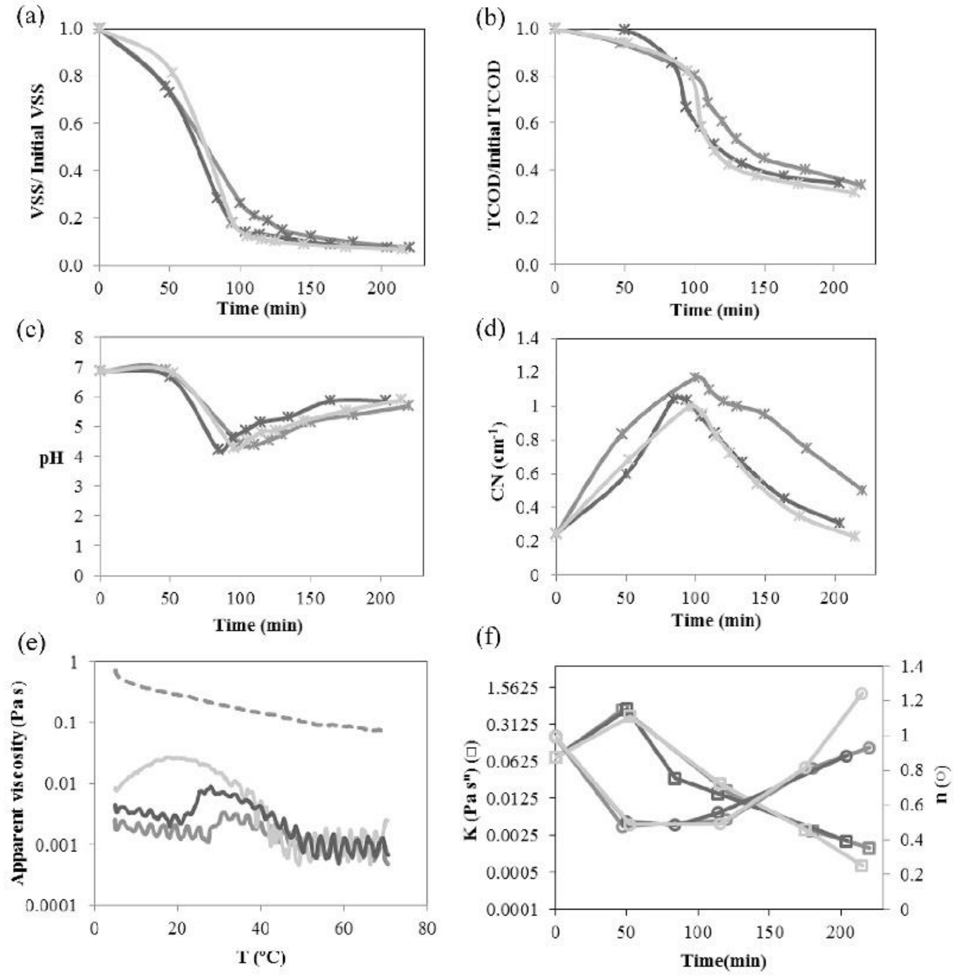


Fig. 3. Effect of pressure on the wet oxidation of sludge (a) VSS, (b) TCOD, (c) pH, (d) CN, (e) apparent viscosity (after 200 min), (f) *K* and *n*. ‒ ‒ Initial sludge, ▬ 4 MPa, ▬ 6 MPa, ▬ 8 MPa. In all cases $T = 200$ °C, initial VSS = 26 g/L, initial TCOD = 35660 mg/L.

Again, a two-orders-of-magnitude reduction was obtained for the pressure used. Parameters τ_0, K and n obtained from the Herschel-Bulkley model for the three pressures, corroborate the low impact of this variable on the rheology (Fig. 3f). These results are reasonable considering that VSS evolution, which has a big impact on the rheology, is similar for the three pressures.

3.3. Kinetic model

3.3.1. Kinetic for modelling TSS disintegration

As can be seen in Fig. 4, TSS rapidly decreased and then remained approximately constant. It was experimentally proved that this final TSS corresponded mainly to FSS, whose concentration remained constant during the entire treatment. On this basis, the parameter S_{rem} was introduced in the disintegration kinetic model. The assigned value to this parameter was FSS.

The experimental data used for the calculation of the kinetic parameters are collected in Fig. 4a and b. From these data, the order of reaction was determined, the best fit being obtained using a pseudo-second order kinetic model (Equation (3)) (For instance, R^2 in the experiment at 200 °C and 6 MPa was 0.92 and 0.98 for the first and second order respectively).

$$-\frac{\partial \mathrm{TSS}}{\partial t} = k_S\ [\mathrm{TSS} - \mathrm{S_{rem}}]^2 \qquad (3)$$

In turn, k_s can be also expressed as: (where k_{0s} is the pre-exponential factor).

$$k_S = k_{o_s}\ \exp[-\mathrm{Ea}_s/RT]\mathrm{C}_{\mathrm{O}_2}^{\propto_s} \qquad (4)$$

In order to determine the parameters k_{0s}, Ea_s and α_s, the concentration of oxygen in the medium was estimated for each experiment, employing Henry's law and empirical correlations (Himmelblau, 1960). The reaction order with respect to oxygen (α_s) was calculated by linear regression, correlating the oxygen concentration and the kinetic constant at different working pressures ($\ln k_S = \ln k_S^{'} + \propto_s \ln \mathrm{C}_{\mathrm{O}_2}$). After that, from the kinetic constants at different temperatures and using the Arrhenius equation, the Ea was determined by linear regression ($\ln(k_S/\mathrm{C}_{\mathrm{O}_2}^{\propto_s}) = \ln k_0 - (\mathrm{Ea}/\mathrm{RT})$). The result obtained for k_{0s} was 0.244, while the order with respect to oxygen (α_s) reached a value of 1.07 ($R^2 = 0.94$). The activation energy (Ea_s) had a value of 15.9 kJ/mol ($R^2 = 0.99$). This value suggests that the hydrolysis reactions during the WO of the sludge require low energy, therefore, a high degree of solubilization is

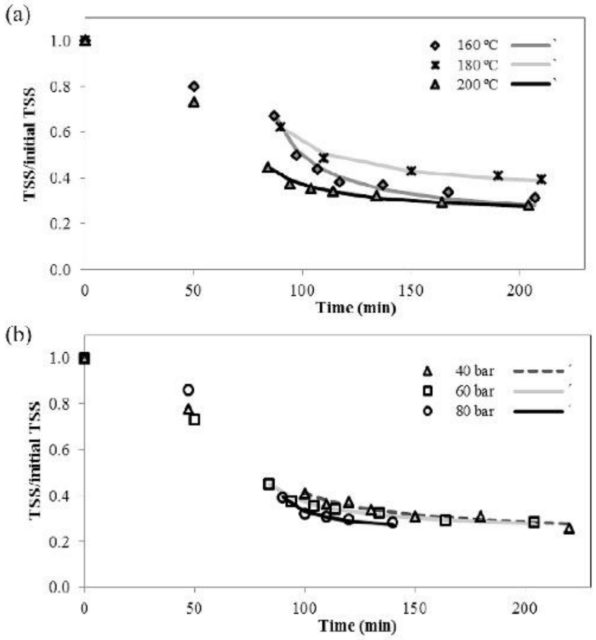


Fig. 4. Adjustment obtained for disintegration of solids. Points - experimental results, curves -theoretical calculations determined from Equation (3). (a) different temperatures ($P = 6$ MPa), (b) different pressures ($T = 200$ °C).

quickly reached. The general term to describe the kinetics of the disintegration of solids is represented in Equation (5).

$$-\frac{\partial TSS}{\partial t} = 0.244\ \exp[-1916.6/T][TSS - S_{rem}]^2[O_2]^1 \tag{5}$$

3.3.2. *COD modelling*

A model that represents the sequence in which the reactions take place was proposed. In this model, initial compounds present in the solid fraction (A), are hydrolyzed to form soluble compounds (R), which are subject to two different oxidation routes: their complete mineralization, forming carbon dioxide and water, or their partial oxidation towards refractory compounds (S), such as low molecular weight acids, mainly acetic acid (Fig. 5).

It can be deduced from Fig. 5 that the refractory character of S leads to a mineralization speed reduction, so that final values of COD tend to remain constant at the end. For this reason, the effectiveness of this treatment is limited and complete oxidation was not achieved during any of the experiments.

The calculation method for the kinetic constants for this process was carried out according to the proposed model. To eliminate the effect of the diffusion of oxygen, a constant high rate of agitation (500 rpm) was maintained during the whole experiment. In this way, the process was kinetically controlled (Mucha and Zarzycki, 2008).

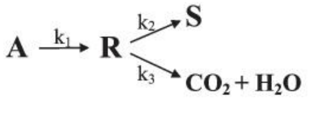


Fig. 5. Diagram of the kinetic model proposed for wet oxidation of an activated sludge. **A** solid fraction, **R** soluble compounds, **S** carboxylic acids, k_1, k_2 and k_3 represent kinetic constants for each step.

First of all, the kinetics model for the initial hydrolysis of the solid compounds (A) was determined using the COD_S data for the different temperatures and pressures used. These data fitted a second order kinetic model well (Equation (6)).

$$-\frac{\partial C_A}{\partial t} = k_1 COD_S^2 \tag{6}$$

Given that oxygen diffuses more easily in the aqueous phase, oxidation reactions of R become more significant than hydrolysis reactions. According to Fig. 5, the following kinetic model for R was proposed.

$$-\frac{\partial C_R}{\partial t} = [k_2 + k_3]\ C_R - k_1\ COD_S^2 \tag{7}$$

The formation of CO_2 and S was adjusted to a first order kinetic and were determined from Equations (8) and (9).

$$\frac{\partial C_S}{\partial t} = k_2 C_R \tag{8}$$

$$\frac{\partial C_{CO_2}}{\partial t} = k_3 C_R \tag{9}$$

Additionally.

On the basis of the difference between the initial TCOD and TCOD for each time, the mineralizated compounds were determined (expressed as the amount of oxygen consumed during the formation of $CO_2 + H_2O$).

$$R + S = SCOD \tag{10}$$

The kinetic constants were determined by simulations using Equations (6)–(10) by means of the Micromath Scientist software. These calculations were performed by adjusting the values obtained with the equations to the experimental data in accordance with the minimum of the sum of squared error.

During the simulation of R and S, where the SCOD measurement represented the sum of both, in order to carry out the calculations it was necessary to establish initial values of R and S. Several tests were carried out with different values, in order to find which best fitted the experimental results. As an example, Fig. 6 shows the experimental data (as symbols) and the theoretical data (lines) for two different experiments, corroborating the successful fit to the proposed model. Table 1 lists the values of kinetic constants obtained.

As shown in Table 1, values for k_3 were around two- or three-fold higher then values for k_2 in all experiments. This reveals that the mineralization reactions taking place during the WO of sludge occur at a higher rate than the formation of carboxylic acids resistant to oxidation. It can also be noted that the impact of temperature or pressure changes on k_3 is more marked that on k_2. This confirms that during the process of WO, an increase in temperature and pressure favoured the oxidation reactions, obtaining a higher degree of mineralization of the treated sludge.

In turn, the kinetic constants that were calculated, can be also expressed as

$$k_i = k_{0_i}\ \exp[-Ea_i/RT][O_2]^{\alpha_i} \tag{11}$$

The parameters k_{0i}, Ea_i and α_i, for each constant (k_1, k_2 and k_3) were obtained by the same procedure as applied with the modelling of TSS disintegration. The results are presented in Table 2.

According to the results shown in Table 2, mineralization was identified as the reaction step that requires the highest energy contribution, obtaining an Ea of 67.4 kJ/mol. Additionally, the order of reaction with respect to oxygen was also higher than in the other

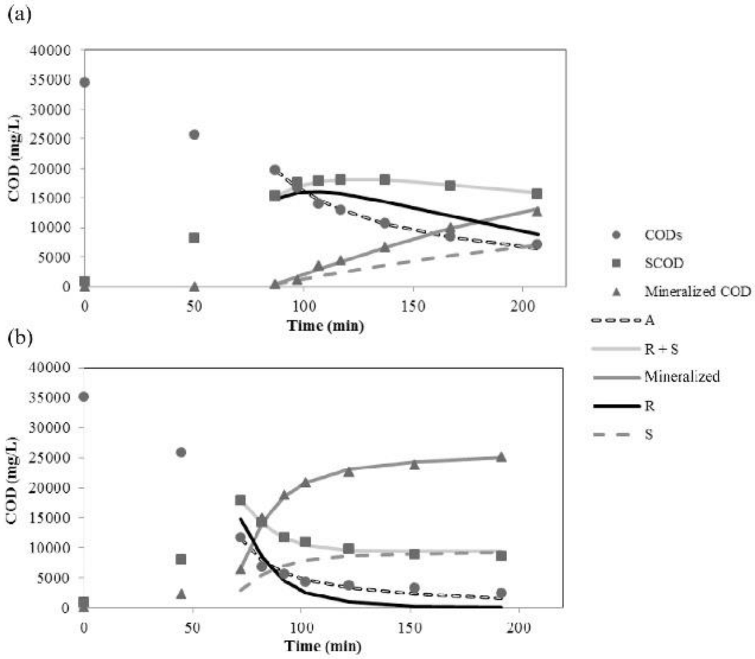


Fig. 6. Experimental data (symbols) and kinetic model (line) according Equations (6)–(10). (a) 160 °C and 6 MPa, (b) 200 °C and 8 MPa.

Table 1
Relevant kinetic data results for wet oxidation of thickened sludge under different operating conditions.

T (°C)	P (MPa)	k_1 (R_1^2)	k_2 (R_2^2)	k_3 (R_3^2)	Initial S (% initial TCOD)
160	6	8.56×10^{-7} (0.9993)	4.07×10^{-3} (0.9997)	8.0×10^{-3} (0.9971)	1.4
180	6	1.35×10^{-6} (0.9971)	8.17×10^{-3} (0.9999)	1.97×10^{-2} (0.9989)	4.7
200	6	3.29×10^{-6} (0.9931)	1.38×10^{-2} (0.9994)	4.49×10^{-2} (0.9994)	9.6
200	4	2.23×10^{-6} (0.9916)	9.50×10^{-3} (0.9989)	2.55×10^{-2} (0.9995)	5.6
200	8	4.26×10^{-6} (0.9893)	2.32×10^{-2} (0.9988)	6.87×10^{-2} (0.9995)	8.3

steps. These results indicate the high sensibility of the mineralization step to the operating conditions. It can be noted that the oxygen concentration had a moderate effect on the solubilization step ($\alpha = 0.67$), indicating that solubilization mainly occurs by hydrolysis, although oxidation reactions are also significant during COD_S reduction.

In other results published for Ea of sewage sludge treated by WO, Foussard et al. (1989) estimated a value of 67 kJ/mol for the mineralization stage. As can be seen, this value is very similar to that obtained in our results, thus supporting the model proposed here. Moreover, Mucha and Zarzycki (2008) reported values of 51.1 kJ/mol for the stage of solubilization. For the stage of mineralization they proposed two pathways: the mineralization of the solid phase, with an Ea 20.5 kJ/mol, and the mineralization of the liquid phase after solubilization, with an Ea of 81.3 kJ/mol. Although the result for the solubilization stage is similar to that obtained in our study (53 kJ/mol), the two results are difficult of compare, since Mucha and Zarzycki first subjected the sludge to thermal hydrolysis and subsequently carried out the WO of liquid and solid fractions separately. In addition, they considered the fit to a first order kinetic. Finally, the Ea (67.4 kJ/mol) determined with the model here proposed, is in agreement with the range of 54–78 kJ/mol reported for the WO of different organic compounds (Li et al., 1991).

Table 2
Parameters of the Equation (11), estimated from the kinetic constants.

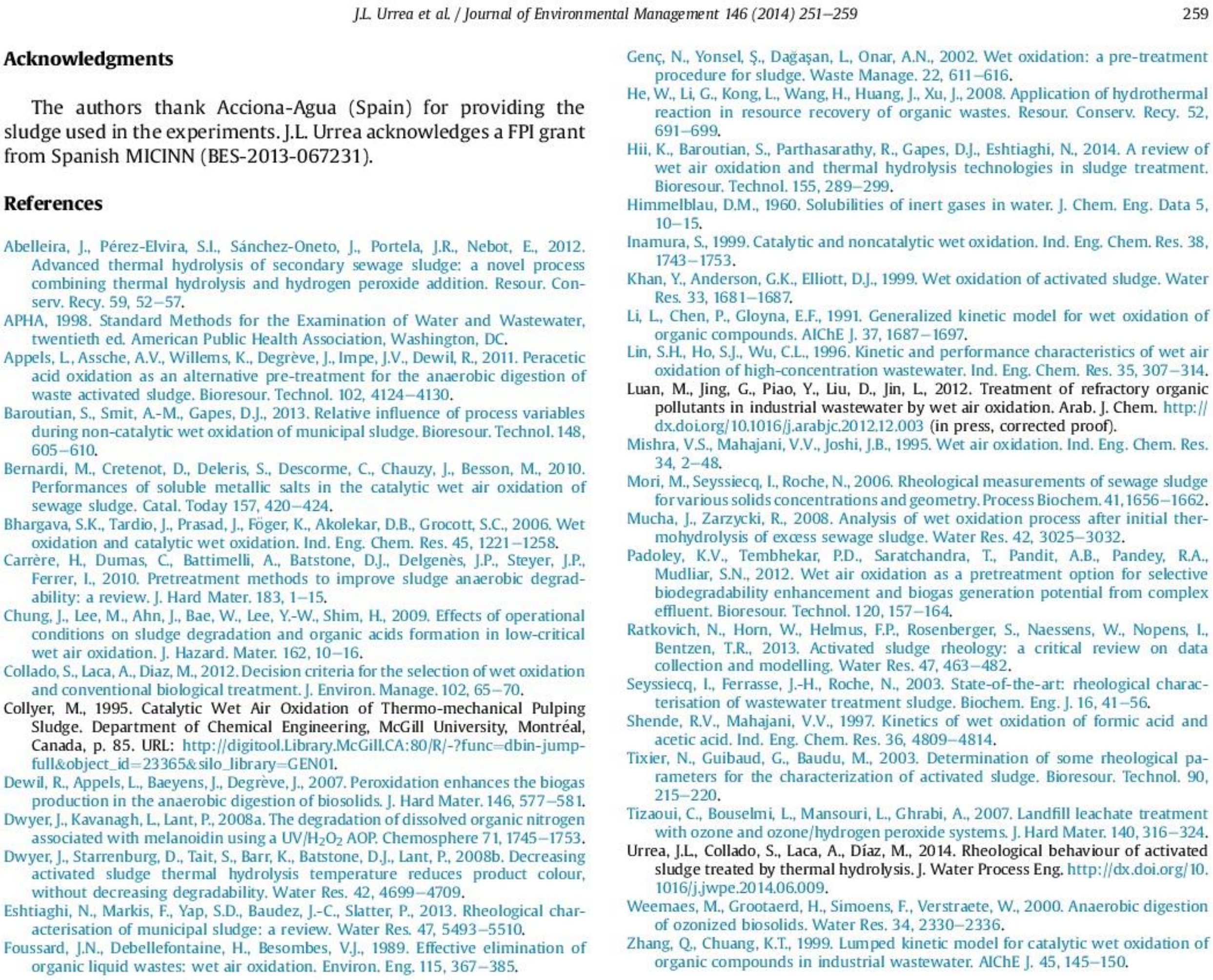
	k_0	α	R^2	Ea(kJ/mol)	R^2
k_1	1.46×10^{2}	0.6697	0.9997	53.00	0.9491
k_2	5.77×10^{5}	0.8984	0.9503	46.57	0.9973
k_3	7.94×10^{8}	1.0191	0.9972	67.41	1

4. Conclusions

WO represents an attractive technology for use in the treatment of sewage sludge. It is able to considerably reduce the solid fraction, as well as the organic load, giving as the final product a liquor composed mainly of organic acids of low molecular weight, which can be easily degraded through a later biological treatment.

Moreover, rheology analysis showed the positive effect of WO treatment in decreasing the apparent viscosity of the sludge, reducing this value by up to two orders of magnitude and changing the initial flow behaviour, corresponding to a Bingham plastic, to Newtonian flow by the end of the treatment process.

Finally, a kinetic model that accurately described the reactions that occur during the WO of the activated sludge has been proposed, achieving a good fit with the experimental data. The model implies an initial hydrolysis of the solid COD, followed by the oxidation of this solubilized COD, finally generating a refractory COD, with degrees of mineralization of around 70% in the best cases (200 °C and 8 MPa).



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4.3. COMPARISON BETWEEN THERMAL HYDROLYSIS AND WET OXIDATION OF SLUDGE

IV. Sludge hydrothermal treatments. Oxidising atmosphere effects on biopolymers and physical properties.

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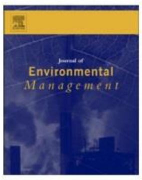

Research article

Sludge hydrothermal treatments. Oxidising atmosphere effects on biopolymers and physical properties

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In this work, the role of an oxidising atmosphere during the hydrothermal treatment of an activated sludge at 160 °C and 40 bar, was determined. The composition and molecular weight sizes of the soluble biopolymers generated during the sludge treatment in presence (wet oxidation "WO") or absence (thermal hydrolysis "TH") of oxygen were compared. Likewise, the characteristics of organic material, settleability, colour and pH of the treated sludge during both treatments were analysed. The thermal treatment in presence of oxygen provided better results in terms of solubilisation, settleability and mineralisation. WO initially favoured a more intense cellular lysis, causing a higher degree of solubilisation than that achieved by TH. Either in presence or absence of oxygen, thermal treatments caused a marked worsening of the settleability of the sludge. However, the degradation of biopolymers during WO led subsequently to an improvement of the settleability properties for longer reaction times. Both treatments caused a fast solubilisation of biopolymers at the beginning by effect of the release of extracellular and intracellular material from sludge. Subsequently, the presence of oxygen produced a significant decrease in the concentration of those biopolymers. In contrast, the proteins were the only one biopolymer that was degraded during TH.

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1. Introduction

Sewage sludge is an inevitable by-product in wastewater treatment plants (WWTP), which management is very complicated due to the high volumes generated and its high water content. Current sewage sludge treatment and disposal methods, such as landfilling, incineration and gasification require drying the sludge, a costly pre-treatment step, and/or risk contaminating the environment (Silva et al., 2016).

In this regard, hydrothermal processing has progressively been recognised as an attractive alternative for the sludge management during the last years. Hydrothermal treatments refer to technologies involving reactions carried out in an aqueous solvent at elevated temperatures and pressures, under inert (thermal hydrolysis) or oxidising (wet oxidation) atmospheres. Both wet oxidation (WO) and thermal hydrolysis (TH) allow the breakage of floc structure by means of solubilisation and degradation of EPS and cellular lysis (Hii et al., 2014).

The potential of hydrothermal treatments to improve the sludge management, as well as, the differences of results achieved with both techniques, have been addressed by some researchers. For instance, Baroutian et al. (2013) studied the effect of WO and TH to degrade wood extractive compounds from pulp mill sludge, such as: phenolic compounds, phytosterols, fatty acids and resin acids. They concluded that although both techniques were effective to reduce the total solid content of pulp mill sludge, the degradation of wood extractives was faster and more complete when WO was applied. Moreover, Yousefifar et al. (2017) studied and compared the effect of oxidative and non-oxidative hydrothermal processes (180–260 °C) on cellulose, which was used as a model compound of organic solid wastes. These authors found that to low temperatures, the capacity of solid degradation and solubilisation of organic matter for both techniques was similar. However, WO showed a major efficiency of solubilisation when higher temperatures of treatment were applied.

In sludge treatment, hydrothermal processing has traditionally had three main goals: the enhancement of a subsequent anaerobic digestion process, the reduction of the solid COD and/or the reduction of waste mass and volume (Barber, 2016; Chung et al., 2009; Genç et al., 2002; Khan et al., 1999). These approaches explain why, although the literature about sludge thermal

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treatments is abundant, the vast majority of these studies are only focused on the measurement of degrees of solubilisation and mineralisation, biodegradability indexes and/or biochemical methane potentials before and after treatment (Abe et al., 2011; Strong et al., 2011; Urrea et al., 2015). Nevertheless, information about the effects of thermal treatments on the composition or on other properties of the hydrolysed sludge is very scarce or does not even exist. As example, thermal treatments involve the release of high amounts of polymeric substances into the bulk medium during the cellular lysis, whose composition, concentration and molecular size will vary with the reaction time, especially under an oxidising atmosphere (Hii et al., 2014; Urrea et al., 2016). Evidently, information about the composition and properties of these biopolymers would be very interesting in order to design the following step of treatment of the hydrolysed sludge. For instance, fouling in membrane bioreactors is greatly affected by the protein and carbohydrates contents as well as their molecular sizes (Judd, 2011). In a similar way, the composition of soluble biopolymers in the medium also has effect on its viscosity, the settleability and wettability of the remaining solids or the biodegradability and chemical properties of the supernatant (Liu et al., 2013; Martins et al., 2011; Ruiz-Hernando et al., 2015; Wang et al., 2014; Zhang et al., 2015). Moreover, taking into account that sewage sludge consists of 61% proteins and 11% carbohydrates (Chen et al., 2007), this gives rise to the following question: is it possible to obtain valuable products from the sludge by means of thermal treatments? Obviously, the first step to answer this question is to know the products obtained and the mechanisms involved in their formation.

Nevertheless, as it was previously mentioned, information about composition and properties of the products formed during thermal treatments (soluble biopolymers, mainly) are very scarce and deals only with TH processes. Thus, Ramirez et al. (2009) pointed out that soluble proteins, carbohydrates and lipids concentrations increased with increasing temperature for a fixed time and that proteins were released easier than carbohydrates from the VSS. However, Bougrier et al. (2008) and Li and Noike (1992) indicated that carbohydrates were more hydrolysable than proteins, and proteins more hydrolysable than lipids in turn. For temperatures ranging from 130 °C to 220 °C, soluble carbohydrates concentration decreased due to reactions between them or with soluble proteins (Bougrier et al., 2008; Ramirez et al., 2009). In the case of proteins, Xue et al. (2015) observed that the rise in their concentration with temperature is accompanied by an increase in the ammonia nitrogen concentration. Bougrier et al. (2008) and Donoso-Bravo et al. (2011) concluded that volatile fatty acids are produced by lipid degradation instead of by protein decomposition. Yin et al. (2015) studied the time and temperature dependence of soluble proteins and carbohydrates and ammoniacal nitrogen, and found that at 220–300 °C, soluble proteins and carbohydrates went through a maximum with the reaction time, and that the higher the temperature, the lower the time at which the maximum appear. At this point, it is important to stress that, although information about the composition of soluble biopolymer during the sludge TH is available, there is no studies dealing with the properties of these biopolymers, such as their molecular weight size.

Regarding the WO of sludge, the majority of the works about this technique are exclusively focused on volatile fatty acids, which are the main chemicals generated by WO of biomass (He et al., 2008; Hii et al., 2014), and specially, on acetic acid, whose concentrations usually exceed those of the other acids. In fact, no studies on the effects of the WO on the composition of soluble biopolymers have been found. Regarding the properties of these polymers, to the best of our knowledge, there is only a work, corresponding to our research group, in which the molecular weight distribution of the solubilised matter during a WO treatment was determined by size exclusion chromatography (Urrea et al., 2016).

Therefore, based on the foregoing, the aim of this work is to study and compare, for the first time, the effects of hydrothermal treatments (TH and WO) on the composition and molecular weight sizes of the soluble biopolymers generated during the treatment as well as on the “classical” parameters of solubilisation, mineralisation and settleability.

2. Material and methods

2.1. Sludge samples

The activated sludge employed in the experiments was obtained from a municipal wastewater treatment plant of the region (Asturias-Spain). The sludge was extracted of a unit of thickening by flotation and stored at 4 °C until its subsequent use. The characteristics of the sludge were as follow: total suspended solids (TSS): 31.9 g/L, volatile suspended solids (VSS): 26.5 g/L, sludge volume index (SVI): 31 mL/g, total chemical oxygen demand (TCOD): 37,200 mg O_2/L, soluble chemical oxygen demand (SCOD): 200 mg O_2/L, soluble total organic carbon (soluble TOC) 400 mg C/L, initial pH: 6.5, soluble protein: 181 mg/L, soluble humic acids: 281 mg/L and soluble carbohydrates: 82 mg/L.

2.2. Experimental setup

The experiments of TH or WO were carried out in a PARR series 4520 reactor with a propeller stirrer (500 rpm). Oxygen (WO) or nitrogen (TH) previously conditioned in a humidifier, were fed since the beginning of the experiment to a constant flow rate of 1.2 L/min. Pressure was adjusted through a backpressure controller located at the end of the gas line, whilst the reactor temperature and the oxygen flow were regulated by means of PID controllers. The operating conditions stablished to carry out the reaction were 160 °C and 40 bar. Eight samples were taken at different times of reaction. The first one was withdrawn when 100 °C were reached in the reactor (45 min). The following samples were extracted periodically from minute 67, when the operating conditions were reached, to the end of the treatment (187 min).

2.3. Analytical methods

The analysis corresponding to total suspended solids (TSS), volatile suspended solids (VSS), fixed suspended solids (FSS), chemical oxygen demand (COD), sludge volume index (SVI) and pH were performed according to Standard Methods (APHA, 1998).

In order to quantify the biopolymers solubilisation, their concentrations were measured by the following colorimetric methods: proteins and humic acids by the modified Lowry method (Frolund et al., 1995), using BSA and humic acids as standards, respectively, and carbohydrates by the Dubois method (Dubois et al., 1956), employing glucose as standard. UV-VIS scans from 190 to 900 nm were performed for supernatants employing a T80 UV/VIS spectrophotometer (PG Instruments Ltd). The colour of the soluble samples was determined by means of the colour number (CN), which is defined according to equation (1) (Tizaoui et al., 2007).

$$CN = \frac{SAC_{436}^2 + SAC_{525}^2 + SAC_{620}^2}{SAC_{436} + SAC_{525} + SAC_{620}} \tag{1}$$

where SAC_i corresponds to the spectral absorption coefficient at a wavelength of i nanometers.

Soluble total organic carbon (TOC) was measured using a TOC analyser (Shimadzu TOC-VCSH, Japan). The mean oxidation number of organic carbon (MOC) was calculated from equation (2) (Vogel et al., 2000).

$$MOC = 4 - 1.5\frac{COD}{TOC} \quad (2)$$

where COD and TOC values are expressed in mg O_2/L and mg C/L, respectively, and MOC takes values from −4 (i.e. for methane) to +4 (i.e. for carbon dioxide).

2.4. Size exclusion chromatographic analysis (HP-SEC)

The size distribution of the solubilised biopolymers, commonly called "fingerprints", was performed by High Performance Liquid Chromatography (Agilent 1200, Agilent Technologies Inc., California, USA), using a Yarra SEC-2000 (300 × 7.8 mm) column. A $NaNO_3$ solution was employed in order to determinate the total column volume (11.8 mL). The mobile phase consisted of a buffer solution of 9 mM NaCl, 0.9 mM Na_2HPO_4 and 0.005% NaN_3, adjusted to pH 7.0 (±0.1) with H_3PO_4, and with an ionic strength of 0.02. The flow rate employed was of 1 mL/min. All samples were filtered through 0.45 μm PVDF filters (Millipore) prior to injection (20 μl). The detection was carried out with a diode array UV detector at several wavelengths (210, 260 and 280 nm).

The calibration of the column was performed by means of a Protein Standard Mix (15–600 kDa) supplied by Sigma-Aldrich (69,385), which was composed of four proteins: Ribonuclease A (13.7 kDa), Albumin (44.3 kDa), γ-Globulin (150 kDa) and Thyroglobulin (670 kDa), as well as a low molecular weight marker, ρ-aminobenzoic acid (0.14 kDa). The coefficient of determination (R^2) of the calibration curve was 0.93.

3. Results and discussion

3.1. Behaviour and characteristics of organic material during hydrothermal treatments

Fig. 1a shows the evolution of total and soluble COD fractions during the TH or WO of an activated sludge. As has widely been commented in the literature, both treatments caused an increase in the soluble COD (SCOD), but WO also provoked a partial mineralisation of the organic load of the sludge, thus observing a steady reduction of total COD (TCOD). In this case, TCOD decreased a 38% after 187 min of reaction in the case of WO, whereas it remained constant for TH.

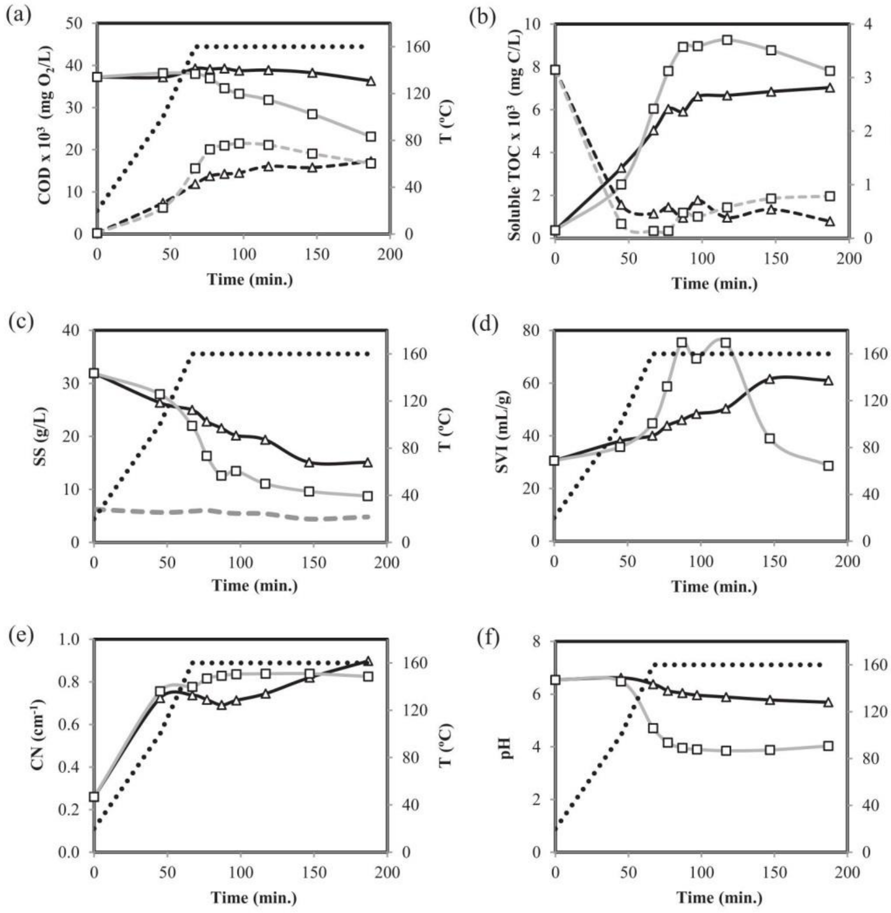

Fig. 1. Effect of the presence (■) or absence (▲) of an oxidising atmosphere on sludge properties during the hydrothermal treatment at 160 °C and 40 bar. (a) COD evolution (solid and dashed lines represent TCOD and SCOD, respectively), (b) Soluble TOC (solid lines) and MOC (dashed lines) evolution, (c) TSS evolution (dashed line corresponds to FSS), (d) SVI evolution, (e) CN evolution and (f) pH evolution. Dotted lines represent the reactor temperature.

It is interesting to point out that initially, when the temperature is not high enough (first 45 min, temperature lower than 160 °C) the degree of mineralisation achieved with both techniques was null. Nevertheless, the presence of an oxidising atmosphere does have effect on the solubilisation of the sludge, even at these low temperatures. The solubilisation of organic compounds from sludge was faster when reactions of oxidation were involved. So, when the operating conditions were achieved (67 min), the SCOD for WO was a 30% higher than for TH, while TCOD remained constant in both experiments. After 187 min of treatment, the solubilisation degrees of solid COD (COD_S) were 83% and 49% for WO and TH, respectively, as Fig. 1a shows. Thus, it seems reasonable to propose that free radicals initially formed tend to attack the solid COD instead of mineralising the soluble COD, thus favouring a higher solubilisation. Therefore, it can be concluded that an oxidising atmosphere offers a higher effectiveness in terms of sludge solubilisation than an inert one.

Obviously, as the CODs decreases, oxidation reactions of the soluble COD prevail, which explain the continuous TCOD and SCOD reductions observed after an hour and a half of treatment by WO, whereas SCOD increased over time for TH experiments. In fact, although the degree of solubilisation was higher for WO (83%) than for TH (49%), similar SCOD were achieved at the end of the both treatments (a 46% of the initial TCOD of the sludge).

Soluble TOC results also corroborate the dual effect of the oxidising atmosphere on the thermal treatment of the sludge, improving the solubilisation of solid matter and, in turn, oxidising the dissolved one (Fig. 1b). The final soluble TOC reduction observed for WO also reveals that part of the COD removal was due to total oxidation of the soluble organic matter towards carbon dioxide and water. As previously commented, free radicals are responsible of the oxidation reactions. These free radicals are generated from the oxidation of the organic molecules into hydroperoxides in a first stage. Hydroperoxides are highly reactive, and if the oxidation continues, they will be transformed into alcohols, ketones and aldehydes, which can be turned into CO_2 and water (Debellefontaine and Foussard, 2000), which explain the reduction observed in TCOD and SCOD for WO but not for TH.

Regarding the mean oxidation number of organic carbon (MOC), results showed that the oxidation state of soluble compounds decreased sharply with both treatments, from an initial value of 3.2 ± 0.1 to 0.45 ± 0.15 after only 45 min of reaction. This reduction of the MOC was attributed to the release of extracellular polymeric substances (EPS) and intracellular material, whose oxidation state is lower than that of soluble biopolymers of raw sludge (higher COD per unit of organic carbon) (Urrea et al., 2016). Likewise, it was also noted that the initial decrease in the MOC was more marked for WO than for TH, probably due to the higher solubilisation of the sludge in presence of an oxidising atmosphere. In a previous work (Urrea et al., 2016), the relationship between more hydrophobic characteristics of organic compounds and lower MOC was theoretically proved. Therefore, the lower MOC obtained in WO experiments is explained by the higher degree of cell destruction attained, as the biopolymers that integrate the cell membrane, such as phospholipids and proteins, have a hydrophobic character. Nevertheless, as the reaction progresses, WO caused a moderated increase in the MOC since minute 97, due to the oxidation of those biopolymers towards more hydrophilic products, such as volatile fatty acids. In contrast, the MOC in TH decreased slightly as a result of the solubilisation but no oxidation mechanism.

3.2. Solubilisation of SS and effect on settleability

The TSS disintegration and its effect on the settleability properties of the sludge for each treatment are showed in Fig. 1c and d. As previously explained, WO caused a stronger impact on the solubilisation of the sludge than TH. In fact, the solubilisation degree of TSS achieved with WO after 77 min of treatment (49%) was almost the same obtained after 187 min of TH (53%). To the end of the reaction, TSS concentrations were 27% and 47% of the initial value for WO and TH, respectively. As expected, FSS concentrations do not change throughout both treatments.

It should be also pointed out that CODs/VSS ratio remained almost constant during the treatments, obtaining values of 1.5 ± 0.2 mg O_2 mg VSS^{-1} and 1.6 ± 0.2 mg O_2 mg VSS^{-1} for WO and TH, respectively. Burger and Parker (2013) reported a value of 1.44 for this ratio in an aerobic sludge without treatment, whereas 1.42 is commonly mentioned in the literature, which is obtained theoretically from the oxidation of the biomass ($C_5H_7NO_2$). No changes in the CODs/VSS ratio discard either the selective solubilisation of some compounds instead of others or the oxidation of the organic matter in the solid phase.

Regarding the effect of the thermal treatments on the settleability, both techniques initially lead to an increase in SVI, which means a worsening of its management during that stage (Fig. 1d), as a result of an increase in the repulsion between particles by electrostatic forces. When 100 °C were reached, the flocculate structure of the sludge was destroyed by both thermal treatments, causing the release of extracellular polymeric substances and the formation of smaller particles. At this time, an initial slight increase of SVI was detected. Afterwards, while the concentration of TSS continued to decrease, the amount of solubilised biopolymers increased in the medium, which goes hand in hand with an increase in the SVI. These changes suggest that a higher concentration of solubilised biopolymers led probably to an increase in the negative surface charge of remaining solid particles, which caused a worse compaction of the latter such, as clearly reflected in the SVI evolution. In fact, the SVI remained constant between minutes 147 and 187 during the TH, coinciding with the period in which the solubilisation reactions had concluded. Zhen et al. (2012) reported that the zeta potential of an activated sludge decreased from -12.6 to -18.0 mV after its treatment by TH at 80 °C, being that result an evidence of the abovementioned.

In accordance with the previous explanation, the higher solids disintegration caused by the oxidant also involved a higher deterioration of the settleability, expressed as SVI. Nevertheless, as WO reactions progress, the SVI, after achieving a maximum in minute 117, began to drop. This is caused by the oxidation of the soluble biopolymers, thus reducing the negative surface charge of the solid matter and, therefore, the repulsion by electrostatic forces. Therefore, in the case of WO, it is noteworthy to stress that the evolution of SVI is strongly linked to the balance of reactions developed during the process. That is to say, SVI increased when the reactions of thermal hydrolysis were predominant. Subsequently, SVI remained stable when the magnitude of the reactions between thermal hydrolysis and oxidation were similar. At the end, SVI began to decrease from the time in which the oxidation reactions prevailed over the hydrolysis ones (see SCOD evolution in Fig. 1a).

3.3. Effects on colour and pH

The effects of WO or TH on the colour number (CN) and on pH of the solubilised samples from sludge are showed in Fig. 1e and f. For both treatments, the CN experienced a sharp increase in its value at the beginning of the reaction, then remaining approximately constant around 0.85 since minute 45. Comparing CN and SCOD, it can be concluded that their trends are analogous, particularly for HT. In this case, the higher SCOD concentration, the higher CN was achieved, as expected. This trend is also observed for the first part of WO, where solubilisation reactions prevail. In fact, CNs between 45 and 147 min of treatment were higher for WO than for TH, since during this period the former caused a greater level of lysis cellular, achieving in turn higher SCOD concentration than the latter. Nevertheless, when oxidation reactions became dominant, reducing the SCOD, no significant decrease in the CN was observed. At the end of treatments (187 min), the CN obtained with TH was a bit higher (8% more) than that with WO, despite the SCOD concentrations were very similar between the final effluents of both treatments. The ability of WO to reduce the colour on different wastewater or sewage sludge has been widely proved (Fu and Kyzas, 2014; Oulego et al., 2016; Urrea et al., 2014) as well as the generation of high coloured intermediates during the oxidation of phenol-like compounds, such as quinhydrones (Collado et al., 2010). So, the no CN change observed for WO was probably due to a balance between the formation of highly coloured intermediates from the initially solubilised compounds and the oxidation of them to non-coloured products, such as low molecular weight acids (LMWA). The pH evolution also supports the higher formation of LMWA in presence of an oxidising atmosphere. While TH caused a minimum effect on the pH of sludge, WO produced a remarkable acidification of the medium (Fig. 1f). During TH, the pH decreased slightly from 6.5 to 5.7, being this the result of a small formation of volatile fatty acids (VFA). Eskicioglu et al. (2006) reported the increase in the concentration of VFA from 281 to 936 mg/L for an activated sludge subjected to TH at 96 °C, which was withdrawn from the reactor exactly when this temperature was reached. Similarly, they observed that the pH slightly decreased with the treatment from 7.5 to 7.0.

In the case of WO, an abrupt drop in the pH values (4.7) was obtained when reaching the operating conditions (67 min). In that point of the reaction, no decrease in TCOD was noticed. Therefore, this indicates that the attack of the hydroxyl radicals during the initial solubilisation step of WO caused the formation of biopolymers with a stronger acid character than those formed only for TH. Afterwards, the oxidation reactions take place, causing a further decline in the pH value (4.0), which remained stable during the rest of the experiment. The acidification of the medium during WO is a common result of this process due to the production organic acids, which are considered as the main final product of reaction (Genç et al., 2002).

In addition to this, the changes in UV-VIS spectra obtained with each of the treatments (see Fig. S1 in the supplementary material) allowed to stablish more detailed differences between the characteristics of the coloured compounds solubilised either by WO or TH. According to the evolution of the spectra and considering only the VIS zone (380–780 nm), it can be noted that the absorbance values for 530–780 nm decreased with both treatments after reaching the operating conditions, being this decrease more marked in presence of oxygen. Subsequently, the VIS spectrum for TH conserved its shape, being observed a continuous increase in the absorbance in the entire region with the advance of the reaction, that is to say, with the increase in the SCOD. On the other hand, the solubilised biopolymers by WO showed higher levels of absorbance to lower wavelengths (380–528 nm) than those by TH, specifically, during the first 147 min of reaction. Besides, the absorbance values increased up to be completed 117 min of WO, decreasing after this time. At the end of the treatments, the spectra area between 380-528 nm and 530–780 nm was of 143 and 146 μA nm for TH, and of 132 and 43 μA nm for WO, respectively.

3.4. Solubilised biopolymers. Yield and size distribution

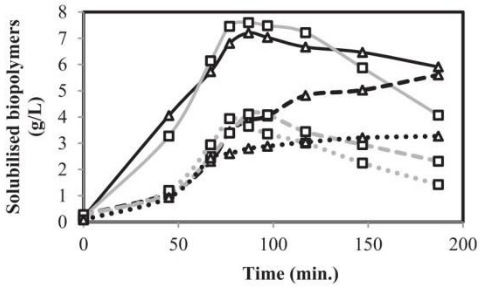

Fig. 2. Solubilised biopolymers by effect of the presence (■) or absence (▲) of an oxidising atmosphere during the hydrothermal treatment of the sludge at 160 °C and 40 bar. Solid, dashed and dotted lines represent proteins, humic acids and carbohydrates, respectively.

The solubilisation of biopolymers from sludge and their size distribution during WO or TH treatments are collected in Figs. 2 and 3, respectively. Both treatments caused a considerable increase in the concentration of soluble biopolymers, with the proteins being identified as the main components. Initially, a fast increase in the soluble biopolymers concentrations was observed for both TH and WO during the first 67 min of reaction. This preliminary solubilisation was the result of the destruction of the flocculate structure of the sludge, which caused the release of extracellular polymeric substances towards the supernatant, as well as of intracellular material from of the cell lysis. In the following minutes, the rate of solubilisation of biopolymers decreased for both treatments, being this due to the lower VSS concentration, as well as to oxidation reactions in the case of WO. Nevertheless, the biopolymers evolution was different for both treatments since minute 87. On one hand, when 87 min of WO was reached, the concentration of biopolymers achieved a maximum with values of 291, 164, 141 mg/g VSS for proteins, humic acids and carbohydrates respectively, but afterwards, their concentrations began to continuously decrease. On the other hand, although proteins concentration also achieved a maximum during TH in minute 87 (272 mg/g VSS), their removal after this minute was significantly slower than in the case of WO. As expected, protein decomposition is faster in presence of an oxidising atmosphere (oxidative plus thermal decomposition) than in its absence (only thermal decomposition). Another difference between both treatments is that humic acids and carbohydrates concentrations increased throughout the reaction of TH, meaning that these biopolymers were less sensitive to thermal degradation than proteins. At the end of TH (187 min), the concentrations of proteins, humic acids and carbohydrates was of 223, 212 and 123 mg/g VSS, respectively. The values obtained by WO for the same time were 158, 97 and 57 mg/g VSS for proteins, humic acids and carbohydrates, respectively. These values correspond to 54, 59 and 40% of the maximum soluble proteins, humic acids and carbohydrates concentrations, that is to say, those achieved in the minute 87 of WO.

In order to compare the changes in the size distribution of the solubilised biopolymers caused by each of the treatments, the evolution of their fingerprints was analysed (Fig. 3). To carry out a more exhaustive analysis, the fingerprints area was divided into four categories as follows: three of them were stablished in the size exclusion zone for low (0–35 kDa), medium (35–150 kDa) and high (>150 kDa) molecular weights, whilst the fourth one corresponded to the area out of the column total volume. The presence of peaks out of the column total volume has been related to molecules with hydrophobic characteristics that interact with the filling material of the column (Görner et al., 2003). After an hour of treatment, the areas for low (0–35 kDa), medium (35–150 kDa) and high (>150 kDa) molecular weight biopolymers were 4, 8 and 1.2 AU s for TH and of 9, 14 and 1.5 AU s for WO, respectively. These results indicate that WO produced a higher solubilisation of low and

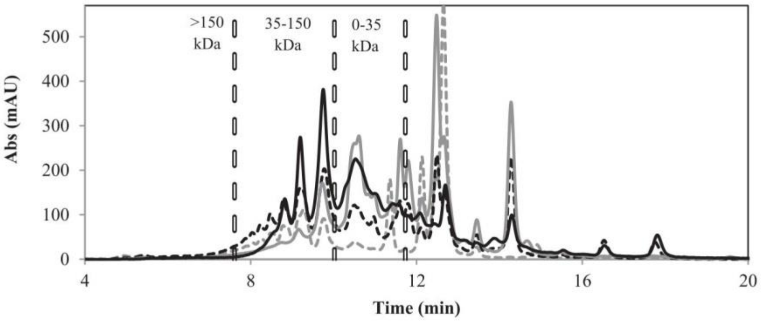

Fig. 3. Changes on the fingerprints of supernatants by effect of the presence (black lines) or absence (grey lines) of an oxidising atmosphere during the hydrothermal treatment of the sludge at 160 °C and 40 bar. Dashed and continue lines correspond to time sampling of 67 and 187 min, respectively.

medium size polymers than TH. Regarding to hydrophobic biopolymers, areas corresponding to times higher than that for total elution were 13.4 and 14 AU s for TH and WO, respectively. Taking into account that the polymers eluted in the zone of size exclusion should be those with hydrophilic characteristics, and that this kind of polymers is mainly located in the cell cytoplasm, it seems reasonable to suggest that the presence of an oxidising atmosphere favours a higher degree of cellular lysis in the initial stage of thermal treatment of sludge, rather than the complete hydrolysis of the cellular components, that is to say, the solubilisation of the cellular wall and membrane.

Finally, when both treatments were concluded, the peaks located at lower elution times in the fingerprints disappeared, whilst peaks corresponding to medium and low size biopolymers increase their height. These changes demonstrated that both reactions, thermal hydrolysis and oxidation, caused the hydrolysis of the larger polymers, which were initially solubilised from sludge, to form other of lower molecular weight. According to the stablished categorisation, the areas of the fingerprints for final effluents were of 15, 7, 0.7 and 24 AU s for TH and 15, 15, 0.4 and 15 AU s for WO. As it can be observed, a higher proportion of hydrophobic polymers was obtained by TH than by WO. This is because oxidation reactions make the oxidised compounds more hydrophilic, due to the fact that these reactions transform organic hydrophobic compounds such as proteins or lipids into alcohols, ketones, aldehydes or small organic acids, for instance, acetic acid (Debellefontaine and Foussard, 2000). The increase in hydrophilic compounds was not observed for TH, because there are not oxidation reactions, only hydrolysis of polymers into oligomers or monomers of the same nature. For instance, proteins will be transformed into amino acids or peptides that maintain some characteristics (Wilson and Novak, 2009). In contrast, the oxidising reactions that occur during WO led to changes in molecules nature. One example is the transformation of phenylalanine to tyrosine due to a hydroxylation in the side chain altering its absorbance properties (Biondi et al., 2006; Zhang et al., 2014). This is only an example, and if the oxidation continues, amino acids can be turned into small organic acids, as previously explained.

4. Conclusions

TH and WO showed a high efficiency to reduce the volume of activated sludge, causing a high solubilisation degree of the extracellular and cellular components of the sludge. However, the use of an oxidising atmosphere offered a more complete solution of treatment, providing better results of solubilisation, settleability and mineralisation of the sludge than an inert one.

The free radicals initially formed by WO favoured a higher solubilisation of the sludge instead of its mineralising. Likewise, these radicals showed particularly high affinity to cause the cellular lysis at the beginning of the reaction, rather than the complete hydrolysis of the cellular components such as cellular wall and membrane.

Despite of that WO caused a higher degree of solids solubilisation than TH, the yield of solubilisation of the main component of the sludge, the protein, was similar between both techniques. This result was a factor of the degradation of proteins caused by free radicals formed under an oxidising atmosphere.

The presence of oxygen during the hydrothermal treatment of sludge favoured a higher solubilisation of low and medium size polymers, as well as a higher hydrophilic character in the composition of the effluent. In addition, an oxidising atmosphere caused the degradation of each solubilised biopolymers from sludge, whilst an inert one only caused the degradation of proteins, demonstrating thus that proteins were more sensitive a thermal decomposition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2017.10.043.

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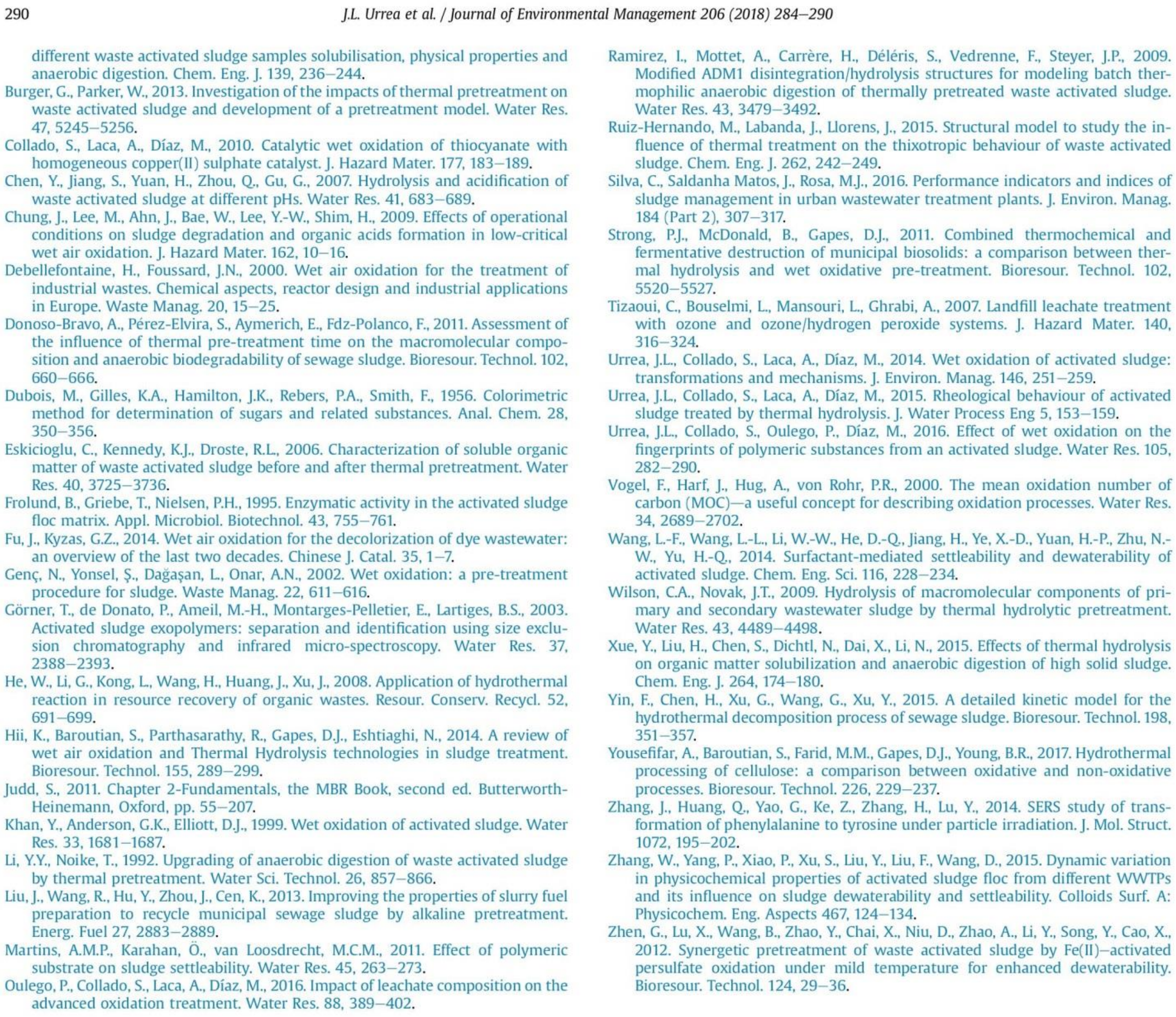

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4.4. EFFECT OF WET OXIDATION ON STRUCTURAL FRACTIONS FROM SLUDGE

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Wet oxidation of the structural sludge fractions

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ABSTRACT

In this study, the effect of wet oxidation on the main properties of the structural components of the sludge were analysed for the first time ever. For this purpose, an activated sludge was separated into its structural fractions: soluble microbial products (SMP), loosely bound extracellular polymeric substances (LB-EPS), tightly bound extracellular polymeric substances (TB-EPS) and naked cells, and these were treated, either individually or simultaneously (raw sludge), by wet oxidation at 190 °C and 65 bar.

Findings revealed that naked cells were the fraction oxidised to a greatest extent followed by TB-EPS, LB-EPS and, finally, SMP, thus concluding that the more internal the biopolymer is located in the cell, the more effectively it is attacked by free radicals.

LB-EPS fraction, whose initial biodegradability (10%) was the lowest one of all the fractions, also showed the most intense colour per unit of total organic carbon. Nevertheless, once the EPS fractions were subjected to the wet oxidation separately, the increase in the proportion of readily biodegradable material was particularly remarkable for this fraction (65%), observing higher biodegradabilities after the treatment for those components which are closer to the cell centre. A similar behaviour was observed for decolourisation capacity of wet oxidation.

Finally, for the raw sludge and the naked cells, wet oxidation caused a fast initial worsening of settleability and dewaterability in both samples, although this effect was more marked when EPS were previously extracted. However, such properties were improved once a high degree of solubilisation and oxidation was reached. In addition, synergetic effects between the structural fractions during the wet oxidation of raw sludge were discarded.

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1. Introduction

Activated sludge is a system commonly applied in wastewater treatment plants (WWTP) to reduce the organic load of the influents (Zang et al., 2015). The main drawback of this system is the management of the excess of sludge caused by cellular growth during the degradation of the organic matter (Ahmad et al., 2016). Besides, the humidity content of the sludge, which can reach values above 90%, increases the complexity of its management (Cieślik et al., 2015).

Today, the legislative framework in force in Europe introduces quite severe restrictions on the properties of residual sewage sludge, both when it is landfilled or used in agriculture, in terms of removal efficiencies of pathogens, toxic compounds and odours (Bertanza et al., 2015). These restrictions have resulted in the adoption of advanced sludge treatment technologies. In particular, wet oxidation allows achieving, beyond a reduction in sludge volume, the stabilization of the sludge and dissolved metals, the reduction of the microbial load, the partial or full mineralization of the sludge, and the absence of hazardous gaseous compounds. In fact, several authors have reported that WO technology can be a sustainable alternative to conventional sludge stabilization and incineration, especially for large WWTP or for centralised sludge treatment plants (Slavik et al., 2015).

The biological sludge is a complex mix formed by extracellular polymeric substances (EPS) and cells, with the EPS being a matrix of high-molecular-weight polymers generated by bacterial excretion, cell lysis and compounds from incoming wastewaters (Sheng et al., 2013). These EPS are responsible for keeping the organisms together in a three-dimensional gel-like hydrated matrix, preventing the desiccation and acting as an ion-exchange resin, thus controlling the ionic movement from the medium to the inside of the cell (Neyens and Baeyens, 2003).

In basis to their cellular location, EPS have been classified in either soluble microbial products (SMP) (dissolved and without

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direct contact with the cells) or bound EPS (as gel and associated with the cells). The latter fraction, in turn, has been divided into loosely (LB-EPS) or tightly bound (TB-EPS) extracellular polymeric substances, depending on the strength of the attachment between cells and EPS (Wang et al., 2014). The composition of EPS includes mainly proteins, carbohydrates and humic acids. However, the distribution of these components varies in function of the operating conditions at WWTP (Sheng et al., 2010). Fig. 1 shows a scheme of the distribution of the various structural fractions within microbial aggregates.

According to Neyens et al. (2004), EPS can account for up to 80% of the total mass of activated sludge, when the water retained within the EPS structure is considered. EPS have been identified as the main responsible for the difficult dewaterability of sludge, due to their ability to bind water (More et al., 2014). Hence, there is a clear need to gain in-depth knowledge of the final fate of these biopolymers during sludge treatment by means of the corresponding minimization technique.

From this viewpoint, recent studies have focused on the analysis of the effect of several of these techniques on the characteristics of EPS from sludge. For instance, Ye et al. (2012) observed that the use of potassium ferrate had a negative impact on the capillary suction time (CST) of an activated sludge, although, in turn, it slightly enhanced the sludge volume index and the dewaterability measured as dry solids content. They indicated that the increase in LB-EPS concentration was the main cause of these changes. Likewise, Ruiz-Hernando et al. (2015) analysed the effect of ultrasound, thermal hydrolysis and alkali treatments on EPS. Although an increase in the CST of the treated sludge was also observed, the release of interstitial water due to the EPS solubilisation caused a higher removal of water from the sludge by centrifugation. Zhang et al. (2016) studied the effects of the combined process of peracetic acid (PAA) pre-oxidation and chemical re-flocculation, on the morphological properties and distribution and composition of EPS. The results showed that PAA produced the solubilisation of bound EPS, whilst chemical flocculation allowed for the reconstruction of the floc structure.

Meng et al. (2016) found that carbohydrates were more stable than proteins during the treatment of EPS by ozonation. In fact, while proteins reached a reduction of 30 ± 12% after 4 h of treatment, the concentration of carbohydrates remained almost flat. He et al. (2015) tested the effect of Fenton-like treatment on sludge dewatering. They suggested that the bound water was released from the floc due to the degradation of EPS and cellular lysis, thereby fostering the dewaterability of the sludge.

Although the changes in the sludge features and the role played by the EPS have been widely studied using various treatment methods, there is a lack of knowledge about these changes for wet oxidation. A better understanding of the effect of this technique on the EPS properties would be very interesting, because these polymers are the main contributors to the difficult management of raw sludge. Hence, there is a clear need for knowledge of the mechanisms involved in the oxidation of the different fractions, as a first step toward optimizing the process, thus resulting in a more sustainable WWTP exploitation.

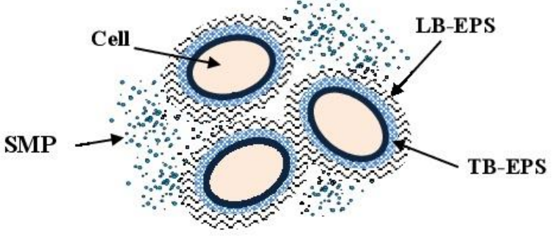

Fig. 1. Scheme of the distribution of the different structural fractions in microbial aggregates: cells, loosely (LB-EPS) or tightly bound (TB-EPS) extracellular polymeric substances and soluble microbial products (SMP).

Wet oxidation is considered as an effective hydrothermal technology for the treatment of sewage sludge as well as effluents bearing a high concentration of organic matter of biorefractory nature (Bertanza et al., 2016). In contrast to the treatment by incineration, wet oxidation does not produce harmful emissions to air (Johansson et al., 2008). During treatment, organic and oxidisable inorganic compounds are degraded in the liquid phase at high temperatures (150–320 °C) and pressures (20–150 bar) with the use of air or oxygen (Menoni and Bertanza, 2016). This technique improves the characteristics of settleability and filterability of the sludge, while reducing its organic content (Genç et al., 2002). In addition to this, the liquid fraction obtained after the treatment can be used as a carbon source for denitrification processes in WWTP (Houillon and Jolliet, 2005).

Several authors have focused their research on the wet oxidation of sludge, but the analysis of the effects of this treatment on the EPS is practically non-existent. Although the most contemporary reviews on sludge pretreatment are focused on thermal and thermochemical process (Zhang et al., 2017), only two works dealing with this topic were found (Urrea et al., 2016, 2017). Both articles were centred on the formation and characterization of soluble biopolymers during the treatment, but not on the characterization of the whole structural fractions. Therefore, the aim of this work was to assess the influence of wet oxidation on the main structural components of the sludge, that is, SMP, LB-EPS, TB-EPS and "naked" cells. Thus, each of the fractions were oxidised individually and the results were compared with those obtained using raw sludge.

2. Material and methods

2.1. Sludge samples

The activated sludge used in the experiments was withdrawn from a thickening unit of a municipal wastewater treatment plant located in Asturias, Spain. The sludge was stored at 4 °C in order to preserve its properties until the extraction of EPS or its direct use in the wet oxidation experiments. The initial characteristics of the sludge were: total chemical oxygen demand (TCOD): 25,694 mg O_2 L^{-1}, soluble chemical oxygen demand (SCOD): 1,034 mg O_2 L^{-1}, total suspended solids (TSS): 21.5 g L^{-1}, volatile suspended solids (VSS): 15.5 g L^{-1}, sludge volume index (SVI): 46.6 mL g^{-1}, pH: 6.9, and capillary suction time (CST): 205 s.

2.2. EPS extractions

A thermal extraction was employed to separate the EPS fractions from sludge. The procedure has already been described in Urrea et al. (2016). A detailed explanation of the method was included in the supplementary material.

2.3. Experimental setup

The wet oxidation experiments were carried out in a series 4520 PARR reactor, which was equipped with a stirrer to maintain continuous mixing inside (500 rpm), as well as a PID controller for the temperatures of the reactor, the humidifier and the gas flow (1.2 L O_2 min^{-1}). Before introducing the gas into the reactor, it was previously conditioned in a humidifier built out of steel. A schematic of the experimental setup can be found in Urrea et al. (2016).

The reactor was initially fed with 500 mL of sample, then fixing the operating conditions (190 °C and 65 bar). Eight samples were grabbed during each experiment; the first one was withdrawn when a temperature of 100 °C was reached in the reactor, thus before reaching the operating conditions, in order to find out if the effects of the initial heating period on the properties of the main structural components of the sludge are negligible or not. The rest of samples were taken during the next 2 h, after reaching the operating conditions (85 ± 5 min). The first experiments were carried out with naked cells and raw sludge, in order to avoid changes in the suspended solids concentration during the storage stage.

2.4. Analytical methods

The analyses corresponding to: total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total suspended solids (TSS), volatile suspended solids (VSS), fixed suspended solids (FSS), sludge volume index (SVI) and pH were carried out according to the Standard Methods (APHA et al., 2005). UV-VIS scans for supernatants at wavelengths from 190 to 900 nm were performed employing a T80 UV/VIS spectrophotometer (PG Instruments Ltd). The colour of the liquid samples was measured by means of the colour number (CN), which is defined according to Equation (1) (Tizaoui et al., 2007).

$$CN = \frac{SAC_{436}^2 + SAC_{525}^2 + SAC_{620}^2}{SAC_{436} + SAC_{525} + SAC_{620}} \qquad (1)$$

where: SAC is the spectral absorption coefficient at a specific wavelength.

The polymeric content in initial EPS fractions is showed in Table 1. These concentrations were determined as follows: proteins and humic acids were measured through the modified Lowry method, using bovine serum albumin (BSA) and humic acid as standards, respectively. Carbohydrates were quantified by Dubois method, employing glucose as standard (Kunacheva and Stuckey, 2014; Urrea et al., 2017).

2.5. Capillary suction time (CST)

In order to analyse the effect of the treatment on the filterability of naked cells and raw sludge, the capillary suction time (CST) was measured, employing an OFITE Capillary Suction Timer (294-50). During the analyses, a filter paper (Whatman #17) was located between two blocks. In the upper block, three radially separated electrodes are connected to a control box, in which the results are displayed. A sample holder with a 1 cm diameter opening was inserted into the upper block and then 5 mL of sample were added. The CST corresponds to the time elapsed from the instant the liquid of the sample begins to filter and reaches the first pair of electrodes to the instant that it reaches the third electrode.

Table 1
Characteristics of EPS fractions extracted from activated sludge.

	SMP	LB-EPS	TB-EPS
Proteins (mg L^{-1})	31 ± 2	36 ± 4	597 ± 40
Humic acids (mg L^{-1})	70 ± 2	49 ± 3	1062 ± 28
Carbohydrates (mg L^{-1})	11.3 ± 0.2	18.3 ± 0.2	367 ± 1
COD (mg O_2 L^{-1})	173 ± 3	176 ± 2	3346 ± 58
RBCOD (mg O_2 L^{-1})	62	18	1061
pH	8.04	7.82	7.05
CN (cm^{-1})	0.03	0.09	0.65

2.6. Readily biodegradable COD (RBCOD) analysis

The method is based on determining the effect of introducing a liquid sample on the oxygen uptake rate (OUR) of an activated sludge. Briefly, 170 mL of activated sludge at endogenous conditions was charged in a 250 mL jacketed vessel, which was maintained at 20 °C. The system was agitated at 250 rpm and five drops of nitrification inhibitor were added. Then, 50 mL of sample, previously centrifuged and filtered, was introduced in the vessel. The aeration of the medium was carried out in feeding cycles (1 min feeding and 6 min turned off). A more detailed description of the experimental protocol has been included in the supplementary material.

3. Results and discussion

3.1. Mineralization (as COD)

Fig. 2a shows the effect of wet oxidation on the different EPS fractions of the sludge. As can be observed, the oxidation was more intense in the following order: TB-EPS > LB-EPS > SMP. In fact, during the heating period necessary to reach the operating conditions (90 min), the COD reduction was almost unappreciable in both the SMP and LB-EPS fractions, whereas 22% COD removal was measured at the same reaction time on the TB-EPS fraction. It is noteworthy that the SMP fraction presented a very high resistance to oxidation, obtaining a COD reduction of only 14% at the end of the experiment (210 min), in contrast to the COD reductions of 32 and 50% for the LB-EPS and TB-EPS fractions, respectively, for the same time period.

Hence, it can be concluded that the more internal the location of the EPS in the structure of the floc, the more intense their oxidation was. Therefore, this behaviour suggests that biopolymers, which make up the TB-EPS fraction are more easily oxidised by free radicals than biopolymers on the SMP and LB-EPS fractions, which require higher activation energies for their mineralization to start. The more external biopolymers in the floc are exposed to the oxidant environment in the WWTP, either during the secondary treatment or during the storage of the sludge after thickening. So, these conditions could have a screening effect on SMP and LB-EPS fractions, with only the more refractory biopolymers remaining. At the same time, the LB-EPS would also act as a physical barrier for the attack of the free radicals in the medium, protecting the more internal biopolymers (which are forming the TB-EPS layer) and promoting their stability against oxidation.

Finally, as can also be seen in Fig. 2b, the TCOD as well as the SCOD followed a similar behaviour throughout the reaction on either naked cells or raw sludge. Both samples achieved a TCOD reduction around to 25 ± 2% before reaching the operating conditions (90 min). This fact indicates that the polymeric material, which composes cell membrane, organelles and cytoplasm, reacts quickly with the free radicals formed at early reaction times, denoting greater affinity for them than EPS or SMP. Moreover, the maximum rate of oxidation on both samples occurred between 100 and 110 min of treatment, reaching at that latter time a TCOD reduction of 47.5 ± 0.3%. From that moment on, the degree to which oxidation reactions occur declined sharply, yielding TCOD concentrations practically constant at the end of the treatment (54.5 ± 0.7% TCOD reduction).

Additionally, in order to identify any synergistic effects between the sludge fractions during the wet oxidation, their individual COD evolutions were added up into a single value, which was compared with the COD corresponding to raw sludge (dotted line in Fig. 2b). Results showed that the overall degradation in sludge fractions was almost identical to that obtained in raw sludge. Consequently, it

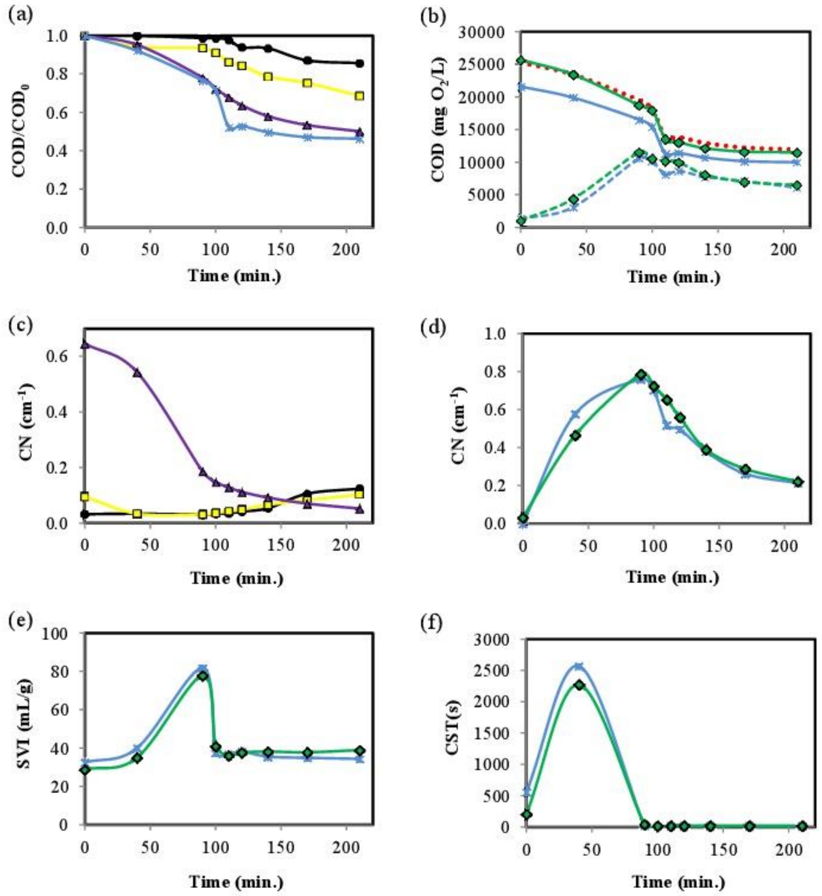

Fig. 2. Effect of wet oxidation at 190 °C and 65 bar on structural components of an activated sludge. (a) TCOD reduction from sludge fractions, (b) COD evolution for naked cells and raw sludge (solid and dashed lines represent TCOD and SCOD, respectively. Dotted line represents the sum of TCOD of all sludge fractions), (c) CN evolution from EPS fractions, (d) CN evolution from naked cells and raw sludge, (e) effect on settleability of naked cells and raw sludge, (g) effect on dewaterability of naked cells and raw sludge. (●) SMP, (🟨) LB-EPS, (▲) TB-EPS, (×) naked cells and (◆) raw sludge. Initial TCOD for each EPS fractions showed in Table 1.

was demonstrated that there were no significant interactions between the oxidation mechanisms of the different sludge fractions.

The results revealed that the naked cells were the fraction oxidised to a greatest extent, followed by TB-EPS, LB-EPS and, finally, SMP. Therefore, it can be concluded that the more internal the biopolymer is located in the cell, the more effectively it is attacked by free radicals.

3.2. Biodegradability (as readily biodegradable COD)

The effect of the wet oxidation on the biodegradability of each fraction is shown in Fig. 3, where the percentages of readily biodegradable COD (RBCOD) for initial and final samples of EPS after being treated for 210 min are depicted.

Prior to the wet oxidation treatment, around one third of the initial COD of both the SMP and the solubilised TB-EPS fractions corresponded to readily biodegradable materials, which could be easily assimilated during a hypothetical subsequent anaerobic digestion treatment, for example. Nevertheless, the percentage of RBCOD on the LB-EPS fraction was just 10%, suggesting that TB-EPS are made up of polymeric substances of lower complexity than those present in LB-EPS. In a previous study (Urrea et al., 2016), it was found that the proportion of low molecular weight polymers was higher on the TB-EPS fraction than on the LB-EPS one, thus corroborating the behaviour here observed.

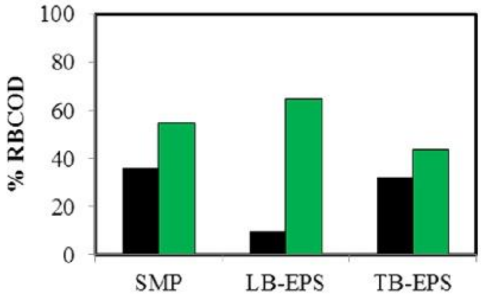

Fig. 3. Effect of wet oxidation at 190 °C and 65 bar on the readily biodegradable COD (RBCOD) of EPS fractions (■ initial and 🟩 final samples).

As previously stated, TB-EPS represents the fraction of biopolymers adjacent to the cells; thus, it seems reasonable to assume that the storage of the most easily biodegradable substrates is carried out mainly in this zone. Besides, LB-EPS forms a dispersible slime layer of biopolymers that can be easily released (Sheng et al., 2010), from the matrix of the sludge to the medium, particularly those of lower molecular weight, which end up lost in the surroundings of the cell. According to the considerations above, the percentage of RBCOD of SMP should be the lowest of them, a fact which has not been validated experimentally. This is due to the presence of cellular death products and compounds from incoming wastewater as well as SMP in the original supernatant of the sludge (Potvin and Zhou, 2011).

Once the EPS fractions were subjected to the wet oxidation treatment separately, an increase in the proportion of readily biodegradable material was observed in all of them (Fig. 3). This increase in the RBCOD content is particularly remarkable in the case of the LB-EPS fraction, achieving a final percentage of 65%, as opposed to 10% before treatment. By contrast, the results of RBCOD obtained at the end of the treatment (210 min) for SMP and TB-EPS were of 55 and 44%, with initial values before treatment of 36 and 32%, respectively. This fact is in line with the finding of the TB-EPS fraction being more easily oxidised by free radicals than biopolymers on the SMP and LB-EPS fractions, as reported previously. This involves greater breakdown of the biopolymers into smaller polymers and low molecular weight organic acids, which are usually considered as the main final products in wet oxidation treatments (Urrea et al., 2016) which, in turn, is reflected in an increase in the percentage of RBCOD.

In terms of production of readily biodegradable material, the percentage obtained by wet oxidation in either naked cells or raw sludge was not as positive as it was for the EPS fractions, either soluble or bounded. So, after 210 min of treatment, the RBCOD in both naked cells and raw sludge was around 33% of their soluble COD, which exhibited values of 6090 and 6500 mg O_2 L^{-1}, respectively.

Other studies have also analysed the effect of different pretreatment techniques on the formation of RBCOD from sludge. Burger and Parker (2013) studied the effect of thermal hydrolysis at 150 °C for 30 min on an activated sludge. They hypothesized that the SCOD generated by the thermal treatment (approximately 40% of the solid COD) was completely composed of RBCOD. Kianmehr et al. (2010) reported a similar result during the ozonation of an activated sludge. In that study, 42% of TCOD was transformed into RBCOD after 15 min of reaction.

Although these techniques showed a good yield in the production of RBCOD from sludge, they proved incapable of attaining any TCOD reduction, by contrast with what occurred with wet oxidation. In this sense, wet oxidation provides a more comprehensive solution to the treatment and reduction of the volume of sludge produced during sewage treatment, as it is able to improve the dewaterability properties of the sludge, in turn, reducing its TCOD and producing an easily biodegradable final effluent.

At this point, it is interesting to point out that the RBCOD concentrations observed after the wet oxidation of the raw sludge make the obtained effluent a cheap *in situ* carbon source for denitrification processes. The general reaction for denitrification indicates that 1.9 g of CH_3OH (equivalent to 2.86 g COD) are required to accomplish the reduction of 1 g of $N{-}NO_3^-$ into molecular nitrogen (N_2). Basing on this equivalence, each litter of wet oxidation effluent obtained could reduce approximately 750 mg of $N{-}NO_3^-$ in a conventional nitrification-denitrification process. The suitability of a wet oxidation liquor from mixed sludge to be used as a carbon source in the denitrification stage was proved by Strong et al. (2011). These authors showed that wet oxidation liquor was able to generate denitrification rates comparable to those obtained using acetate as carbon source.

3.3. Colour

Fig. 2c and d shows the effect of the wet oxidation on the colour number (CN) of each of the fractions as well as the raw sludge. As expected, the CN for initial TB-EPS was quite higher than those observed for SMP and LB-EPS, whose values were similar to one another. In fact, while TB-EPS presented an initial dark brown colour, SMP and LB-EPS were almost colourless. The LB-EPS fraction, whose RBCOD was the lowest of all the fractions, also showed the most intense colour per unit of total organic carbon (see Fig. 4). The presence of colour is usually related to conjugated molecules (alternating single and double bonds), these molecules (phenol, quinones …) having a low biodegradability (Collado et al., 2010). Hence, these facts are consistent with those observed on LB-EPS.

Obviously, the CN value is a function of the content of polymeric substances as well as their capacity of absorbance. Therefore, TB-EPS, which had a higher concentration of proteins, carbohydrates and, especially, humic acids, reached a six-fold higher CN than the other EPS fractions. However, when the CN is expressed per unit of organic carbon, LB-EPS was the fraction with the highest value, being up to three-fold higher (1.4 cm^{-1} g C^{-1} L) than those obtained for either SMP or TB-EPS (0.55 ± 0.5 cm^{-1} g C^{-1} L) (see Fig. 4).

Fig. 2c also shows a small drop in both TB-EPS and LB-EPS colour during the initial heating period (40 min). Nevertheless, when the operating conditions were reached (90 min), the CN on SMP and LB-EPS fractions remained approximately constant, whereas it showed a significant decrease on the TB-EPS one, achieving 71% reduction with respect to its initial value. This result reflects a strong impact of the initial oxidation stage (22% COD reduction during the first 90 min) on the colour of TB-EPS, which also experienced an initial fast shift in colour from brown to reddish-yellow. The conjugated molecules responsible for the colour also have great affinity for the free radicals (Luan et al., 2017). This explains why the TB-EPS, which make up the most coloured fraction, are also the fastest to be oxidised, as observed experimentally.

Subsequently, with the advance of the reaction, when the conjugated molecules were oxidised, the CN of TB-EPS showed a progressive and slight decrease until the end of the treatment (210 min), contrary to what occurred with SMP and LB-EPS, for which CN experienced a slight increase after 140 min of reaction. At the end of the experiment, a pale yellow colour in each one of the treated EPS fractions was observed. The formation of coloured compounds during thermal treatments of effluents containing proteins and carbohydrates has been attributed to the formation of substances known as melanoidins, which produce brown or yellow colour in function of their molecular weight (Wang et al., 2011). These substances, which are generated through Maillard reactions,

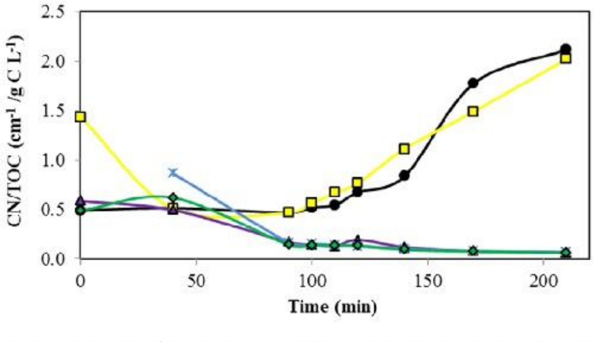

Fig. 4. Evolution of CN/TOC of supernatants during wet oxidation treatment at 190 °C and 65 bar. (● SMP, □ LB-EPS, ▲ TB-EPS, ✳ naked cells and ◆ raw sludge).

were identified as the main cause of colour in supernatants obtained from an activated sludge treated by thermal hydrolysis at 140–165 °C (Dwyer et al., 2008b).

Likewise, it is noteworthy that absorption spectra revealed the presence of two peaks in the UV zone (at 260 and 366 nm) from SMP and LB-EPS fractions (see Figures S1a and S1b). Those peaks, in turn, showed a continuous increase in the absorbance values with the advance of the treatment, also coinciding with the intensification of the yellow colour in the samples. Therefore, these results suggest that both facts are interrelated. Taking into account that the absorbance in the UV zone is usually correlated with the aromaticity of the dissolved organic material (Dwyer et al., 2008a), the behaviour in the UV zone could be due to the formation of melanoidins, whose composition includes heterocyclic and aromatic structures. Nevertheless, another possible explanation could be the hydroxylation of the phenylalanine aminoacid by effect of the attack of hydroxyl radicals (Zhang et al., 2014). This reaction, which has been well documented (Biondi et al., 2006; Devlin, 2002), transforms the phenylalanine into tyrosine, another aromatic aminoacid whose molar absorptivity in UV zone is five times higher than that of phenylalanine.

On the other hand, a slight and continuous decrease in absorbance values in the near-UV region was observed in the case of TB-EPS (Figure S1c). This difference in behaviour with respect to the other EPS fractions was probably due to the greater amenability to oxidation exhibited by the TB-EPS fraction during the wet oxidation. In fact, wet oxidation treatment caused TB-EPS to have the least intense colour, as can be observed in Fig. 1c.

According to Fig. 1d, it can also be observed that CN in supernatants during the wet oxidation of naked cells or raw sludge initially increased and then progressively decayed. The initial colourisation of the medium was attributed to the predominance of thermal hydrolysis reactions at the beginning of the experiment, which caused the increase in the CN due to the high solubilisation of polymeric substances during the first 90 min, as evidenced by the SCOD evolution (Fig. 2b) (Urrea et al., 2014). Later on, when the amount of suspended solids decreased, the oxidation reactions prevailed, causing the degradation of the biopolymers, which positively affected the colour of the effluent. The decolourisation capacity of wet oxidation has been profusely proved in the treatment of both different wastewaters and sludge (Fu and Kyzas, 2014; Oulego et al., 2016; Urrea et al., 2014). Similarly to colour, UV absorbance of supernatants in both samples experienced a sharp increase just after reaching a reaction temperature of 100 °C in the reaction (Figures S1d and S1e), presenting maximum values in such conditions. Nevertheless, since this time, the UV spectrum remained unaltered and no other peaks were identified, unlike for the EPS fractions. The high values of absorbance observed in the UV zone probably resulted from the solubilisation of a wide variety of compounds such as proteins, humic acids, lipids, DNA, etc, which usually present unsaturated bounds or aromatic rings.

Finally, it was observed that the colour per gram of organic carbon increased in both SMP and LB-EPS during the treatment, in contrast to what occurred with the rest of the fractions, in which such ratio decreased (Fig. 4).

3.4. Settleability (as SVI) and dewaterability (as CST)

Fig. 2e shows the evolution of the sludge volume index (SVI) during the wet oxidation of the naked cells and the raw sludge. During the first 90 min of treatment, it was observed a fast increase in SVI values for both samples. This worsening of the settling capacity of the sludge is probably due to the presence of a high concentration of dissolved biopolymers, which increase the electrostatic repulsion between the solid particles and cause a more stable suspension. In fact, the lowest settleability of both the raw sludge and the naked cells occurred when the highest degree of solubilisation had been reached. This finding is supported by the work of Zhen et al. (2012), who determined that the zeta potential of an activated sludge (20.4 g VSS/L) decreased from −12.6 mV to −18 mV after being treated thermally at 80 °C.

For both the raw sludge and the naked cells, surprisingly, the SVI showed a fast reduction between 100 and 110 min of treatment, remaining constant after that time. As well as the reduction in the suspended solids concentration, a plausible explanation for this fact is the oxidation and breakdown of the initially complex biopolymers, thus favouring the decrease in the electrostatic repulsion forces between the solid particles.

Regarding the wet oxidation effects on dewaterability, Fig. 2f shows the evolution of the capillary suction time (CST) during the wet oxidation of the raw sludge before and after the extraction of the EPS. Initially, the CST in the naked cells was 2.7 fold higher than in the raw sludge, meaning that the sludge dewaterability worsened after the EPS extraction. This behaviour can be due to the fact that the methods for EPS extraction do not permit to achieve a 100% yield. Therefore, during the resuspension of the naked cells fraction (once a high percentage of EPS have been extracted), the cells and the remaining EPS formed again an aggregated structure that binds into the water even more tightly. A similar result was reported by Jin et al. (2015) during the treatment of a biological sludge (8 g TSS L^{-1}) by ultrasound. They found that the treatment was effective in increasing the degree of disintegration of the sludge. However, the distribution of moisture in the sludge changed, observing that the percentage of bounding water (bound water plus surface water) increased. They suggested that the sludge flocs had been decomposed into smaller size ones, thus proving greater total surface area for water adhesion.

When the naked cells and the raw sludge were subjected to wet oxidation, a considerable increased in CST was observed in both samples during the first 40 min of reaction (upon reaching 100 °C). It is noteworthy that under these conditions, the EPS were released from the matrix while the cell walls and membranes began to lose its integrity, discharging intracellular components into the surroundings. According to Prorot et al. (2011), when the sludge is processed at temperatures above 80 °C, a re-flocculation phenomenon may occur due to the release of intracellular or extracellular polymers, which could favour the creation of new chemical bonds, especially when a high concentration of solids is still available. Chu et al. (2001) observed that the bound water content of the sludge increased by four-fold when it was treated by ultrasound (0.33 W/mL), suggesting that a great amount of water was attached onto the new surface of the small particles generated.

Therefore, a new distribution in the physical state of the water was probably the cause of the larger resistance to filtration observed at this time of treatment (40 min) for the naked cells and the raw sludge, as a greater amount of bounding water could have been retained either inside the formed flocs or on the surface of the particles. In fact, a change in the physical properties of the sludge had already been observed in a previous work with sludge samples treated by wet oxidation at 100 °C. In that study, the apparent viscosity for the aforementioned samples was more stable to the temperature scans in the rheometer than for the raw sludge, whose apparent viscosity decreased more sharply (Urrea et al., 2014). Therefore, the observed behaviour can be explained considering that the interstitial water in the raw sludge, which is bound by capillary forces, was released during the increase in temperature of the rheological analyses. Thus, a greater fluidity in the sludge was achieved. For the treated sludge, the increase of surface water (bound by adhesive forces) in its structure caused a low release of water during such analyses.

Afterwards, the sludge dewaterability was improved substantially, obtaining CST values of 31 and 44 s for naked cells and raw sludge, respectively. These results were attributed to an important reduction in the TSS concentration (58 ± 3%) as well as a high degree of hydrolysis and oxidation of the solubilised polymeric material that had been achieved by that time. After 100 min of wet oxidation, the CST in both samples showed a small decrease (19 ± 1 s) and, from this time on, the values plateaued.

Similar behaviours of CST have been reported during thermal treatments of various types of sludge. Bougrier et al. (2008) observed that thermal hydrolysis at temperatures below 130 °C caused an increase in CST of an activated sludge, for instance, from 1300 s to 2030 s. Nevertheless, the CST largely decreased and reached very low values (31 s) at higher temperatures. Andersen et al. (1994) found that the CST from a paper mill sludge increased from 93 s to 133 s after 15 min of wet oxidation at 150 °C, although a slight decrease (116 s) was subsequently observed at 30 min. Finally, they reported a CST reduction of 62 and 51 s after 15 and 30 min of wet oxidation at 200 °C, respectively.

4. Conclusions

This work provides a better understanding of the effect of the wet oxidation on the mechanisms involved in the oxidation of the different structural fractions of sludge, as a first step toward optimizing the process, thus resulting in a more sustainable WWTP.

The analysis of the structural components of the sludge during wet oxidation revealed that the inner fractions of the microbial aggregates are more amenable to oxidation than the outer ones. This behaviour was attributed to two factors: the screening effect of the oxidative environment in the WWTP on SMP and LB-EPS fractions and the role of the LB-EPS as a physical barrier against the attack of the free radicals in the medium, protecting the more internal biopolymers and promoting their stability against oxidation.

Once the EPS fractions were subjected to the wet oxidation treatment separately, an increase in the proportion of readily biodegradable material was observed for all of the fractions (Fig. 3). This increase in biodegradability is particularly remarkable in the case of the LB-EPS fraction, and it was accompanied by a high decolourisation of the sample, proving that the conjugated molecules responsible for the colour also have great affinity for the free radicals.

Short treatment times of the sludge by wet oxidation caused a marked worsening of some important properties, such as settleability and dewaterability. These results were attributed to the increased electrostatic repulsion forces between the solid particles due to the solubilisation of polymers, as well as to the increase of the bound water content in such solids. Nevertheless, once a high degree of solubilisation and oxidation was reached, the characteristics of settleability and dewaterability were largely improved.

Finally, it was proved that there are no synergistic/inhibitory effects between the different sludge fractions during the wet oxidation process.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2017.09.033.

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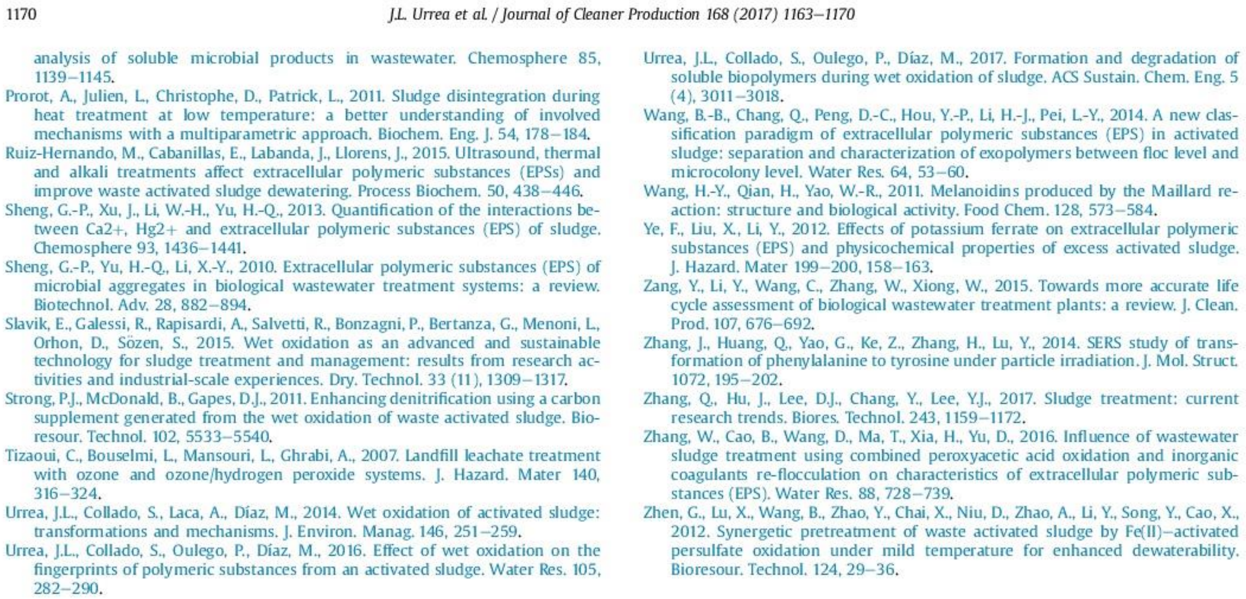

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Effect of wet oxidation on the fingerprints of polymeric substances from an activated sludge

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ABSTRACT

Thermal pre-treatments of activated sludge involve the release of a high amount of polymeric substances into the bulk medium. The molecular size of these polymers will largely define the subsequent biological treatment of the liquid effluent generated. In this work, the effects of wet oxidation treatment (WO) on the fingerprints of the polymeric substances which compose the activated sludge, were analysed. For a better understanding of these transformations, the sludge was separated into its main fractions: soluble microbial products (SMP), loosely bound extracellular polymeric substances (LB-EPS), tightly bound extracellular polymeric substances (TB-EPS) and naked cells, and then each one was subjected to WO separately (190 °C and 65 bar), determining the fingerprints evolution by size exclusion technique. Results revealed a fast degradation of larger molecules (over 500 kDa) during the first minutes of treatment (40 min). WO also increases the absorptive properties of proteins (especially for 30 kDa), which is possibly due to the hydroxylation of phenylalanine amino acids in their structure. WO of naked cells involved the formation of molecules between 23 and 190 kDa, which are related to the release of cytoplasmic polymers, and more hydrophobic polymers, probably from the cell membrane. The results allowed to establish a relationship between the location of polymeric material and its facility to become oxidised; thus, the more internal the polymeric material in the cell, the easier its oxidation.

When working directly with the raw sludge, hydrolysis mechanisms played a key role during the starting period. Once a high degree of solubilisation was reached, the molecules were rapidly oxidised into other compounds with refractory characteristics. The final effluent after WO showed almost 90% of low molecular weight solubilised substances (0–35 kDa).

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1. Introduction

Sludge produced in wastewater treatment plants is considered as a problem of great interest due to the large volumes generated and its difficult dewaterability. In order to reduce the volume of sludge and improve its handling characteristics, different techniques are already being implemented. One of the most attractive technologies is hydrothermal processing, which takes advantages of the unique characteristics of water at elevated temperatures and pressures to degrade organic and inorganic components of sludge (Neyens et al., 2004). Hydrothermal technologies in sludge treatment can be broadly categorised into two main groups, namely oxidative (wet oxidation) and non-oxidative (thermal hydrolysis) techniques. In this respect, wet oxidation (WO) represents a potential alternative to be applied to the treatment of sludge, due to the good results that have been achieved using this technique (Strong et al., 2011). WO is usually carried out at elevated temperatures (100–320 °C) and pressures (0.5–20 MPa) under a flow of pure oxygen or air as oxidising agent (Riedel et al., 2015). In this way, high temperature accelerates sludge solubilisation, whereas high pressure increases the solubility of the oxidant and avoids the transference of pollutants to air (Chung et al., 2009). This technique is frequently applied under moderate conditions as pretreatment for sludge conditioning before a subsequent biological treatment (Abe et al., 2011).

Biological sludge is mainly composed of microbial aggregates, which, in turn, are made up of cells embedded in a matrix of extracellular polymeric substances (EPS). EPS are the main components of activated sludge, granular sludge and biofilms (Bezawada et al., 2013). Based on their association with cells, EPS have been classified into soluble microbial products (SMP) when they are in the dissolved form in the surrounding environment

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(Zuriaga-Agustí et al., 2013), and bound EPS when they are bound to cells. The latter can also be divided into loosely bound (LB-EPS) or tightly bound (TB-EPS) to the cell (Yu et al., 2008; More et al., 2014). They are originated from secreted substances during cellular metabolism, as well as by products of cellular lysis, and compounds present in the wastewater. EPS are composed of either hydrophobic or hydrophilic groups. It is believed that the formation of hydrophobic areas due to EPS in microbial aggregates could be beneficial for the adsorption of organic pollutants (Sheng et al., 2010). Their composition mainly includes proteins, carbohydrates and humic acids, along with other minority substances such as DNA and uronic acids (Frolund et al., 1996; Liu and Fang, 2002; Guibaud et al., 2005). In fact, the amount of EPS and their composition play a key role in cell–cell and cell–surface interactions. Such characteristics largely determine the main properties of sludge, such as cell adhesion, floc aggregation and cohesion, retention of water, sorption of organic compounds and inorganic ions (Ni et al., 2009; Zhu et al., 2015). It is assumed that the biosorption capacity of bacteria involves a physicochemical interaction between the adsorbate and the functional groups of EPS on the cell surface (Comte et al., 2008).

Hydrothermal treatments, either WO or thermal hydrolysis, allow the breakage of floc structure by means of solubilisation and degradation of EPS and cellular lysis. Both mechanisms involve the release of high amounts of polymeric substances into the bulk medium, whose composition, concentration and molecular size will vary with the reaction time, especially under an oxidant atmosphere. Metals are commonly linked to the polymeric substances of sludge. In WO, the fate of most of these metals present in the sludge, such as Cd, Mn, Ni, Pb, and Zn, is associated with the solid residue obtained after the treatment. However, in the case of Cu, its presence have been reported in the soluble material (Mishra et al., 1995). It should be noted that pH play an important role in the solubility of metals. Hence, this parameter is carefully taken into account in WO studies, especially in those including the use of homogeneous catalysts (Bhargava et al., 2006). During the WO of sludge, the pH of the medium turned to acid ($pH \approx 4$), which occured while the solubilisation step is developed. Subsequently, the oxidation reactions led to a re-alkalinization of the effluent (Urrea et al., 2014). In the case of sludge, another factor that influences the final fate of the metals is the sorption by particulate material (Bernardi et al., 2010).

Curiously, even though numerous papers are available regarding thermal treatment of sludge, these are focused on the degrees of solubilisation or mineralization and/or the improvement of biodegradability. Nevertheless, to the best of our knowledge, there are no available studies on the nature of the polymeric compounds generated during thermal treatment. Taking into account that the molecular size of these polymers largely determines, among other properties, the biodegradability, filterability, settleability and rheology of the final effluent, this information could be very useful in order to select the most appropriated post-treatment for either the stabilised sludge or the supernatant obtained after the hydrothermal processing (Wang and Li, 2015).

In the last few years, sophisticated methods have been implemented to better characterise pools of polymeric substances. One of them is size exclusion chromatography (SEC), which is a rapid molecular weight screening technique aimed at determining the average molecular weight range and polydispersity of a polymeric sample. This technique has been successfully applied to the characterisation of different samples such as proteins (Uversky, 1993) and humic substances (Bhatia et al., 2013), natural organic matter (Sánchez-González et al., 2012), lignin and carbohydrates (Wong and de Jong, 1996), glycogen (Sullivan et al., 2014), and even more complex analysis, including peptide mixtures quantification (Bodin et al., 2015). In fact, the characterisation of extracellular polymeric substances in microbial aggregates by SEC has received considerable attention in recent years. Thus, Görner et al., 2003 characterised the EPS of an activated sludge by SEC and Fourier transform infrared micro-spectroscopy. They identified seven groups of EPS containing protein in each of them, whereas carbohydrates were observed in only three of these groups. Comte et al., 2007 analysed the effect of EPS extraction method on their molecular size. They observed that the use of chemical agents caused a change in the fingerprints of EPS, probably due to either the contamination of samples with these reagents or a selective separation. However, after an in-depth bibliographic review, the conclusion was that the available works, dealing with EPS, have focused on the characterisation of these substances in raw and non-stabilised sludge. Nevertheless, there is no information on the modification of EPS when a sludge minimisation technology is applied.

On the basis of the remarks made in the foregoing paragraphs, the aim of this study was to determine, for the first time ever, the effect of WO on the fingerprints of polymeric substances generated from the sludge, either by EPS solubilisation or by cellular lysis. To that end, sludge was separated into its structural components (SMP, LB-EPS, TB-EPS and naked cells), which were subsequently oxidised in order to compare the resulting fingerprints between them and with those obtained from the raw sludge.

2. Material and methods

2.1. Sludge samples

To perform the experiments, activated sludge obtained from a municipal wastewater treatment plant located in Asturias (Spain) were used. This sludge was thickened in a dissolved air flotation unit from which it was collected, being subsequently stored at 4 °C until further use. The sludge had the following characteristics: total suspended solids (TSS): 21.6 ± 1.3 g/L, volatile suspended solids (VSS): 15.6 ± 0.9 g/L, sludge volume index (SVI): 46.3 mL/g ± 2.7, total chemical oxygen demand (TCOD): 22963 ± 1377 mg O_2/L and initial pH: 6.9.

2.2. EPS extractions

The extraction method applied is mainly based on the one proposed by Li and Yang, 2007. A volume of 450 mL of sludge was collected in a centrifuge bottle of 500 mL and subjected to centrifugation at 4000 g (4 °C, 5 min), employing a Kubota 6500 centrifuge. The supernatant obtained, corresponding to the SMP fraction, was removed and stored, whilst the pellet deposited at the bottom of the bottle was re-suspended with distilled water until its initial volume. Then, the sample was centrifuged again (6000 g, 4 °C and 15 min) and the resulting supernatant, which corresponds to LB-EPS, was also extracted and stored. Finally, the pellet was again re-suspended to its original volume with distilled water at 65 °C and placed in a thermostatically controlled water bath at the same temperature, for 30 min. Afterwards, the sample was centrifuged for the third time (8000 g, 4 °C and 15 min). The supernatant, corresponding to TB-EPS, was removed and stored, while the residue, that is to say, the “naked cells”, was also re-suspended and stored at 4 °C.

So as to remove any material in suspension, SMP, LB-EPS and TB-EPS were filtered through a gravimetric filter paper after extraction, and then centrifuged at 12000 g and 4 °C and 15 min. Finally, these samples were stored at −20 °C until being used in WO experiments.

2.3. Experimental setup

WO experiments were carried out in a PARR series 4520 reactor (Fig. 1), which is equipped with a propeller stirrer (500 rpm) to maintain mixed the sample. Since the beginning of the experiment, a stream of pure oxygen, previously conditioned in a humidifier, was fed into the reactor with a flow rate of 1.2 L min^{-1}. The reactor temperature and the oxygen flow were regulated by means of a proportional integral differential controller. The operating conditions employed were 190 °C, 65 bar and two hours of reaction, also considering the initial heating period before the reactor reaches the reaction conditions (85 ± 5 min).

Eight samples were taken from the reactor throughout each experimental run; the first one was withdrawn when temperature reached a value of 100 °C in order to check changes during the heating period, whereas the following samples were collected when the operational conditions were reached, remaining constant until the end of the experiments.

The first WO experiments were conducted with the raw sludge or with the naked cells, in order to prevent any changes in its structure during the storage phase that could distort the results.

2.4. Analytical methods

Various analysis were performed in order to characterise the sludge used in the experiments. Such analysis included the determination of total suspended solids (TSS), volatile suspended solids (VSS), fixed suspended solids (FSS), total chemical oxygen demand (TCOD), sludge volume index (SVI) and pH and were performed according to Standard Methods (APHA, 1998).

The initial EPS fractions were characterised according to their content of proteins, humic acids and carbohydrates. In order to determine the content of proteins and humic acids, the modified Lowry method was employed, using BSA and humic acids as standards, respectively (Frolund et al., 1995). Dubois method was employed for carbohydrates determination, glucose being used as standard (Dubois et al., 1956). All of the analyses were performed in triplicate, obtaining a standard deviation lower than 6% with respect to mean values. The composition of each EPS fraction is shown in Table 1.

Table 1
Characteristics of EPS fractions extracted from activated sludge.

	SMP	LB-EPS	TB-EPS
Proteins (mg/L)	31 ± 2	37 ± 3	453 ± 27
Humic acids (mg/L)	70 ± 5	50 ± 3	1131 ± 57
Carbohydrates (mg/L)	11.1 ± 0.6	18 ± 1	291 ± 17
Total[a] (mg/L)	112 ± 8	103 ± 7	1876 ± 101
COD (mg O_2/L)	173 ± 10	176 ± 12	3346 ± 200
TOC (mg C/L)	65 ± 4	66 ± 4	1104 ± 66
MOC	0.04	0.03	−0.55

[a] Sum of the content in proteins, humic acids and carbohydrates.

Total organic carbon (TOC) analysis were measured by means of a TOC analyzer (Shimadzu TOC-V_{CSH}, Japan). Soluble COD concentration was determined by closed reflux, colorimetric method, using a DR2500 spectrophotometer (Hach Company, USA).

Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) measurements of initial and final fractions of SMP, LB-EPS and TB-EPS were performed on a Varian 670-IR spectrometer with 32 scans at a resolution of 4 cm^{-1}. All spectra were recorded in the range of 4000 to 600 cm^{-1}. In all cases, the fractions were lyophilised previously to the performance of ATR-FTIR analyses. However, the information obtained using this technique was less significant than initially expected. This is because of the great degree of complexity in the polymeric matrix, caused by the simultaneous presence of high concentrations of carbohydrates, proteins, humic acids, DNA … Besides, each of these compounds presents a high number of different functional groups that can vibrate at similar wavenumbers. For instance, proteins and peptides have broad bands, which are difficult to differentiate due to considerable overlap. The reason for this is the high number of amino acids, which, in turn, form the great range of different proteins in the samples. A more detailed discussion of the FTIR spectra obtained can be found in the Supplementary Material.

Finally, the mean oxidation number of organic carbon (MOC) was calculated on the basis of equation (1) (Vogel et al., 2000).

$$\text{MOC} = 4 - 1.5\frac{\text{COD}}{\text{TOC}} \quad (1)$$

where MOC takes values from −4 (i.e. for methane) to +4 (i.e. for carbon dioxide) and COD and TOC values are expressed in mg O_2/L and mg C/L, respectively.

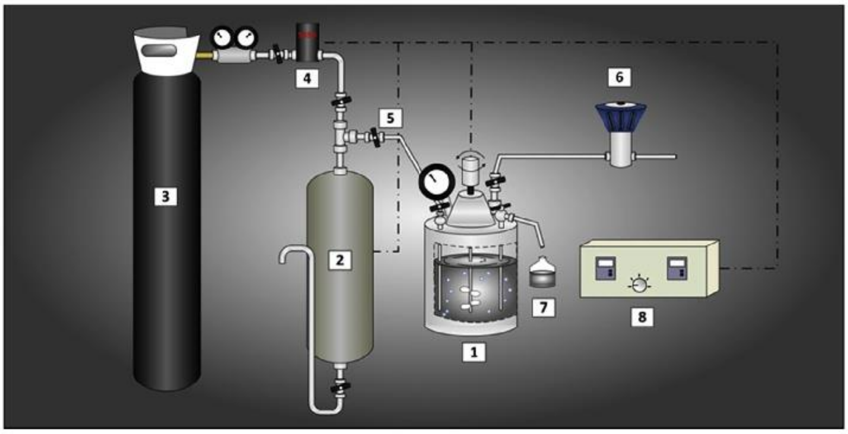

Fig. 1. Scheme of the experimental setup. (1) Reactor, (2) humidifier, (3) oxygen cylinder, (4) flow controller, (5) control valve, (6) back pressure valve, (7) sampling port and (8) PID controller.

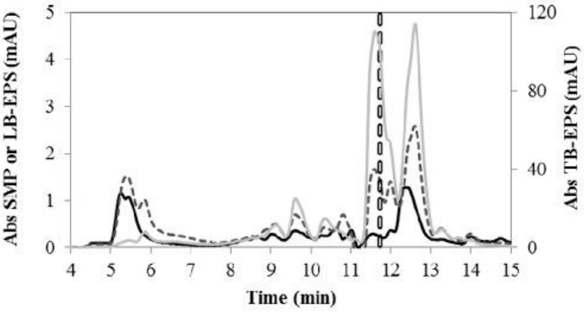

Fig. 2. Initial EPS fingerprints ▬SMP, - - - LB-EPS, —— TB-EPS, ▭ ▭ ▭ total column volume. UV absorbance at 260 nm.

2.5. *Size exclusion chromatographic analysis (HP-SEC)*

The monitoring of the fingerprints of each sample was performed by high performance liquid chromatography (Agilent 1200, Agilent Technologies Inc., California, USA), employing a Yarra SEC-2000 (300 × 7.8 mm) column. The total column volume was determined using a $NaNO_3$ solution, obtaining a value of 11.8 mL. The mobile phase was composed of a buffer solution of 9 mM NaCl, 0.9 mM Na_2HPO_4 and 0.005% NaN_3, adjusted to pH 7.0 (±0.1) with H_3PO_4, and with an ionic strength of 0.02, selecting a steady flow rate of 1 mL/min. All the samples were filtered through 0.45 μm PVDF filters (Millipore) prior to injection (20 μl). The detection was carried out with a diode array UV detector at several wavelengths (210, 260 and 280 nm) and at room temperature.

The column was previously calibrated employing a Protein Standard Mix 15–600 kDa supplied by Sigma-Aldrich (69385), which contains four proteins: Ribonuclease A (13.7 kDa), Albumin (44.3 kDa), γ-Globulin (150 kDa) and Thyroglobulin (670 kDa), as well as a low molecular weight marker, ρ-aminobenzoic acid (0.14 kDa). The resulting calibration curve showed a coefficient of determination (R^2) of 0.93. The samples obtained during the treatment of TB-EPS, naked cells and raw sludge, which contained higher concentrations of polymeric substances, were diluted with mobile phase before injection. Analysis for each of the samples were conducted in triplicate and the differences observed between them were negligible, either for peak retention times or total areas.

It was observed that the hydrophobic and/or electrostatic nature of the interactions between the molecules of the EPS and the packing of the column impeded the correct determination of the molecular mass of EPS in accordance with other studies (Simon et al., 2009). All of the samples were tested again using a mobile phase containing methanol with dual purpose. Firstly, the verification of the interactions between the molecules in the samples and the filling material of the column. Secondly, the identification of the peaks affected by these phenomena.

The chromatograms obtained with both mobile phases for a given sample were compared. The disappearance or displacement of some of the peaks when running the tests with the mobile phase containing methanol revealed the hydrophobic interactions between such peaks and the packing material of the column. The second mobile phase was prepared containing 5% (v/v) of methanol, 9 mM NaCl, 0.9 mM Na_2HPO_4 and 0.005% NaN_3, adjusted to pH 7.0 (±0.1). A description of these results has been included in the supplementary material.

3. Results and discussion

3.1. *Initial EPS fingerprints*

The fingerprints for the extracted EPS before WO treatment were shown in Fig. 2. Obviously, initial fingerprints for non-solubilised naked cells cannot be measured, whereas the fingerprint for raw sludge has to coincide with that for SMP.

As can be seen in Fig. 2, the fingerprints for SMP and LB-EPS fractions exhibited a high similarity between them, with two main peaks, one obtained at a retention time of around 5.5 min, which corresponded to large polymers of 500 kDa, and the other one being associated with small polymers of 14 kDa, at 11.5 min. Some other low intensity peaks were also detected for elution times of approximately 9.0, 9.7, 10.5 and 11.5 min (65, 43, 26 and 14 kDa) in both chromatograms. The similarity observed in the molecular distributions and compositions of these two fractions (Table 1) can be easily explained taking into account that LB-EPS are of highly hydrated matrix, and tend to form a dispersible and loose slime layer without an obvious edge. This involves that LB-EPS can be easily released from suspended or attached cells by either dissolution/hydrolysis or shearing-off from the cell surface, thus turning into SMP. In the same way, some SMP can be adsorbed by the biomass flocs and then, become loose bound EPS. This dynamic equilibrium between SMP and LB-EPS is even more marked in our case, in which the sludge was preconditioned to reduce its moisture content. So, the free water in the sludge, where SMP is present, was reduced considerably during the thickening, and the transformation of the SMP and LB-EPS fractions (found in direct contact) proceeds faster with a lower water content, obtaining similar characteristics between both fractions.

At this point, it is also worthy to note that peaks at times longer than the one corresponding to the total column volume, that is, 11.8 min, were observed for every fingerprint. Several authors also noted similar behaviors during the characterisation of other pools of polymers. They reported that this is probably due to the presence of interactions between the molecules corresponding to such peaks and the filling material of the column, which causes the polymers to be retained for times longer than those corresponding to their sizes. As can be seen in Fig. 2, fingerprints suggest a higher proportion of hydrophobic polymers in the LB-EPS fraction than in the SMP one.

When comparing the height of the SMP and LB-EPS peaks, and taking into account that EPS compositions are very similar (see Table 1), it can be suggested that the proportion of high molecular weight polymers is higher in the SMP fraction than in the LB-EPS one. Hence, we have established three categories for the classification of polymers according to their size: low (0–35 kDa), medium (35–150 kDa) and high (>150 kDa) molecular weight molecules. Then, after discarding the peaks located outside the measuring range of the column, it can be proposed that SMP were composed of 14, 13 and 73% polymers of low, medium and high molecular weight, respectively, whereas the proportions found in the LB-EPS fraction were 44, 10 and 46%, respectively. The higher proportion of high size polymers in SMP than in LB-EPS is probably caused by their easier shedding from the cell surface and their lower biodegradability when compared to smaller polymers.

However, the differences between TB-EPS fingerprint and that of SMP or LB-EPS are easily observable. Firstly, the absorbance values detected for the TB-EPS are around twenty-times higher than those for SMP or LB-EPS, according to the expected behaviour, considering the higher concentrations of proteins, humic acids and carbohydrates detected in this fraction (see Table 1). Secondly, the small peak at 6 min (400 kDa) suggests that a small proportion of high molecular weight polymers is present in this fraction. In fact, the proportions of low, medium and high molecular weight polymers were 83, 15, 2% respectively. Thirdly, around 52% of fingerprint area was obtained at times higher than that corresponding to the elution volume, against the 38 or 47% observed for SMP and LB-EPS, respectively. This fact suggests that TB-EPS has a greater hydrophobic character than SMP or LB-EPS.

According to these results, it can be identified a clear transition on the size distribution of polymers as a function of their nearness to cell. Hence, lower molecular weight polymers with greater hydrophobic character were more predominant in the proximity to cell. It seems reasonable to assume that the more hydrophobic and less soluble EPS tends to be found in an inner layer around the cellular surface, as far as possible from the aqueous medium, as it was observed experimentally. Finally, MOC results also suggested differences in the oxidation state of the polymers, as well as in the size and hydrophobicity. The MOC was similar for SMP and LB-EPS fractions, obtaining values of 0.04 and 0.03 respectively, whereas TB-EPS exhibited a lower value (−0.55). Consequently, the oxidation state for TB-EPS is lower than for SMP or LB-EPS fractions, which are in direct contact with the aggressive environment and also acts as an external barrier, protecting TB-EPS from oxidation.

3.2. Effects of WO on fingerprints of activated sludge fractions

Figs. 3 and 4 show the evolution of the fingerprints obtained for each sludge fraction during its treatment by WO at 190 °C and 65 bar, and the mean oxidation number of organic carbon, respectively.

3.2.1. SMP fraction

Fig. 3a and b shows the evolution of the SMP fingerprints as the areas corresponding to low medium and high size polymers during WO treatment, respectively. It can be easily seen that during the first 40 min of reaction (T = 100 °C), the largest molecules (>150 kDa) disappeared rapidly, even when the final temperature (190 °C) had not been achieved yet. On the other hand, the area of the peaks corresponding to lower molecular weight polymers (<65 kDa) clearly increased during all reaction times. Prominent among them was the peak which corresponds to a group of molecules of 30 kDa (10.3 min), which maintained a continuous and fast increase in absorbance capacity until the end of treatment. This behaviour was caused by the progressive increase detected in the area of the polymers of 0–35 kDa. On the other hand, the area of the polymers of 35–150 kDa and those of greater hydrophobicity (located after total column volume) showed a slight decrease after reaching the reaction conditions in the WO (Fig. 3b). It is also remarkable the low intensity peaks that emerged after 11.6 min. All of these results suggest that WO processes cause the fast breaking of long polymers, yielding molecules with molecular weights mainly below 35 kDa (10 min).

MOC evolution was also measured in order to determine the nature of these changes on fingerprints during WO. This parameter can be used to establish if the transformations that took place were due to physical degradation (thermal hydrolysis), chemical (oxidation) or both, considering at the same time the changes on COD and TOC. As can be observed in Fig. 4, MOC from SMP fraction remained almost constant during the course of the treatment, only showing a slight increase at the end of the experiment. Regarding COD and TOC concentrations, slight reductions were also detected after the treatment, namely around 14% and 10%, respectively. However, the values of both parameters did not change during the first 110 min of reaction, indicating that the initial changes of fingerprints, that is to say, the breakage of the longer polymers, were caused exclusively by thermal hydrolysis reactions. After 110 min, a small increase in the MOC (caused by the COD and TOC reductions) is observed, suggesting that oxidation mechanisms began to take place during the last minutes of the reaction, although at a low proportion. It is also remarkable that the fingerprints remained approximately constant during this period, except for the peak corresponding to 30 kDa. At first sight, these polymers should be generated from larger polymers, but the corresponding peak was not detected. This suggests the modification of one or several functional groups in the polymer, which causes a higher light absorption coefficient of the polymer without the occurrence of polymer break-up. This behaviour was due to the oxidation turns phenylalanine amino acid into tyrosine in proteins, as it will be explained in more detail in the section below.

3.2.2. LB-EPS fraction

The changes caused by WO treatment in LB-EPS fingerprints were quite similar to those observed for the SMP (see Fig. 3c and d). As in the previous case, a reduction in the area of the peaks corresponding to the higher molecular weights (over 150 kDa) was observed during the first minutes of reaction (Fig. 3c). In turn, medium size molecules particularly from 65 to 45 kDa, i.e., from 9 to 9.6 min, gradually disappeared whereas the area of 0–35 kDa in fingerprints showed an increased up to 6 times its initial value. In fact, two news groups of molecules emerged, which correspond to 35 (10.0 min) and 30 (10.3 min) kDa, respectively. The latter also showed an increase in its absorbance capacity with the course of the treatment, as it happened with SMP fingerprints. At the end of the WO treatment, LB-EPS were made up of mainly 35 and 17 kDa polymers (10–11.2 min).

Examining the MOC evolution for the LB-EPS fraction (Fig. 4), a small increase can be identified, which was a bit more marked than that observed for SMP fraction; suggesting that oxidation reactions were more significant for LB-EPS than for SMP. In this case, COD and TOC reductions around 32%, and 24%, respectively, were achieved at the end of treatment. It is worthy to note that COD gradually decreased since the beginning of the reaction, whereas TOC reduction was only observed from 100 min onwards. According to these results, it can be deduced that during heat-up period, the thermal hydrolysis reactions took place to a certain extension at the same time that oxidation, both being responsible for the greatest initial changes in fingerprints. When 100 min of reaction was reached, the partially oxidised organic material began to be mineralised, yielding CO_2 and producing a reduction in TOC concentrations. Simultaneously, a decline in the area of polymers of higher hydrophobicity was also observed, suggesting that the mineralization in LB-EPS was mainly developed in the polymers belonging to this group.

3.2.3. TB-EPS fraction

In the WO of TB-EPS, the major changes in the fingerprints were registered after reaching the reaction conditions (see Fig. 3e). So, when temperature reached 100 °C in the reactor, the fingerprints had already lost the peaks corresponding to molecules over 500 kDa (5.5 min) and 65 kDa (9 min). In turn, two peaks located outside the volume of the column were partially degraded, being probably the origin of three new peaks that appeared in the same zone. Hence, after the first 90 min of reaction, the total area of the fingerprint reached a minimum (Fig. 3f), suggesting a low concentration of polymers, either of high, medium or low size.

With the increase in the reaction time, no more changes in the number of peaks in the fingerprint were observed. Nevertheless, some of these peaks showed a continuous increase in their absorbance values during this period, thus leading to a considerable increase in the area of the fingerprints. Particularly, the areas most affected by this phenomenon were the corresponding to polymers of 0–35 kDa and those of higher hydrophobicity (Fig. 3f). In contrast with the SMP and the LB-EPS fractions, the area attributed to polymers of 0–35 kDa and hydrophobic polymers in TB-EPS

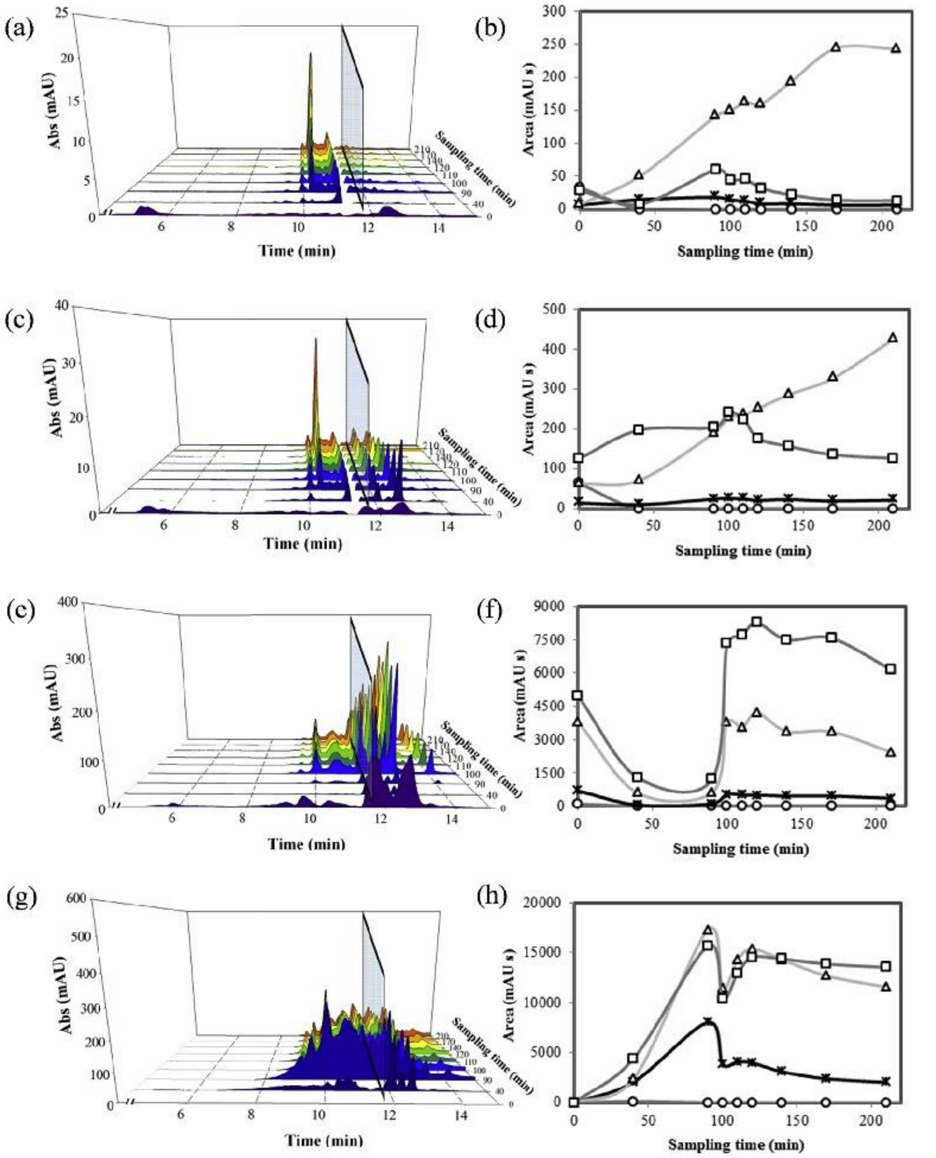

Fig. 3. Evolution of the fingerprints of activated sludge fractions. (a) SMP, (c) LB-EPS, (e) TB-EPS and (g) naked cells. ▭ total column volume. UV absorbance at 260 nm. Evolution of the fingerprints area of activated sludge fractions. (b) SMP, (d) LB-EPS, (f) TB-EPS and (h) naked cells. -○- >150 kDa, -✳- 35–150 kDa, -△- 0–35 kDa and -□- after total column volume. WO conditions, 190 °C and 65 bar.

showed a continuous decrease during the last minutes of reaction.

Observing the MOC evolution (Fig. 4), this fraction also showed the highest MOC after treatment of all the fractions, even when its initial value was the lowest one. This finding implies that TB-EPS were more sensitive to the attack by free radicals than LB-EPS, which, in turn, were also more reactive than the SMP. A COD reduction of approximately 50% was obtained after oxidation, which evolved gradually since the initial time. However, TOC reduction was clearly lower, around 26%, and noticeable only after 90 min. These results, which were quite similar to those obtained for the LB-EPS fraction, also suggest the fast hydrolysis and partial oxidation of the larger polymers during the first minutes of reaction. In this sense, initial reactions had a screening effect, reducing the number of peaks. For longer reaction times, WO seems to have no effect on the polymeric molecular weight distribution since the number of peaks was the same. However, the results revealed the modifications in the absorbance capacities of some polymers, probably caused by the partial oxidation of some of their functional groups. Therefore, the position of the peaks corresponding to these molecules in the fingerprint remains unaltered, undergoing only an increase in their intensity.

There are several studies that have determined that in samples containing proteins, the attack of hydroxyl radicals turns phenylalanine into tyrosine (Biondi et al., 2006; Zhang et al., 2014), which

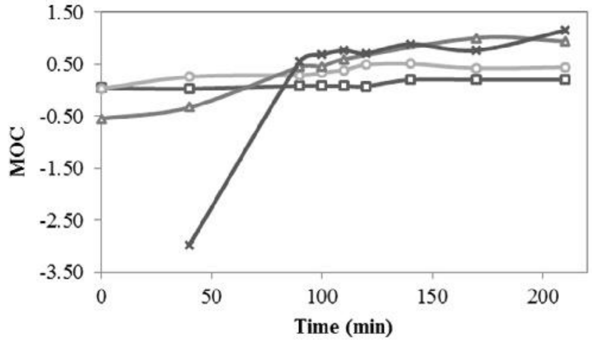

Fig. 4. Evolution of the mean oxidation number of organic carbon (MOC) of activated sludge fractions during WO at 190 °C and 65 bar. (a) –□– SMP, (b) –○– LB-EPS, (c) –△– TB-EPS and (d) –✱– naked cells.

is another amino acid with an aromatic side chain, but with a molar absorptivity five times higher than that corresponding to phenylalanine (Devlin, 2002). Although in those works, hydroxyl radicals have been generated by means of other techniques, it seems reasonable to propose the hydroxylation of phenylalanine in the fractions of the sludge as one of the main causes of the observed results after wet oxidation, whose oxidation mechanism also involves hydroxyl radicals (Bhargava et al., 2006). In addition to this, the increase in the area of hydrophobic polymers was greater in comparison to the one of the small size polymers, which could be explained taking into account that proteins have hydrophobic tendencies whereas carbohydrates are more hydrophilic. This suggests that such peaks due to proteins, which contain phenylalanine, tend to appear at longer retention times (Liu and Fang, 2003).

3.2.4. Naked cells fraction

Fig. 3g shows the fingerprints obtained for the naked cells after different WO times. As might be expected, the fingerprint area increases rapidly (Fig. 3h). This increase is the result of the initial release of the intracellular material caused by the lysis of the cells during the heating period. Observing the fingerprint after 40 min of oxidation (T = 100 °C), it can be deduced the initial fast solubilisation of high molecular weight polymers, ranging from 23 (10.7 min) to 190 kDa (7.2 min). Regarding the MOC (Fig. 4), this parameter achieved a value of −3, indicating that the polymers that initially composed the cells have a low oxidation state.

When the operating conditions were reached, the fingerprint area was maximum, indicating a high and fast degree of cellular solubilisation, and a total of fourteen peaks were detected, obtaining eight of them at times higher than the one corresponding to the total volume of the column. Nevertheless, the area of those peaks, that is to say, the one which corresponds to the most hydrophobic polymers, was 38% of the total fingerprint, being very similar to those obtained for 0–35 kDa polymers (42%). Meanwhile, the MOC increased considerably (0.6) while the soluble COD (10590 ± 130 mg O_2/L) and TOC (4610 ± 90 mg C/L) achieved a maximum.

The presence of a significant number of molecules outside of the column volume could be associated with the solubilisation of biopolymers that integrate the cell membrane. These substances consist of phospholipids and proteins, both presenting in their structure either hydrophobic or hydrophilic regions, the hydrophobic ones being more predominant (Madigan et al., 2003). So, it is also reasonable to assume that the hydrophilic properties are predominant in the biopolymers which compose the cell cytoplasmic region, thus allowing their separation based on their size. After the reaction conditions were reached, the fingerprint area started to decrease, indicating that the oxidation and/or hydrolysis mechanisms of the dissolved polymeric material prevail over the solubilisation of the suspended biomass. This fact was also proven through the continuous reduction of COD and TOC of soluble material, which, in turn, promoted a slight increase in MOC. Such increase meant that the oxidation of the polymers occurred progressively until they are eventually mineralised (partially converted into CO_2). Thus, after 210 min of treatment, the soluble COD and TOC reductions were 42% and 30% of the maximum values achieved in the medium by solubilisation.

By following the course of the oxidation treatment, it was observed a reduction in the size of the peaks, especially in the zone where the separation of the polymers occurred by size exclusion. In fact, this effect seems to be more marked for the shorter retention times, when the polymers with molecular weights above 54 kDa (<9.3 min) completely disappeared after WO treatment. However, there are two exceptions to the decrease in the absorbance: 35 kDa (10 min) and 16 kDa (11.3 min). In short, while the initial hydrolysis reactions observed at the beginning of the treatment increase the area of the peaks in the chromatogram due to the solubilisation, the oxidation reactions, prevailing at high temperatures, had a screening effect, which led to a narrowing of the range of polymer sizes observed in the medium.

Moreover, these results revealed that the naked cells, especially the polymeric molecules arising from cytoplasm, seem to be the most easily oxidisable material of all the fractions tested. This characteristic could be related to the lower oxidation state observed on these molecules. According to the results of the fingerprints of initial EPS fractions, it can be observed that the composition of EPS had a more hydrophobic character when its location was closer to the cells, contrary to what happened with the MOC, which decreased. This fact suggests that the more hydrophobic EPS, the lower MOC. This makes sense and can be demonstrated theoretically through some simple calculations (see Supplementary material).

3.3. WO of sludge explored through of their fingerprints

Once the effects of WO on the molecular weights of the polymeric material, including the separate discussion on each structural fraction of the flocs, were discussed, the next step was to determine its effect on a raw sludge.

The changes in the fingerprints of an activated sludge for different WO treatment times were depicted in Fig. 5. Obviously, the fingerprint for t = 0 is the same as that obtained for SMP, so its area is the smallest of the obtained ones. When starting the heating step, an increase in the solubilised polymeric substances (100 °C, 40 min) took place, because of the detachment of LB-EPS and TB-EPS fractions, as well as the beginning of the release of intracellular contents caused by lysis. At that stage of the reaction, the presence of the largest size molecules about 260 kDa (6.7 min) was detected, which probably stemmed from the cell itself and not from the EPS, as it was explained before. Just as occurred with the naked cells fraction, the MOC decreased at this time, obtaining a value of −1.54. As it can be noted, the MOC for raw sludge was higher than for the naked cells fraction. This result is reasonable, considering that MOC for raw sludge included the effect of all fractions together.

When the reaction conditions were reached after 90 min, the high degree of solubilisation obtained (78% VSS reduction) involved either an increase in the absorbance or a wider range of molecular weights detected in the fingerprint. Meanwhile, COD and TOC of

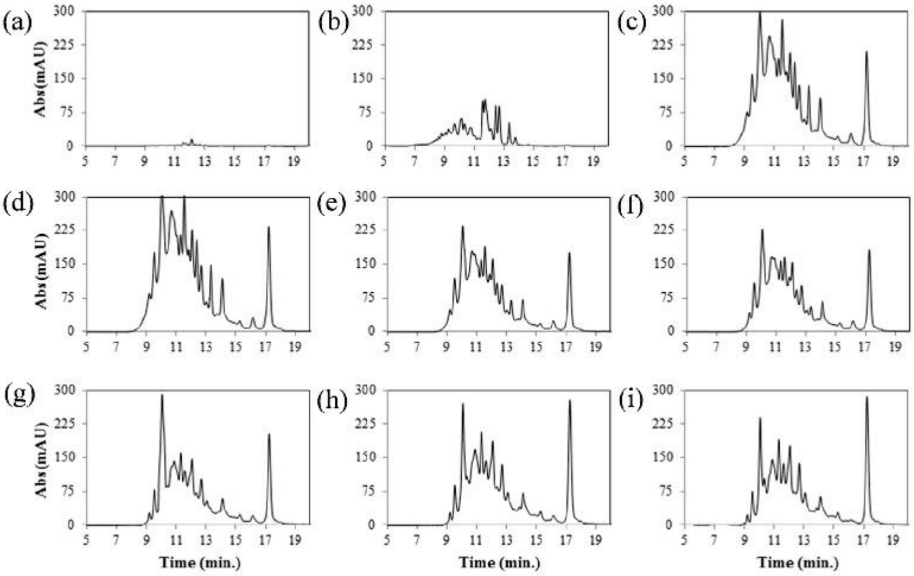

Fig. 5. Evolution of activated sludge fingerprints during WO at 190 °C and 65 bar. (a) Initial sample, (b) 40 min (100 °C), (c) 90 min (190 °C, 65 bar), (d) 100 min, (e) 110 min, (f) 120 min, (g) 140 min, (h) 170 min and (i) 210 min. UV absorbance at 260 nm.

the supernatant reached a maximum of 11530 ± 120 mg O_2/L and 5630 ± 120 mg C/L, respectively, with the MOC increasing up to a value of 0.9. At this time, the thermal hydrolysis and oxidation reactions were able to degrade the larger molecules, reducing their maximum size to 60 kDa (9.1 min).

After 100 min of reaction, the decreases in the concentration of soluble COD (8%) and TOC (6%) in the supernatant began to be significant, while the fingerprints maintained their shape with a slight increase in the height of some of the peaks. These facts suggest a greater effect of oxidation reactions on the absorptive properties of the polymers during the preliminary stage of WO.

Nevertheless, after 110 min of reaction, the fingerprints experienced a significant drop in the absorbances for all the molecular weights. This is probably due to the degradation of a large fraction of the polymers, including those whose absorbance capacities had been increased. However, the fingerprints of subsequent samples showed minimal changes, except for a slight increase in the intensity of the last peak (17.2 min). This behaviour could be attributed to the formation of intermediate compounds with refractory characteristics.

Finally, the oxidation was interrupted because of the formation of these refractory intermediates, which require stronger operating conditions to be oxidised by the treatment here studied. Final soluble COD and TOC reductions of 44% and 34%, with regard to the maximum concentrations of solubilised material were obtained. Final MOC value, 1.4, was very similar to those observed after the oxidation of each fraction by separate.

According to the fingerprint, the final supernatant was composed of a high concentration of low molecular weight solubilised material. So, considering only the peaks eluted in the total volume of the column and assuming that all the molecules absorb the same amount of light in proportion to its concentration, the size distribution of the material solubilised in the sample after 40 min of treatment (when 100 °C were reached) was 4, 42.2 and 53.8% for high (>150 kDa), medium (35–150 kDa) and low (0–35 kDa) molecular weight, respectively. Upon completion of the oxidation reactions (about 110 min), a decrease in the size distribution corresponding to 11.2 and 88.8% for medium (35–150 kDa) and low (0–35 kDa) molecular weight polymers was achieved, respectively.

4. Conclusions

According to the fingerprints for initial EPS fractions, it was established that LB-EPS gathers characteristics of the other EPS fractions in terms of molecular size distribution. This behaviour was attributed to the exchange of polymers that occurs between LB-EPS and the others EPS fractions, due to its intermediate location in the arrangement of the floc. Considering the fingerprint peaks present only in the size exclusion stretch, SMP was composed of 14, 13 and 73% polymers of low, medium and high molecular weight, respectively. Likewise, the content of these polymers in LB-EPS and TB-EPS was 44, 10, 46% and 83, 15, 2%, respectively.

The overall balance in WO of activated sludge fractions was mainly the degradation of larger molecules by reactions of thermal hydrolysis and oxidation, as well as the modification of proteins by hydroxylation of phenylalanine, thus generating polymers with a higher absorbance capacity in the UV zone. Additionally, it was observed that the more internally the polymers are located in the floc, the higher their reactivity and more significant the effects of WO on their molecular weight. That is, the molecules were more refractory to oxidation following this order: SMP > LB-EPS > TB-EPS > naked cells. This behaviour was related to the initial mean oxidation number of organic carbon (MOC) of the fractions (see Supplementary material), thus concluding that higher COD and TOC reductions were attained when the initial MOC was lower.

During WO of activated sludge, a high and fast degree of solubilisation was observed during the first minutes of reaction, followed by a second period where oxidation reactions became more evident on fingerprints, causing a decrease in either the amount or the molecular size of polymers. The results also showed that the oxidation reactions occurred quickly, and finished approximately 20 min after reaching the reactions conditions (190 °C and 65 bar). Once treatment was completed, a final effluent containing almost 90% of low molecular weight solubilised substances (0–35 kDa) was obtained.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.09.004.

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Formation and Degradation of Soluble Biopolymers during Wet Oxidation of Sludge

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Supporting Information

ABSTRACT: In this paper, wet oxidation was applied to an activated sludge as a potential technique for the recovery of products of industrial value. With this end, the effect of the treatment on the polymeric composition of the sludge was analyzed. To establish differences due to the origin of the biopolymers (extracellular or intracellular), the sludge was separated into fractions: soluble microbial products (SMP), loosely bound extracellular polymeric substances (LB-EPS), tightly bound extracellular polymeric substances (TB-EPS) and naked cells and each was treated at 190 °C and 65 atm. A model that describes the evolution of soluble biopolymers during the wet oxidation of sludge was proposed and successfully fitted to the experimental data. Results showed a rapid solubilization of polymers during the first minutes of reaction, achieving values of 23.1%, 14.7%, 8.3%, 0.9% and 0.5% for proteins, humic acids, carbohydrates, uronic acids and DNA, respectively, with regard to the initial volatile suspended solids concentration. Afterward, the oxidation reactions led to a considerable reduction in these substances, which underwent a degree of decomposition higher than 70%. Finally, it was demonstrated that the mineralization of soluble biopolymers depended on their location within the floc structure, mineralization being higher when the polymer was more internal.

KEYWORDS: *Activated sludge, Carbohydrates, Extracellular polymeric substances, Proteins, Soluble microbial products, Thermal treatment, Wet oxidation*

INTRODUCTION

The use of activated sludge is an efficient method for treating sewage in wastewater treatment plants.[1] Biological sludge consists of bioaggregates composed of bacterial cells and extracellular polymeric substances (EPS), the latter being considered as the key component in activated sludge, granular sludge and biofilms.[2] EPS have been defined as a complex mixture of high-molecular-weight polymers generated by bacterial excretion, cell lysis and compounds from the incoming wastewater.[3] Although their composition varies according to the wastewater characteristics and the operating conditions of the treatment plant, the main components of EPS include proteins, humic acids (nonsecreted fraction) and carbohydrates, whereas other compounds such as uronic acids and DNA are also present, though in lower quantities.[4,5]

EPS have been classified into two groups: soluble microbial products (SMP) and bound EPS, the SMP being defined as the pool of organic compounds that are in solution, whereas bound EPS consist of organic compounds closely bound to the cells.[6,7] In turn, bound EPS have been divided into tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS).[8]

One of the most important functions of EPS in the microbial aggregates, is keeping the organisms together in a three-dimensional gel-like hydrated matrix by means of weak physicochemical interactions, and it is this which is mainly responsible for the difficulties found in the management of sludge.[9] In this sense, several techniques have been developed in order to destroy the floc structure, solubilizing either the cells or EPS and improving the sludge dewaterability.[10]

Among these techniques, wet oxidation (WO) is an effective thermal treatment that accomplishes oxidation at elevated temperatures (150−325 °C) and pressures (10−200 atm), using air or pure oxygen as oxidant, which is being successfully implemented in sludge management.[11−13] The advantages of this process with respect to incineration include low operating costs and minimal air pollution discharges.[14] Besides, WO requires no additional heat and can be self-sustaining or even useful for producing heat from wastes.[15]

Taking into account that many characteristics of the sludge, such as filterability, dewaterability, biodegradability, hydrophobicity, etc., are defined by the composition of the EPS, it is evident that the changes experienced by the polymeric materials during WO have a great effect on the final characteristics of the treated effluent. On this basis, several research studies have focused on investigating the effect of different treatments on EPS composition. Thus, Ruiz-Hernando et al.[16] analyzed the

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effect of sonication, thermal hydrolysis and alkali treatments on the EPS solubilization in an activated sludge, whereas Neyens et al.[17] studied the effect of thermal hydrolysis, thermochemical processes (acid or alkaline) and chemical oxidation using hydrogen peroxide on the dewaterability properties of the sludge. Both Farno et al.[18] and Baudez et al.[19] pointed out that thermal history irreversibly affected sludge rheology, due to the irreversible denaturation of solubilized proteins, which also caused an increase in the amount of water bound by the protein molecules. If the proteins were cooled once more, the increased water binding reduced the solubility. All these treatments caused the solubilization and degradation of EPS, generating a final effluent with lower viscosity and better dewaterability.

Nevertheless, to the best of our knowledge, the impact of WO on polymeric substances from sludge is still unknown. Although the research effort on hydrothermal treatments of sludge has been substantial, these works have mainly focused on VSS, COD or TOC reductions, but none of them have attempted to characterize the reaction products, even though this information will be critical to the design of subsequent techniques of purification or revalorisation of the hydrolyzed and oxidized effluent.[20] Therefore, the aim of this work was to analyze the effect of WO on the main species of polymeric substances from activated sludge. To this end, the sludge was separated into its components (SMP, LB-EPS, TB-EPS and naked cells) in order to check the differences during treatment according to the origin of the biopolymers (extracellular or intracellular), and each was subjected to WO. The results were compared to those obtained from the raw sludge.

■ MATERIAL AND METHODS

Sludge Samples. The experiments were performed with activated sludge obtained from a municipal wastewater treatment plant in Asturias (Spain). The sludge was extracted from a thickening unit that operates by dissolved air flotation, and stored at 4 °C until further usage. The initial characteristics of the sludge were the following: total suspended solids (TSS), 21.5 g/L; volatile suspended solids (VSS), 15.5 g/L, sludge volume index (SVI); 46.6 mL/g; pH, 6.9; total chemical oxygen demand (TCOD), 25 694 mg O_2/L.

EPS Extractions. The EPS extraction was carried out according to a modified method based on Li and Yang.[21] This procedure has been described in Urrea et al.[22]

The initial composition of EPS fractions is shown in Table 1.

Table 1. Characteristics of EPS Fractions Extracted from Activated Sludge

	SMP	LB-EPS	TB-EPS
proteins (mg/L)	31.3 ± 1.5	35.6 ± 4.3	597.1 ± 40.0
humic acids (mg/L)	69.8 ± 2.0	48.9 ± 3.4	1061.9 ± 28.0
carbohydrates (mg/L)	11.3 ± 0.2	18.3 ± 0.2	366.7 ± 0.6
uronic acids (mg/L)	0.8 ± 0.2	1.9 ± 0.2	43.2 ± 0.5
DNA (mg/L)	3.3 ± 0.6	2.1 ± 0.3	40.7 ± 1.0
total[a] (mg/L)	116.5	106.8	2109.6
COD (mg O_2/L)	173 ± 10	176 ± 12	3346 ± 200

[a] Sum of the content in proteins, humic acids, carbohydrates, uronic acids and DNA.

Experimental Setup. The WO experiments were carried out in a PARR series 4520 reactor. A detailed description of the characteristics of the equipment has already been given in Urrea et al.[23] A typical experiment was as follows: 500 mL of sample (EPS fraction, naked cells or raw sludge) were loaded into the reactor and then the operating conditions (190 °C and 65 atm) were adjusted. These conditions were selected because they are within the typical ranges of temperatures (150−365 °C) and pressures (30−250 bar) reported in the literature for the WO of sewage sludge.[24] A stream of pure oxygen was continuously fed into the reactor, from the beginning to the end of the reaction, at a flow rate of 7.2×10^{-2} Nm^3/h and the stirring speed was set at 500 rpm. The first sample was taken when the temperature reached 100 °C, in order to determine whether any change occurred during the heating period (first 90 min of treatment). The next samples were periodically extracted once the operating conditions had been achieved. The first experiments were performed with the sludge and the naked cells to avoid any change in structure that might take place during storage, since this could distort the results.

Analytical Methods. The analyses carried out to determine the sludge features were: total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total suspended solids (TSS), volatile suspended solids (VSS), fixed suspended solids (FSS), sludge volume index (SVI) and pH. These analyses were performed according to Standard Methods.[25] Soluble total organic carbon (TOC) was measured using a TOC analyzer (Shimadzu TOC-VCSH, Japan). The polymeric compositions of the initial and oxidized samples were determined by means of different colorimetric methods. Proteins and humic acids were measured by the modified Lowry method,[26] using bovine serum albumin (BSA) and humic acids as standards, respectively. Carbohydrates were determined by the Dubois method,[27] employing glucose as standard. Uronic acids were measured by the Blumenkrantz and Asboe-Hansen method,[28] using glucuronic acid as standard. Finally, the DNA content was estimated by the diphenylamine method,[29] employing DNA calf thymus as standard. All the analyses were performed in triplicate, always obtaining standard deviations lower than 6%.

■ RESULTS AND DISCUSSION

Decomposition of Polymeric Substances by WO: Analysis of the Sludge Fractions. *SMP Fraction.* The evolution of biomolecules during the WO of SMP is shown in Figure 1a. As can be seen, a rapid decrease in their concentration occurred during the initial stage of the treatment, a high degree of degradation being obtained upon reaching the reaction conditions (90 min). At this time, the proteins, which had an initial concentration of 31.3 ± 1.5 mg/L, reached a stable value of 8.3 ± 0.5 mg/L, whereas carbohydrates and humic acids, with initial contents of 11.3 ± 0.2 and 69.8 ± 2.0 mg/L, dropped to values of 4.6 ± 0.2 and 7.4 ± 1.1 mg/L, respectively. After the first 90 min, slight decreases were detected in carbohydrate and humic acid concentrations, whereas protein concentration remained constant. At the end of the treatment, reductions of 73% in both proteins and carbohydrates were obtained, while humic acids reached 95%. Uronic acids and DNA, which were present at very low initial concentrations, were not detected in any of the samples collected during WO. Moreover, the TOC of SMP showed few changes during the treatment. Initially, the TOC remained steady during the first 110 min of reaction, decreasing slightly afterward (see Figure S1 in the Supporting Information). The behavior observed revealed that during the initial step of WO, the decomposition of the SMP biopolymers led mainly to the formation of intermediate compounds of refractory characteristics. A similar behavior has previously been reported during WO of different compounds and wastes, suggesting the presence of a significant proportion of material that cannot be oxidized, even when the reaction time is increased.[30,31]

LB-EPS Fraction. During the WO of LB-EPS (Figure 1b), the fastest decomposition of biopolymers was attained during the first minutes of reaction, before the operating conditions were achieved, as occurred with the SMP fraction. During the first 90 min, proteins, humic acids and carbohydrates, with initial concentrations of 35.6 ± 4.3, 48.9 ± 3.4 and 18.3 ± 0.2

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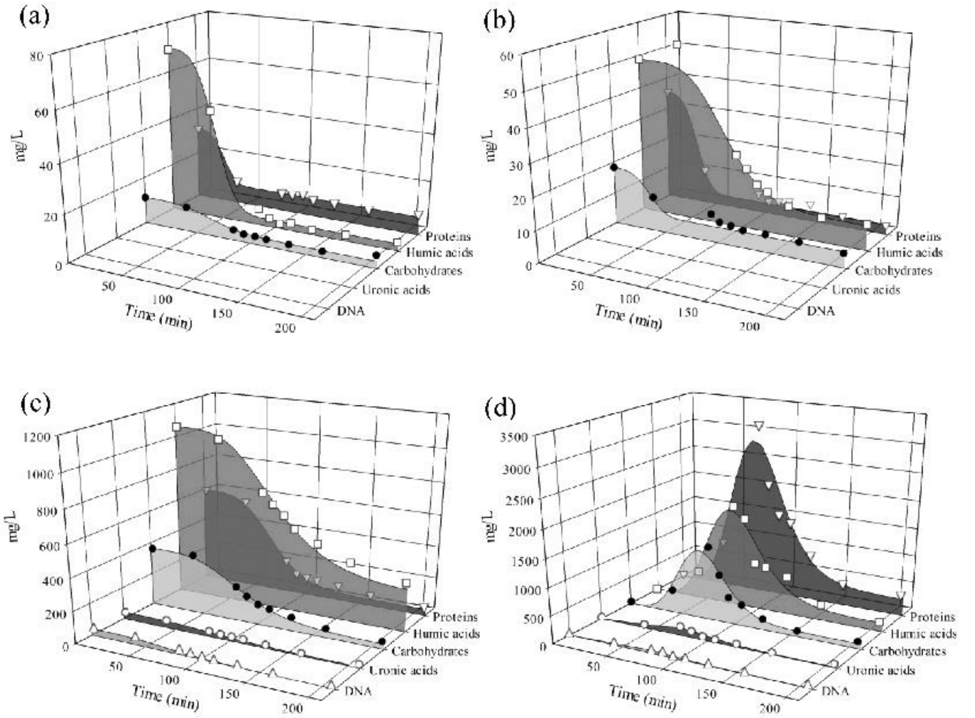

Figure 1. Evolution of polymeric substances from sludge fractions during WO at 190 °C and 65 atm. (a) SMP, (b) LB-EPS, (c) TB-EPS and (d) naked cells. Experimental data (symbols) and kinetic model (lines) according to eqs 1 and 2.

mg/L, decreased significantly, yielding removal percentages of 84, 53 and 55%, respectively. Subsequently, with the advance of the reaction, the decomposition of polymeric substances progressed slowly, until eventually constant concentrations for each biopolymer were reached. The values of the concentrations of proteins, humic acids and carbohydrates at the end of the WO (after 210 min) were 2.6 ± 1.2, 8.3 ± 2.6 and 4.6 ± 0.6 mg/L, which meant a reduction of 93, 83 and 75% with respect to their initial concentration, respectively. Furthermore, the analysis of DNA and uronic acids in the samples taken at different reaction times showed that these concentrations were negligible, as in the case of SMP. Likewise, the TOC evolution of LB-EPS was quite similar to that obtained in SMP, although it should be noted that the mineralization achieved in LB-EPS (24%) was higher than that of SMP (10%) (see Figure S1).

TB-EPS Fraction. As can be seen in Table 1, TB-EPS clearly presented the greatest content of biopolymers of all the extracellular fractions. In fact, uronic acids and DNA, whose origin is usually considered intracellular, had a significant concentration in this fraction, reaching values around 16 and 8 times higher, respectively, than those corresponding to the sum of SMP and LB-EPS. Nevertheless, this seems logical given that when the cells lyse their components are released into the outside environment and retained in the closest layer of EPS, that is to say, the TB-EPS fraction. Besides, it is to be expected that some of the bacteria would have been lysed during the extraction.

Another difference with respect to the other fractions studied is that the biopolymers from TB-EPS were more refractory to oxidation when they were subjected to WO (Figure 1c). For instance, only a marginal effect of the treatment was observed at 100 °C (40 min). Once the operating conditions (90 min) were reached, however, the degradation was evident, leading to a decrease of 66, 32, 41, 38 and 56% in proteins, humic acids, carbohydrates, uronic acids and DNA, respectively, these values being, nonetheless, lower than those obtained for either SMP or LB-EPS.

When the treatment concluded (210 min), the final concentrations were 46.4 ± 9.5, 300.8 ± 6.0, 35.2 ± 1.3, 9.2 ± 1.7 and 1.1 ± 0.2 mg/L for proteins, humic acids, carbohydrates, uronic acids and DNA, which meant removal efficiencies of 92, 72, 90, 79 and 97%, respectively. As can be seen from these data, the uronic acids and DNA, whose concentrations were too low to be measured during the WO of SMP and LB-EPS, were strongly affected by the treatment. So, DNA showed the highest degree of elimination during the WO of TB-EPS. In addition to this, it was observed that in the case of TB-EPS, mineralization began just after reaching the operating conditions (90 min). A higher degree of mineralization was attained at the end of the treatment in comparison to the other EPS fractions (see Figure S1). Therefore, these results indicate that despite the fact that the degradation of biopolymers in TB-EPS was slower than in SMP and LB-EPS, the intermediate compounds formed from the former had a higher rate of conversion toward CO_2 and H_2O. In this way, taking into account the amount mineralized in each of the EPS fractions during WO, it can be established that the more internal the location of biopolymers in the floc structure was, the higher was the degree of oxidation achieved. These results demonstrated the effectiveness of WO in decomposing TB-EPS from the sludge and, bearing in mind that this fraction is

Table 2. Kinetic Parameters Obtained from eqs 1−4 for SMP, LB-EPS and TB-EPS after WO at 190 °C and 65 atm

	parameter	proteins	humic acids	carbohydrates	uronic acids	DNA
SMP	k_i (190 °C) (L mgO_2^{-1} min^{-1})	994	242	67	N/A[a]	N/A
	Ea_i (kJ/mol)	47.3	52.7	56.7	N/A	N/A
	k_{0i} (L mgO_2^{-1} min^{-1})	2.16×10^8	2.14×10^8	1.66×10^8	N/A	N/A
	$Crem_i/C_{0i}$	0.25	0.04	0.27	N/A	N/A
	R^2	0.996	0.997	0.987	N/A	N/A
LB-EPS	k_i (190 °C) (L mgO_2^{-1} min^{-1})	830	31	564	N/A	N/A
	Ea_i (kJ/mol)	49.1	59.7	50.2	N/A	N/A
	k_{0i} (L mgO_2^{-1} min^{-1})	2.87×10^8	1.67×10^8	2.61×10^8	N/A	N/A
	$Crem_i/C_{0i}$	0.09	0.16	0.27	N/A	N/A
	R^2	0.990	0.991	0.980	N/A	N/A
TB-EPS	k_i (190 °C) (L mgO_2^{-1} min^{-1})	50	19	26	25	41
	Ea_i (kJ/mol)	58.0	61.5	60.4	60.6	59.0
	k_{0i} (L mgO_2^{-1} min^{-1})	1.73×10^8	1.65×10^8	1.74×10^8	1.73×10^8	1.86×10^8
	$Crem_i/C_{0i}$	0.07	0.23	0.08	0.19	0
	R^2	0.994	0.996	0.997	0.995	0.993

[a] N/A: not applicable.

principally responsible for the bound water, it can be concluded that WO, as a sludge management technique, greatly improves the dewaterability of the final solid.[32]

Naked Cells Fraction. The evolution of biopolymers generated from the disruption of the cell itself (in other words, not considering the EPS fractions) is shown in Figure 1d, where the data from the WO of the intracellular materials are depicted. The results indicated a fast solubilization of the naked cells at the beginning of the reaction. In fact, the highest concentrations of dissolved biopolymers were achieved during the heating period, with maximum values of 3133 ± 40.2, 1866 ± 92.4, 1323 ± 49.7, 156 ± 13.5 and 57 ± 0.4 mg/L (which is equivalent to 241.5, 144, 102, 12 and 4.4 mg/g VSS) for proteins, humic acids, carbohydrates, uronic acids and DNA, respectively, after 90 min of reaction. Likewise, it was noted that the TOC concentration achieved a maximum in the supernatant at this time, providing approximately 440 mg C per gram of solubilized VSS.

On the basis of these results, it seems reasonable to consider that proteins are the main cellular component, reaching a concentration in the supernatant 2.5 times higher than that of the carbohydrates. The initial solubilization is caused by the damage suffered by the structure of the cell membrane due to the thermal conditions. When a temperature above 100 °C is reached, the membrane loses its integrity and becomes a permeable element, thus facilitating the release of intracellular components to the outside environment, where they are more soluble.[33] At this stage, it should be pointed out that, although humic acids are not part of the substances that make up the cells, their presence in the solubilized material is to be expected, as the separation techniques of EPS are not 100% effective. This is especially true when this separation is carried out on a sludge with high solids concentration and at moderate temperatures, in order to avoid cellular lysis during the process.

Subsequently, during the course of the reaction, it was seen that the biopolymer concentration in the supernatant began to decrease, this probably being due to two factors. First, the low solubilization of volatile suspended solids, caused by the decrease in their own concentration. Second, the more intense radical attack on a higher concentration of solubilized compounds, leading to their rapid elimination. Finally, in all the polymeric substances, a reduction of 89−93% with regard to the maximum solubilized concentrations was obtained after 210 min of WO treatment.

Moreover, Figure S1 shows the percentage of TOC removed during WO of naked cells after reaching maximum solubilization. It can be seen that just after achieving a high concentration of soluble biopolymers, mineralization took place, causing a progressive fall in TOC, which was reduced by 30%. This indicates that the intracellular biopolymers are more easily oxidized than the extracellular ones.

Kinetic Modeling of Biopolymer Profiles. To analyze the effect of WO on each biopolymer and simplify the comparison of the results between the sludge fractions, a new kinetic model was proposed and validated to describe the evolution of each biopolymer in each of the fractions during WO.

The proposed kinetic model is based on the assumption that VSS are initially solubilized by hydrolysis reactions, generating soluble biopolymers which, in turn, are subsequently oxidized to CO_2, NH_3 and low molecular weight organic acids.[34] Obviously, the solubilization step must be discarded for the initial soluble fractions, such as SMP, LB-EPS and TB-EPS, but not for the naked cells or the raw sludge. Both the solubilization and oxidation steps were assumed to follow pseudo-first-order kinetic models. Additionally, the presence of small concentrations of biopolymers which are refractory to oxidation was experimentally observed in all of the samples at the end of the treatment. For this reason, it was necessary to include the parameter $C_{i,rem}$, which accounts for the remaining concentration of these biopolymers. Therefore, the following kinetic equations were proposed:

$$-r_{VSS} = k_{VSS}(C_{VSS} - C_{VSS,rem})C_{O_2} \tag{1}$$

$$-r_i = k_i(C_i - C_{i,rem})C_{O_2} - Y_{i/VSS}k_{VSS}(C_{VSS} - C_{VSS,rem})C_{O_2} \tag{2}$$

In turn, k_{VSS} and k_i can be also expressed as

$$k_{VSS} = k_{0_{VSS}}\exp[-Ea_{VSS}/RT] \tag{3}$$

$$k_i = k_{0_i}\exp[-Ea_i/RT] \tag{4}$$

Where i is the biopolymer (protein, carbohydrate, humic acid, uronic acid or DNA), k_i, k_{0_i} and Ea_i are the pseudo-first kinetic constant, the pre-exponential factor and the activation energy, respectively, for biopolymer i removal, k_{VSS}, k_{0VSS} and Ea_{VSS} are

Table 3. Kinetic Parameters Obtained from eqs 1−4 for Naked Cells and Raw Sludge after WO at 190 °C and 65 atm

	parameter	proteins	humic acids	carbohydrates	uronic acids	DNA	VSS
naked cells	k_i (190 °C) (L mgO_2^{-1} min^{-1})	38	33	54	41	51	N/A[a]
	k_{VSS} (190 °C) (L mgO_2^{-1} min^{-1})	N/A	N/A	N/A	N/A	N/A	95
	Ea_i (kJ/mol)	58.5	58.7	58.4	58.1	57.9	N/A
	Ea_{VSS} (kJ/mol)	N/A	N/A	N/A	N/A	N/A	55.8
	k_{0i} (L mgO_2^{-1} min^{-1})	1.52×10^8	1.41×10^8	2.07×10^8	1.47×10^8	1.74×10^8	N/A
	k_{0VSS} (L mgO_2^{-1} min^{-1})	N/A	N/A	N/A	N/A	N/A	1.87×10^8
	Crem (mg/L)	300	150	90	10	5	1.4
	$Y_{i/VSS}$ (mg/g VSS)	430	266	222	23	10	N/A
	R^2	0.980	0.969	0.985	0.967	0.966	0.999
raw sludge	k_i (190 °C) (L mgO_2^{-1} min^{-1})	36	28	50	26	53	N/A
	k_{VSS} (190 °C) (L mgO_2^{-1} min^{-1})	N/A	N/A	N/A	N/A	N/A	98
	Ea_i (kJ/mol)	59.0	59.9	58.2	60.1	57.9	N/A
	Ea_{VSS} (kJ/mol)	N/A	N/A	N/A	N/A	N/A	55.7
	k_{0i} (L mgO_2^{-1} min^{-1})	1.62×10^8	1.62×10^8	1.85×10^8	1.60×10^8	1.81×10^8	N/A
	k_{0VSS} (L mgO_2^{-1} min^{-1})	N/A	N/A	N/A	N/A	N/A	1.87×10^8
	Crem (mg/L)	450	500	90	20	10	1.5
	$Y_{i/VSS}$ (mg/g VSS)	492	233	200	13	15	N/A
	R^2	0.984	0.992	0.997	0.993	0.946	0.999

[a] N/A: not applicable.

the pseudo-first kinetic constant, the pre-exponential factor and the activation energy, respectively, for VSS removal, C_{O_2} is the concentration of oxygen, and $Y_{i/VSS}$ is a yield which indicates the grams of biopolymer released per gram of VSS solubilized. Clearly, k_{VSS} for SMP, TB-EPS and LB-EPS is equal to zero. At this point, it is important to explain that the existence of an initial heating period made it necessary to include the effect of temperature on the kinetic model. Hence, an Arrhenius-type dependence for both k_{VSS} and k_i was assumed (eqs 3 and 4). The concentration of oxygen during each experiment was calculated from Henry's law and empirical correlations.[35]

The fitting of the model to the experimental data was performed employing the Micromath Scientist software, thus determining the kinetic parameters of the reaction. The most relevant kinetic data for each of the sludge fractions and the raw sludge are shown in Tables 2 and 3. Solid lines in Figures 1 and 2 denote model curves according to eqs 1 and 2. The adjustment of eq 1 to experimental data can be seen in the Supporting Information (Figure S2).

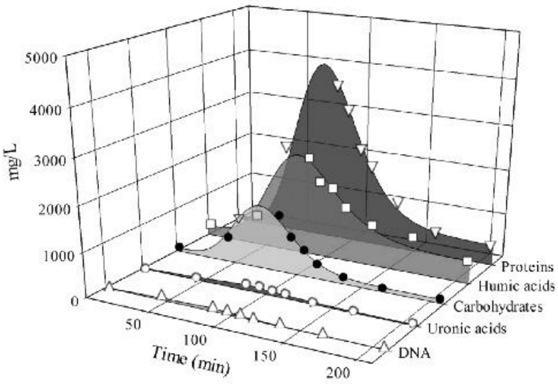

Figure 2. Evolution of polymeric substances from raw sludge during WO at 190 °C and 65 atm. Experimental data (symbols) and kinetic model (lines) according to eqs 1 and 2.

The results clearly showed that proteins were the most easily oxidizable biopolymers in the sludge, particularly when they are dissolved, as in the case of SMP, or located in the most external part of the bacteria, like in LB-EPS. In fact, the more external the location of the protein in the cell, the higher its oxidation kinetic constant. So, the value of $k_{protein}$ for the SMP fraction is around 25 times higher than that obtained for the naked cells. This indicates that during WO, the degradation rate of proteins is affected by factors that are probably linked to specific features in their structure, which, in turn, are determined by the location of the protein in the bacterial structure. Nevertheless, this trend was less marked in the case of humic acids and carbohydrates, in which the effects of biopolymer location on k_i were not so evident, although a slight increase in the kinetic constants was observed for SMP and LB-EPS when compared to TB-EPS and naked cells. Obviously, concepts such as "humic acids" or "carbohydrates" refer to a large group of compounds which, in spite of sharing some characteristics, show a great heterogeneity in terms of composition and structure. These heterogeneities will likely be responsible for the differences observed in the oxidation kinetic constants for the same biopolymer depending on its position in the floc arrangement. For instance, the term "humic substances" generally refers to a mixture of humic and fulvic acids, the latter being more resistant to WO.[36]

Regarding $Y_{i/VSS}$ parameters obtained for naked cells or sludge, the results are consistent with the concentrations of the dissolved biopolymer measured. As expected, the higher the concentration of the biopolymer in the medium, the higher the value of this fitting parameter. This yield revealed that proteins were the main product solubilized during the hydrolysis of sludge. So, the yield per gram of solubilized VSS was twice as high as that obtained for carbohydrates, both in the naked cells and in the raw sludge. Moreover, the $Y_{i/VSS}$ parameters made it possible to establish an approximate cellular composition based on the soluble biomolecules generated by the WO process. So, it can be considered that the cells from activated sludge are composed of 64% proteins, 31% carbohydrates, 3% uronic acids and 2% DNA.

Decomposition of Polymeric Substances from Raw Sludge. Once the effect of WO on soluble biopolymers from the sludge fractions was studied, the next logical step was to analyze the behavior of these substances when the treatment was performed on the raw sludge.

As can be observed in Figure 2, the evolution of biopolymers during the WO of raw sludge was quite similar to that seen in the case of naked cells. Obviously, this result was to be expected, since the amount of biopolymers contained in the naked cells is much higher than that corresponding to SMP and EPS. The floc structure was destroyed during the first minutes of reaction, leading to the release of EPS and the beginning of cellular lysis. Initially, logical reasoning would suggest that the amount of solubilized biopolymers in the raw sludge should be the sum of the solubilized biopolymers from each of the fractions after the same reaction time. Surprisingly, this was not what was observed experimentally, discrepancies being detected between the concentrations in the raw sludge and the sum of its fractions (see Figure S3). The initial hydrolysis step for the raw sludge proved to be faster than that observed for the sum of each fraction. This behavior is consistent with the fact that the LB-EPS and TB-EPS had been previously solubilized during their extraction, whereas in the experiments with raw sludge they formed a gel-matrix attached to cells. In the case of the sum of the fractions, the presence of a higher concentration of dissolved biopolymers at the beginning of the reaction implies that oxidation reactions can start sooner, leading to lower maximum concentrations of biopolymers, as can be seen in Figure S3. To some extent, these differences may also be explained by the fact that the matrix of the floc in the raw sludge is not only composed of EPS, but also multivalent cations such as Ca^{2+}, Mg^{2+} and $Fe^{(2\text{ or }3)+}$, which contribute to improving the mechanical characteristics of the biological aggregates.[37] Among these cations, Fe^{2+} has been identified as a catalyst that improves the solubilization rates of sludge during WO[38] and so the naked cells, which experienced stripping of the cations during EPS extractions, would not be affected by the catalytic properties of Fe^{2+}. It must also be taken into account that, after the separation of EPS fractions from the sludge, it was necessary to perform on them a subsequent filtration step to remove any suspended material remaining. This also has a slight effect on the mass balance of polymers, thus influencing the results obtained.

Then, when the operating conditions were reached (90 min), the concentration of soluble biopolymers achieved a maximum corresponding to 3589 ± 69.0, 2286 ± 46.5, 1296 ± 2.7, 134 ± 8.0 and 73 ± 1.1 mg/L (equivalent to 231, 147, 83, 9 and 5 mg/g VSS) for proteins, humic acids, carbohydrates, uronic acids and DNA, respectively. In the same way as with the naked cells, the soluble TOC also reached a maximum at this reaction time, although with a lower yield per gram of solubilized VSS (430 mg C/g VSS), disclosing a higher intensity of the oxidation reactions in the raw sludge, as mentioned above. As has already been established, the increase in solubilized material during this phase of the reaction is mainly due to the complete destruction of cellular components caused by reactions of either thermal hydrolysis or oxidation. In previous studies, we have identified that the presence of an oxidizing atmosphere during the hydrothermal treatment of the sludge improves its degree of solubilization.[23,39] This has also been reported by Baroutian et al.[40] The theory that the initial attack of radicals in organic compounds takes place on the α carbon, starting with the abstraction of a hydrogen atom, is commonly accepted. However, other mechanisms based on the rupture of a C−C bond (β or γ carbon position) have also been considered possible,[41] and this would lead to the reduction in the size of the polymers until their solubilization.

Finally, with the progress of the WO, the oxidation reactions became more significant, causing a continuous decline in the concentration of biopolymers. At the end of the treatment (210 min), the reduction obtained was 88% for proteins, 78% for humic acids, 93% for carbohydrates, 85% for uronic acids and 95% for DNA, with respect to the maximum solubilized concentration. Regarding mineralization, Figure S1 shows the percentage of soluble TOC removed after reaching the maximum solubilization in the raw sludge. As can be observed, the behavior of the raw sludge was quite similar to that obtained in the naked cells, due to the larger contribution of such naked cells to the sludge composition.

The degradation rates for solubilized biopolymers in the raw sludge were also modeled using the kinetic model proposed in eqs 1−4, obtaining a good fit to experimental data (Table 3). On account of the fact that the contribution of DNA due to the TB-EPS fraction during the solubilization of raw sludge was significant, it was necessary to exclude the experimental data obtained at 100 °C in the simulations of DNA. According to the fitting parameters obtained, it can be concluded that the kinetic constants for oxidation of the raw sludge and the naked cells were quite similar for each of the biopolymers, as was expected because the naked cells account for 84% of the total COD of the sludge.

It was observed that the lowest degradation rates corresponded to uronic and humic acids, which had k values of around 27 L mgO_2^{-1} min^{-1}. Taking this value as a reference, the oxidation kinetic constants were 30% higher for proteins and 85% and 95% higher for carbohydrates and DNA, respectively.

Moreover, the values of the activation energies for the biopolymers were fairly similar, with mean values around 59 ± 1 kJ/mol. These results are consistent with the values of the activation energies (54−78 kJ/mol) reported by other studies for the noncatalytic wet oxidation of different organic compounds,[41] although a good number of them were determined from COD or TOC data. In conclusion, the degrees of decomposition obtained for the main biopolymers indicated that the conditions chosen for the treatment were severe enough to provide the energy required for their solubilization and partial oxidation. From this perspective, a more detailed explanation is that during the treatment, the biopolymers are oxidized through a chain of reactions involving free radicals, thus yielding intermediate compounds. Then, the oxidation is interrupted by the higher activation energy of the compounds formed, causing the accumulation of recalcitrant molecules in the final effluent. A proof of the magnitude of the increase in the activation energy is provided by acetic acid, which is considered as the main final product after the WO of organic compounds. According to the results reported by Shende and Levec,[42] the WO of acetic acid requires an activation energy of around 178 kJ/mol.

Regarding the values of $Y_{i/\mathrm{VSS}}$ for the raw sludge, it can be seen that such values were not very different to those obtained for the naked cells. Curiously, $Y_{i/\mathrm{VSS}}$ for proteins was even higher in the raw sludge than in the naked cells, whereas the contrary was true for humic acids. It is worth noting that based on the $Y_{i/\mathrm{VSS}}$ values, 52% of the composition of polymers in the activated sludge corresponded to proteins. This result is a bit lower than the value of 61% reported by Chen et al.[43] This difference can be explained considering that this value was determined from COD data rather than $Y_{i/\mathrm{VSS}}$ values. Moreover, the kinetic constant of solubilization, that is to say, the k_{VSS} parameter, was significantly lower than the k_i obtained for biopolymer oxidation of either SMP or LB-EPS. However, it was almost 2 times higher than the majority of k_i of either TB-EPS or the naked cells. This makes sense and it can be easily explained if it is remembered that SMP corresponds to the soluble material in the raw sludge, whereas LB-EPS can be released from the matrix of the sludge with little effort. Therefore, the solubilization is not as important a factor for the oxidation of those fractions as for TB-EPS and naked cells.

■ CONCLUSIONS

Wet oxidation of sludge involves an initial fast solubilization of biopolymers, mainly proteins, followed by their gradual oxidation. The results also showed that the degradation rates for the same family of biopolymers depended on their location in the floc, with higher degrees of oxidation when the biopolymer was more internal.

Moreover, a new kinetic model for sludge solubilization, including the effects of oxygen concentration and temperature on VSS solubilization rates and on the formation and reaction rates of the main solubilized biopolymers, has been proposed and its validity proved.

Finally, the high degree of solubilization of biopolymers obtained (23.1%, 14.7%, 8.3%, 0.9% and 0.5% for proteins, humic acids, carbohydrates, uronic acids and DNA, respectively, with regard to the initial volatile suspended solids concentration) suggested the possibility of applying this technique in order to recover products of potentially high added value, thus also improving the economic appeal of the process.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.6b02664.

> Additional figures including evolution of soluble TOC from sludge fractions, volatile suspended solids and biopolymers solubilized during wet oxidation treatment (PDF)

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Notes

The authors declare no competing financial interest.

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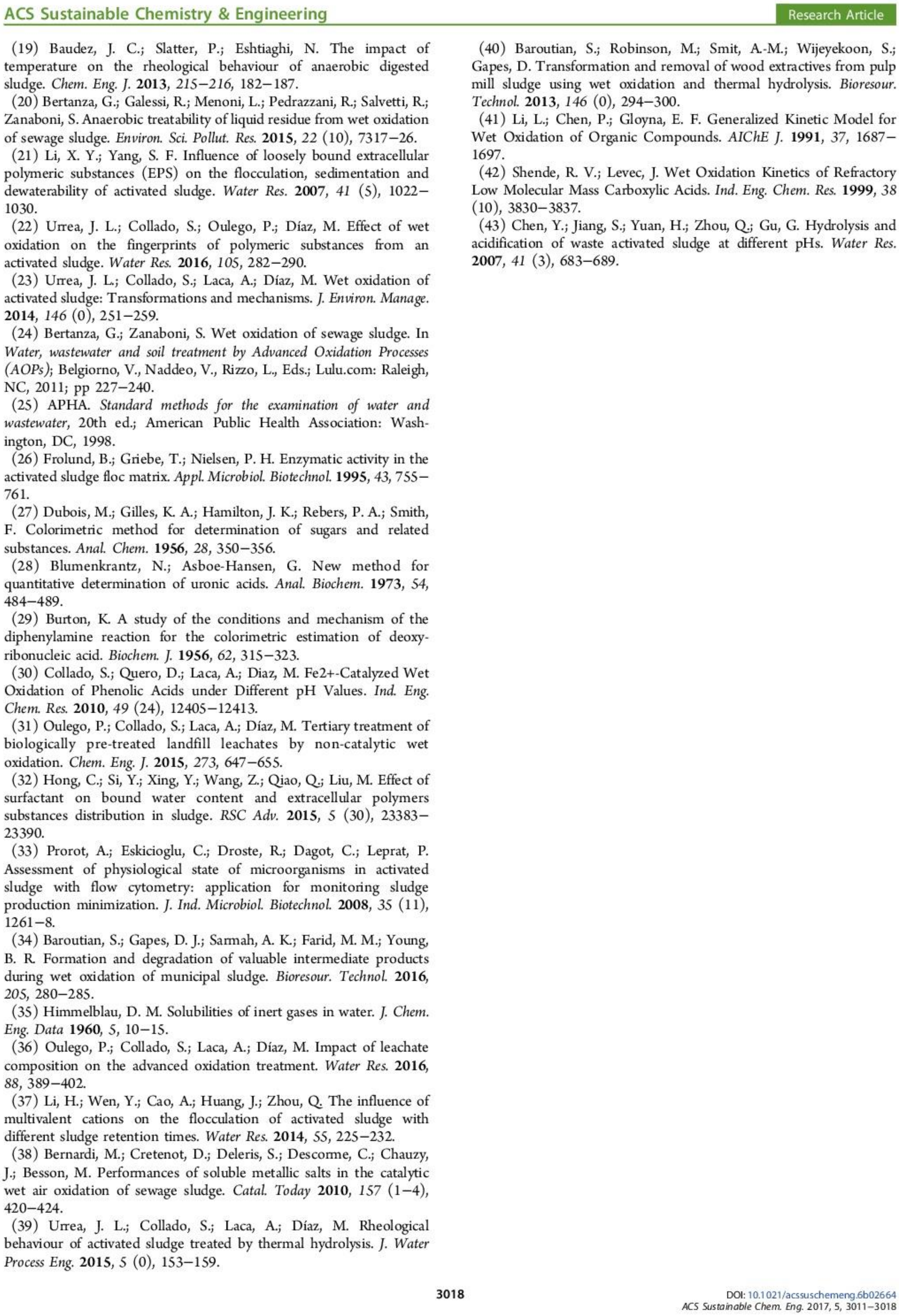

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4.5. EFFECT OF WET OXIDATION ON PHYSICOCHEMICAL CHARACTERISTICS AND COMPOSITION OF DIFFERENT LANDFILL LEACHATES

VIII. Wet oxidation effects on LC-MS fingerprints for different landfill leachates.

WET OXIDATION EFFECTS ON LC-MS FINGERPRINTS FOR DIFFERENT LANDFILL LEACHATES

ABSTRACT

Wet oxidation is an effective technique to degrade recalcitrant organic compounds from different effluents. In this work, the effect of wet oxidation on the composition of different leachates (young, old and biologically stabilised) was analysed by Liquid Chromatography Mass Spectrometry (LC-MS). The mean oxidation number of organic carbon (MOC) for old leachate (0.36) was about half of that corresponding for young (0.61) and stabilised leachates (0.59), indicating that the former presented compounds with lower oxidation state. Further, a higher presence of substances with aromatic characteristics was present in stabilised leachate, whilst old and young leachates had the highest amount of ions detected. Most of the ions detected in old leachate were also observed in young leachate, although with some differences in their abundance. Ions observed in the commercial humic acid, corresponding to [M H]$^{-}$ 112.9, 153.0, 214.9, 209.0 and 209.1 were also present in each leachate. The LC-MS/MS analysis led to the identification of some features in regards to the structure of the ions, being observed functional groups characteristic of alcohols, acids and phenolic compounds. Likewise, the theoretical molecular formula of some ions was established. During wet oxidation at 200 ºC and 50 bar, similar yields of COD removal for each leachate were observed, especially in the first minutes of reaction. TOC decreased 37, 43 and 47%, for and old, young and stabilised leachates, respectively, when the treatment was concluded (280 min). The changes in MOC suggested that the reactions of mineralisation and partial oxidation that occurred during the treatment were balanced. Finally, wet oxidation caused an important decrease in the chromatogram area for almost all ions, achieving reductions between 40 and 80%.

1. Introduction

Despite the evolution of landfill technology to eliminate or minimize potential adverse impacts of the waste on the surrounding environment, generation of contaminated leachate remains as an inevitable consequence of the practice of waste disposal in landfills (Banar et al. 2006). Leachates are liquids resulting from the release of water in the decomposition of municipal solid wastes and the percolation of rainwater into the landfill. They have high concentration of dissolved biodegradable and non-biodegradable compounds, mainly of humic and fulvic nature (Ramírez-Sosa et al. 2013). Likewise, numerous hazardous compounds as aromatics, halogenated compounds, phenols, pesticides, heavy metals and ammonium have also been identified in these (Naveen et al. 2017).

The characteristics of landfill leachate are affected by many factors, such as age, precipitation, weather variation, and waste types and composition. In particular, leachates vary greatly depending on the age of the landfill (Wei et al. 2010). In addition, large variations in leachate quality exist for different landfills, but also at different locations within the same landfill (Banar et al. 2006).

It is evident that the presence of a large variety of pollutants in leachates makes their management complicated, being necessary the application of various treatment processes to achieve appropriate characteristics of discharge (Turki et al. 2013). Conventional treatment technologies, including physical/chemical and biological processes, are not able to yield a final treated leachate with enough quality to be discharged into natural water bodies (Rocha et al. 2013). However, amongst the various types of physical-chemical treatments, advanced oxidation processes has recently been reported as one of the most effective methods to degrade a variety of refractory compounds in landfill leachate (Wei et al. 2010).

Wet oxidation is an advanced oxidation process that has provided good results to remove organic compounds from a large variety of effluents, such as sewage sludge (Urrea et al. 2017), wastewaters from petrochemical companies (Lin et al. 1996), coke wastewaters (Oulego et al. 2014), dye wastewaters (Fu and Kyzas 2014) and leachates (Oulego et al. 2015), amongst others. Typical operating conditions include temperatures of 100-350 ºC, pressures of 5-200 bar and residence time of 1 hour (Kurniawan et al. 2006).

Currently, there are some studies in which the effect of wet oxidation on leachate characteristics has been addressed. Nevertheless, most of these works have been mainly focused on the removal of COD (Rivas et al. 2005), ammonia nitrogen (Karimi et al. 2013) or the improvement of biodegradability (Garg and Mishra 2010), without paying special attention to the specific compounds formed during the process, as previous step in order to get a deeper characterization of the final effluent.

In this context, to the best of our knowledge, there are no studies analysing the effect of wet oxidation on composition of leachates by using high specificity techniques, such as gas chromatography mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS). These techniques have already been used in several works to identify and quantify organic compounds from raw leachates, being GC-MS the most commonly employed, since it has various libraries with a large amount of reference spectra, whilst LC-MS by being a more recent technique, it still has a lack of spectra.

In this way, Ribeiro et al. 2002 proposed an analytical methodology to determinate the presence of chlorophenols and phenols from leachates by using solid-phase microextraction combined with GC–MS. The method was evaluated and optimised to identify 13 chlorophenols from leachates with detection limits from 0.005 to 2.5 µg/L. Paxéus 2000 identified more than 200 individual organic compounds in leachates obtained from three municipal landfills, employing GC-MS. In a similar way, Eggen et al. 2010 detected the presence of some emerging pollutants in leachates by GC-MS and of ionic perfluorinated substances and telomers by LC-MS. Likewise, Öman and Junestedt 2008 used both techniques to analyse organic and metal-organic compounds present in leachates.

In addition, there are a few works in which was employed GC-MS to evaluate the capacity of advanced oxidation processes to remove organic compounds from leachates. For instance, Ramírez-Sosa et al. 2013 determined that the total corrected area from the GC-MS chromatogram of a raw leachate decreased more than 97% after a Fenton–Adsorption treatment was carried out. Rocha et al. 2013 reported that a solar photo-Fenton process applied to a leachate was effective to remove 65% of the identified compounds, whilst the signal decreased between 37 and 56% in 12% of those, and the concentration increased for the other ones. Turki et al. 2013 carried out a series of three treatments on leachates, corresponding to coagulation-flocculation, Fenton and adsorption. They analysed the degradation of organic compounds after each step treatment by GC-MS and observed that a large number of compounds were still present in the leachates treated by the coagulation-flocculation and Fenton processes. However, when the adsorption process was included in the treatment, the total corrected area of the effluent decreased 99.9% compared to the total area of the raw leachate.

On the basis of the above mentioned, the aim of this study was to identify organic compounds present in three samples of landfill leachates (young, old and biologically stabilised ones) by using LC-MS, as well as, to compare and establish differences in their composition and physicochemical characteristics according to their fingerprints during their treatment by wet oxidation.

2. Material and methods

2.1 Leachate samples

To perform the experiments, three leachate samples were collected from a municipal landfill located in Asturias-Spain. The samples corresponding to young, old and biological stabilised leachates, the latter obtained from a membrane bioreactor after ultrafiltration process, were stored at 4 ºC until being used in either characterisation analyses or wet oxidation experiments. The main characteristics of the leachates are summarized in the Table 1.

Table1. Physicochemical characteristics of leachate samples.

Sample		Young leachate	Old leachate	Stabilised leachate
COD (mg O_2/L)		3821	2383	1085
TOC (mg C/L)		1693	982	477
MOC		0.61	0.36	0.59
CN (cm^{-1})		0.88	0.73	0.55
SUVA (L mg C^{-1} m^{-1})		1.90	1.67	2.54
pH		8.92	8.63	7.26
Elemental analysis wt%	**%C**	76.9	71.9	57.4
	%H	11.6	13.0	8.5
	%N	8.5	8.9	29.5
	%S	3.0	6.2	4.7

2.2 Experimental setup

Wet oxidation treatment was carried out in a PARR series 4520 reactor of one litre of capacity, equipped with a propeller stirrer which was maintained at 500 rpm throughout the experiments. Approximately, 600 mL of the corresponding leachate were charged into reactor and then, oxygen, previously conditioned in a humidifier tank, was fed since the beginning of the reaction to a flow rate of 1200 mL/min. The operating conditions established for the experiments were 200 ºC, 50 bar and 280 min of reaction, considering the time at which the reactor reached the reaction conditions (95 min) as the zero-time. The first sample was withdrawn when a temperature of 100 ºC was reached, in order to detect any change on leachate properties during the heating period. The experiments were performed by duplicate and the values obtained of standard deviation for the same samples were lower than 6%.

2.3 Analytical Methods

The following analysis to characterise initial and treated samples were carried out. Chemical oxygen demand (COD) and pH were performed according to Standard Methods (APHA 1998). Total organic carbon (TOC) analysis was measured by means of a TOC analyzer (Shimadzu TOC-V_{CSH}, Japan). UV-VIS spectrum was determined with a T80 UV/VIS spectrophotometer (PG Instruments Ltd). Elemental analysis of carbon, nitrogen, sulfur and hydrogen was carried out with an Elementar Vario EL analyser. Specified ultraviolet absorption (SUVA) was calculated by dividing the absorbance at 254 nm by the TOC concentration. The colour was quantified by means of the Colour Number (CN), which is defined according to equation (1) (Tizaoui et al. 2007).

$$\mathrm{CN} = \frac{\mathrm{SAC}_{436}^2+\mathrm{SAC}_{525}^2+\mathrm{SAC}_{620}^2}{\mathrm{SAC}_{436}+\mathrm{SAC}_{525}+\mathrm{SAC}_{620}} \qquad (1)$$

Where SAC is the spectral absorption coefficient at a specific wavelength.

The mean oxidation number of organic carbon (MOC) was calculated on the basis of equation (2) (Vogel et al. 2000).

$$\mathrm{MOC} = 4 - 1.5\,\frac{\mathrm{COD}}{\mathrm{TOC}} \qquad (2)$$

Where MOC takes values from −4 (i.e. for methane) to +4 (i.e. for carbon dioxide) and COD and TOC values are expressed in mg O_2/L and mg C/L, respectively.

2.4 Extraction and purification of samples

The extraction and purification of organic compounds from leachates and humic acid standard were carried out by solid-phase extraction (SPE), since this method offers a better efficiency and selectivity than the traditional liquid–liquid extraction Ask Reitzel and Ledin 2002. The extraction was carried out using SPE columns (ISOLUTE C18, 200 mg, 3 mL) and a SPE Vacuum Manifold of 24-Ports. Firstly, the SPE columns, located on the SPE Vacuum Manifold, were conditioned by passing 1 mL of methanol and 1 mL of 1% formic acid solution. Secondly, 0.5 mL of sample were added to an SPE column, being retained in the packing both compounds of interest and impurities. Thirdly, the impurities were removed from the packing by washing it with 1 mL of 1% formic acid solution. Fourthly, the interest compounds were recovered by adding 1 mL of methanol to the SPE column.

2.5 LC-MS analysis

LC-MS analysis of the samples extracted and purified was performed trough a Q-Tof Premier mass spectrometer (Waters Corporation, Micromass MS Technologies, Manchester, UK) coupled to an Alliance 2695 HPLC system (Waters Corporation, Milford, MA, USA). The Q-Tof Premier is equipped with a lockspray source where an internal reference compound (Leucine-Enkephalin) was introduced simultaneously with the analyte for more accurate mass measurements. Compounds were separated on an Atlantis T3 C18 column (Waters Corporation, Milford, USA, 100 mm x 2.1 mm; 3 µm particle size), using 0.1% formic acid solution (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Column temperature was maintained at 40 ºC. A stepwise gradient from 10% to 90% solvent B was applied at a flow rate of 0.3 mL/min for 18 min. Electrospray mass spectra data were recorded on a negative ionisation mode (loss of a proton $[M\text{-}H]^{-}$) for a mass range m/z 100 to m/z 1000. Capillary voltage and cone voltage were set at 3 kV and 30 V respectively. Collision induced fragmentation of the analytes was achieved using 12 eV to 20 eV energy with argon as the collision gas.

3. Results and discussion

3.1 Characteristics and composition of initial leachate samples

The physicochemical characteristics of the leachates used in this study were included in Table 1. The more remarkable difference observed amongst the samples was the content of organic matter. COD for young leachate was around 1.6 and 3.5 times higher than that for old and stabilised leachates. However, it was noted that the COD values for young and old leachates were relatively lower than those commonly reported in the literature. This fact can be due to a dilution process by effect of the rainy weather of the zone where the landfill is located. Further, some rainfalls were registered the previous weeks in which the samples were taken, achieving values of accumulated precipitation of 71.5 L/m^2 (Aemet-España 2018).

On the other hand, the MOC for old leachate (0.36) was about a half of that corresponding for young and stabilised leachates (0.61 and 0.59, respectively), which indicated that the former presented a higher presence of organic substances with a lower oxidation state. Regarding to colour, young leachate showed a more intense brown colour than the other leachates samples, as was demonstrated by its highest CN. This result was expected, since a higher amount of organic substances (see TOC values) was present in that sample. Nevertheless, considering the CN as function of the organic carbon content, stabilised leachate had the most intense colour per unit of carbon, with 1.6 L g C^{-1} cm^{-1}, whereas it was 0.74 and 0.52 L g C^{-1} cm^{-1} for old and young leachates, respectively. In order to establish differences amongst the leachates samples according to their aromaticity, the SUVA was determined. This parameter provides a quantitative measurement of the amount of unsaturated bonds or aromaticity of the dissolved organic matter and has also been correlated relatively well with the content of humic acids in raw water (White et al. 1997). The SUVA analysis showed a higher presence of substances with aromatic characteristics in the stabilised leachate (SUVA 2.54 L mg C^{-1} m^{-1}). This finding makes sense, since the substances with aromatic rings present a higher complexity in its structure, and thus a higher refractoriness to be degraded by a biological treatment. Consequently, the biological treatment which this leachate was subjected previously probably removed aliphatic compounds, not having effect on those with aromatic characteristics. Moreover, the SUVA for the old leachate (1.67 L mg C^{-1} m^{-1}) was a bit lower than that for young leachate (1.90 L mg C^{-1} m^{-1}). This result was probably caused by changes in the structure of organic compounds present in old leachates, which have occurred over the years.

In regard to elemental analysis, it was observed that stabilised leachate had a lower percentage of carbon and hydrogen, and a higher content of nitrogen than young and old leachates, which showed a similar elemental composition each other. Although stabilised leachate was treated by means of a pressurised biological nitrification–denitrification process, the high proportion of nitrogen in its composition suggests that the conversion of ammonium to nitrogen gas in the process was limited. The atomic ratios of H/C for young, old and stabilised leachates were of 1.8, 2.2 and 1.8, respectively. These ratios were higher than those reported by Kang et al. 2002 (1.54 and 1.88 for young and old leachates, respectively). However, differences between leachates generated in different landfills are expected, since the characteristics of these are affected by many factors, especially waste types and compositions.

The LC-MS chromatograms of the leachate samples and the humic acid standard are shown in the Figure 1. The highest presence of ions was observed for old and young leachates. Furthermore, a larger amount of peaks eluted after 6 min for each leachate sample, indicating thus that hydrophobic characteristics were predominant in the extracted fraction of these leachates. Likewise, it was observed that there was a quite good correspondence in terms of composition between the fractions of old and young leachates. That is to say, a high proportion of the ions detected were present in both samples, although with some variation in their abundance. It is worth noting that the [M-H]$^-$ ions observed in the humic acid standard corresponding to *m/z* 112.9, 153.0, 214.9, 209.0 and 209.1 were present in each leachate. This finding demonstrates that humic acids are a common component of different types of leachate and also that they persist over the years and the action of microorganisms.

On the other hand, the most prominent peak for all leachate samples corresponded to [M H]$^-$ ion at *m/z* 209.0, which eluted at 9.0 min. Other important [M H]$^-$ ions in basis to their peak area were at *m/z* 186.0, 170.9, 185.0, 214.9, 199.0 and 417.0 for times of 2.1, 3.8, 6.5, 7.5, 8.7 and 13.4 min, respectively, which were present mainly in young and old leachates. At short elution times, trifluoroacetic acid (TFA) ([M H]$^-$ ion at *m/z* 112.9) and methylphenol ([M H]$^-$ ion at *m/z* 107.0) were identified in each leachate sample, although the latter had a very low abundance.

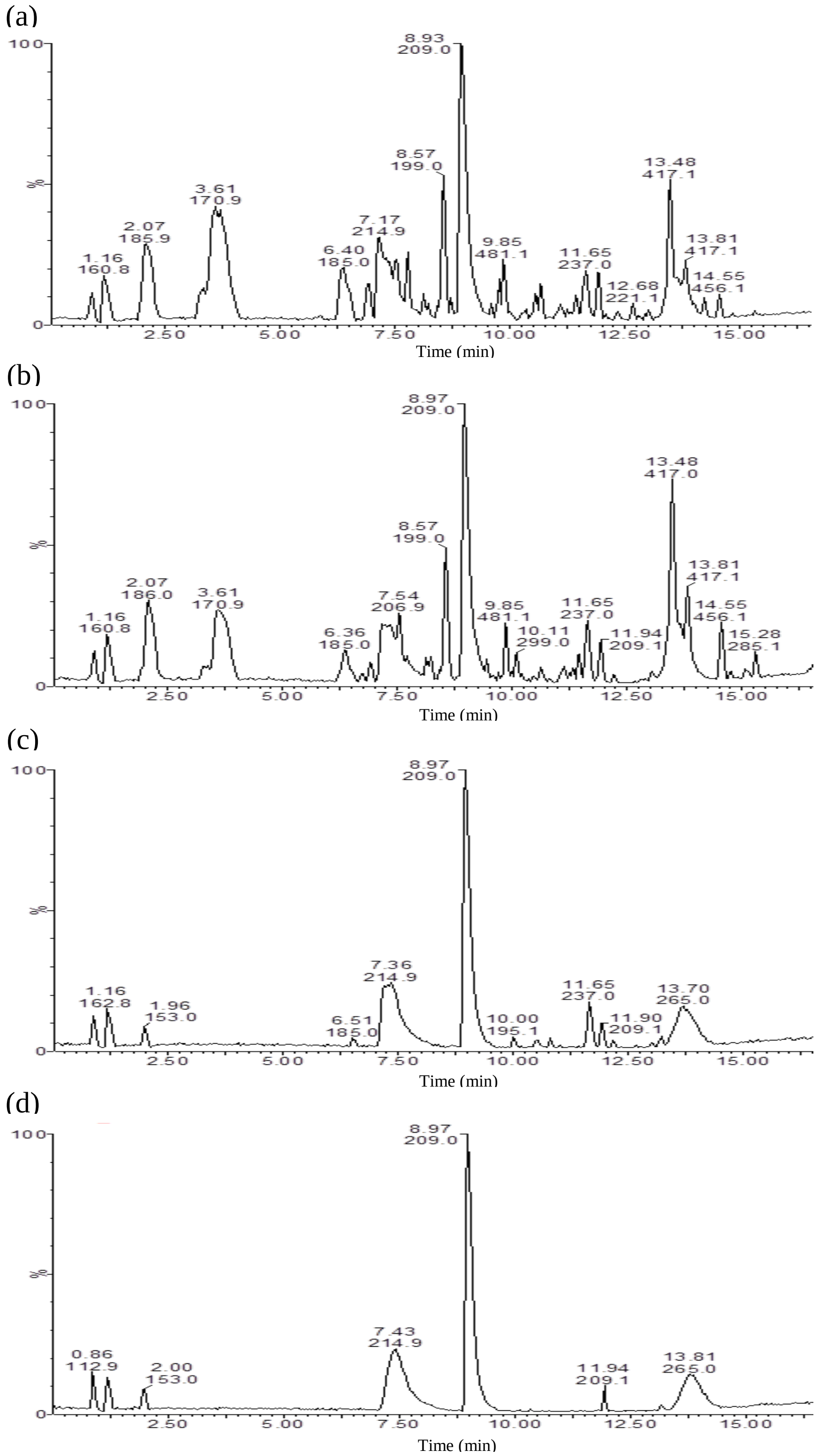

Figure 1. LC-MS chromatograms of leachates. (a) Young, (b), old, (c) stabilised and (d) humic acid standard.

The methylphenol, also known as cresol, is a toluene derivative which has applications as disinfectant and also is used for the production of pesticides, plastics amongst others.

Therefore, its presence in the leachate samples probably comes from packages that contained this kind of products. In contrast, TFA is a compound that is widely employed in organic chemistry for transformation of different compounds, and this is not present as such in the composition of manufactured products. Consequently, TFA was likely formed by the degradation of complex organic molecules containing trifluoromethyl groups.

The identification of the compounds present in the leachates in basis to their ions represented a challenging task, since it is expect to find any kind of substances in this effluent. In addition, databases of reference standards for LC-MS technique are scarce, and unfortunately, GC-MS libraries are not applicable to LC-MS analysis. In order to obtain more information about molecular structure of the leachate ions, LC-MS/MS analyses were performed and the results were collected in the Table 2.

Table 2. Common ions detected for young and old leachate samples.

T_R (min)	$[M\text{-}H]^-$	MS/MS
1.2	186	122
3.8	171	107
6.6	185	121
7.0	257	183
7.8	207	143, 115
8.2	477	433, 415, 331, 313
8.4	271	183
8.7	199	183
9.1	209	153
9.7	285	183
9.8	481	463, 437, 419, 379, 361, 335, 289, 235, 173
10.2	463	405, 361, 317, 299, 162
10.3	299	183
10.6	463	419, 401, 375, 357, 329, 163
11.1	345	329, 301, 285, 257, 206
11.4	463	445, 419, 401, 375, 357, 329, 297, 163
11.6	313	183
11.8	209	164
12.1	237	193, 149, 134
12.9	486	424, 412, 398, 355, 339, 301, 299
13.4	417	399, 373, 355, 327, 311, 301, 299
13.7	417	399, 373, 355, 327, 311, 301, 299
14.5	456	412, 381, 339, 299, 111
14.6	373	329, 311
14.9	498	398, 301, 197
15.1	285	270, 214, 201

For $[M\ H]^-$ ions at *m/z* 207, 345 and 417 (7.8, 11.1 and 13.4 min, respectively) was observed a neutral loss of 28 Da corresponding to CO, which is characteristic of phenolic groups. [M H]$^{-}$ ion at *m/z* 237 (12.1 min) had two decarboxylations, being CO_2 a common loss for compounds with acid characteristics. Furthermore, [M H]$^{-}$ ions at *m/z* 237 and 417 (9.8 and 13.4 min) had three and two dehydrations, respectively, which indicated that these ions presented functional groups characteristic of alcohol compounds. On the other hand, [M H]$^{-}$ ions at *m/z* 477, 463 and 373 (8.2, 10.2 and 14.6 min, respectively) showed decarboxylation and dehydration losses, whilst [M H]$^{-}$ ion at *m/z* 463 (10.6 min) had those same losses as well as CO. In the case of [M H]$^{-}$ ion at *m/z* 456 (14.5 min), its loss of CH_2CO suggested that this ion could belong to benzylic esters family. Finally, with the purpose of improving the identification of the extracted compounds from the leachates, the accurate mass of the main ions was determined and the theoretical molecular formula more probable for some of them was established from the equipment software (Table 3).

Table 3. Theoretical chemical formula determined for some ions of leachate samples.

T_R (min)	[M-H]$^{-}$	Theoretical chemical formula
0.9	112.9836	$C_2 O_2 F_3$
2.153	186.0208	$C_{10} H_4 N O_3$
	156.9946	$C_9 H O_3$
3.971	107.0484	$C_7 H_7 O$
6.814	185.0261	$C_6 H_7 N_3 O_2 S$
6.994	289.1272	$C_{12} H_{16} N_4 O F_3$
	172.0381	$C_8 H_5 N F_3$
7.124	393.1919	$C_{21} H_{29} O_7$
7.166	393.1932	$C_{19} H_{26} N_4 O_4 F$
	171.0656	$C_8 H_{11} O_4$
	127.0746	$C_7 H_{11} O_2$
7.787	632.0396	$C_{34} H_{13} O_{12} F$
9.392	209.0927	$C_{10} H_{13} N_2 O_3$
10.599	463.2684	$C_{21} H_{38} N_3 O_7 F$
11.866	209.1516	$C_{11} H_{19} N_3 O$
12.467	237.0743	$C_{10} H_{12} O_3 F_3$
	193.0845	$C_7 H_{10} N_3 F_3$
	149.0944	$C_8 H_{11} N_3$
12.658	357.1873	$C_{11} H_{27} N_5 O_8$
	285.1525	$C_9 H_{23} N_3 O_7$
12.704	221.1502	$C_9 H_{21} N_2 O_4$
13.267	417.2266	$C_{24} H_{33} O_6$
	373.2381	$C_{23} H_{33} O_4$
13.359	417.2238	$C_{19} H_{33} N_2 O_8$
14.93	359.1863	$C_{21} H_{27} O_5$
	283.1697	$C_{19} H_{23} O_2$
14.965	456.2426	$C_{19} H_{38} N O_{11}$
	412.2534	$C_{18} H_{38} N O_9$
	301.2000	$C_{16} H_{29} O_5$
15.126	285.1655	$C_{10} H_{25} N_2 O_7$

3.2 Oxidation and mineralisation reactions during wet oxidation of leachates

The effect of wet oxidation on the COD for the different leachate samples is shown in Figure 2a. As can be seen, the COD of each leachate remained almost constant when 100 ºC were reached in the reaction (35 min), being only observed a reduction of 1-3%. However, COD decreased considerably (between 19-22%) after 95 min, when the operating conditions were achieved (200 ºC and 50 bar). This same behaviour has been observed during the treatment by wet oxidation of other types of effluents (Urrea et al. 2018), in which the attack of free radicals formed in the medium was not effective until temperatures higher than 100 ºC were achieved. Furthermore, it is worth noting that neither the leachate type nor the initial COD concentration seemed affect the oxidation degree of the treatment, being obtained similar yields of COD degradations for each leachate, especially in the first minutes of reaction. These facts can be explained taken into account the following reasoning. First of all, the presence of an important amount of biorefractary compounds is a common characteristic in young, old and biologically stabilised leachates. A fraction of these compounds are mainly formed by humic acids, which present a lower resistance to be degraded by advanced oxidation processes than other substances as fulvic acids (Oulego et al. 2016). Therefore, humic acids were probably the main species oxidised during the first minutes of wet oxidation, which also suggests a similar distribution of those substances in the composition of each leachate according to the percentage of COD removed. Moreover, the oxidation of organic compounds by wet oxidation is known to be developed through free radical chain reactions. In the initial stage, reactive molecules are formed and, in turn, react with organic compounds present in the samples, forming more reactive molecules, and thus propagating the chain reaction. Consequently, higher COD concentrations mean that there are more molecules to react and that is because a larger organic load was degraded in leachates with higher COD, although similar COD percentages were achieved in these.

After 95 min of wet oxidation, some differences in function of the leachate type were noted in the way COD decreased. Firstly, young leachate had the highest oxidation rate, although the COD level quickly stabilised from 115 min, being observed a slight reduction since that time and achieving a COD removal of 43% at the end of the treatment (375 min). Secondly, old leachate presented a continued COD decline, reaching a COD removal of 42% after 375 min of wet oxidation. Thirdly, COD of stabilised leachate also showed a constant decrease, but with higher degradation percentages than the other samples, obtaining a 48% COD removal when the treatment was concluded.

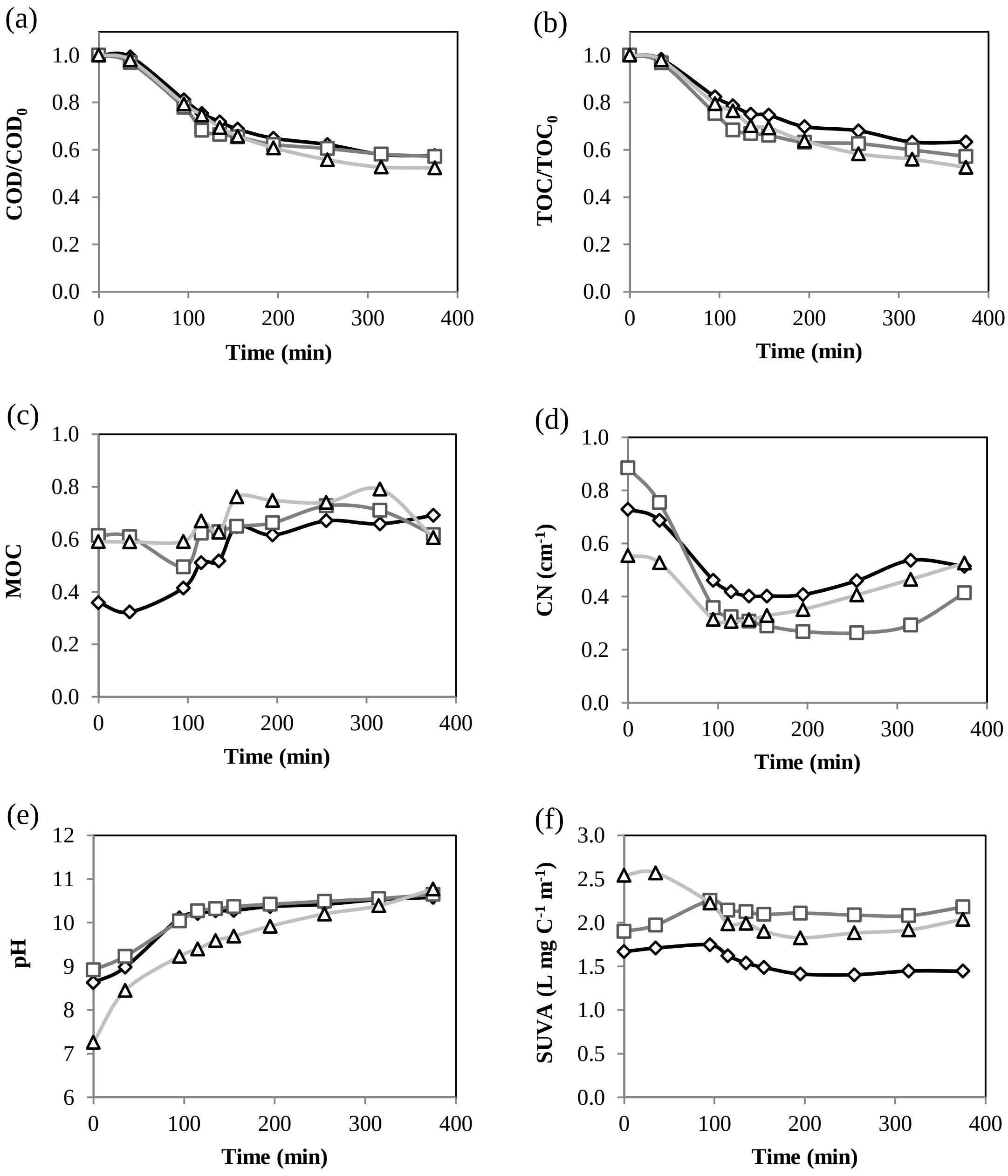

Figure 2. Evolution of properties of (-□-) young, (-◇-) old and (-△-) stabilised leachates during wet oxidation. (a) COD/COD$_0$, (b) TOC/TOC$_0$, (c) MOC, (d) Colour, (e) pH and (f) SUVA.

On the other hand, the changes observed on the TOC (Figure 2b) showed that the mineralisation of organic matter was more important for young leachate than for the rest of leachates during the first 155 min of wet oxidation. However, stabilised leachate reached the largest mineralisation degree at the end of the reaction (47% TOC removed). The TOC removal for young and old leachates once the treatment was concluded was of 43 and 37%, respectively. In addition, it was noted that the evolution of TOC for each leachate followed the same behaviour observed on their COD, thus indicating that the free radicals continuously produced both the partial oxidation and the mineralisation of organic matter.

The relationships between the reactions of partial oxidation and complete mineralisation developed during wet oxidation, can easily be established in basis to the changes experienced by the MOC for each leachate (Figure 2c). Therefore, when MOC decreased, the mineralisation (including the effect of the formation of CO_2 produced by decarboxylation reactions) was more important than the partial oxidation and vice versa. As it can be observed, the variation on the MOC for each leachate was not too high during the treatment, suggesting that both reactions were balanced. A small decline in the MOC was noted for young leachate at short times of treatment (95 min), probably by effect of decarboxylation of a higher amount of humic acids. After the operating conditions were reached, the MOC showed a slight increase for each leachate, indicating that the partial oxidation had more relevance from that treatment time. At the end of wet oxidation, the oxidation state of young and stabilised leachate was similar to that of their initial samples, whilst old leachate had a small increase in its oxidation state.

3.3 Effect of wet oxidation on colour, pH and aromaticity

The effect of wet oxidation on the colour of the leachate samples was quantified by the colour number (CN). Despite a low treatment temperature (100 ºC) was not effective to degrade organic matter from leachates (only 1-3% COD removal), a slight reduction of the CN was observed under this condition for each sample, that being a bit more important on young leachate (Figure 2d). An explanation for this result could be the loss of either a functional group or a molecular fragment of some compounds by effect of thermal hydrolysis reactions, giving rise to other molecules of lower colour. Once the operating conditions were reached, the colour removal was more evident, obtaining an overall decrease in CN of 60, 37 and 43% for young, old and stabilised leachate, respectively. After 95 min of treatment, the colour of the samples changed from a dark brown for initial samples to a very pale brown. It is worth noting that CN for young leachate showed a more significant decline than the rest of leachates at this reaction time, coinciding with the decrease in its MOC. Therefore, this finding seems to indicate that the higher mineralisation degree achieved by the substances that compose this sample had a notorious effect on the reduction of its colour, especially at short reaction times. With the advance of the treatment, the CN of all samples decreased up to reach a minimum value and then increased. During this stage, the colour changed from a pale brown to a pale yellow in the final effluents.

Regarding to the changes on pH (Figure 2e), it was observed that wet oxidation caused an increase on this parameter for each leachate, even when 100 ºC were initially reached in the reaction. The variation of the pH was more important for stabilised leachate, showing in turn a more continuous rise than for the rest of the samples. This result was probably due to a lower alkalinity of this leachate by effect of biological treatment applied previously, such as was reported by Oulego et al. 2015. Eventually, all leachates achieved a pH of 10.7 ± 0.1 when wet oxidation was concluded.

On the other hand, the changes caused by wet oxidation on SUVA of each leachate are shown in Figure 2f. The hydrothermal treatment at 100 ºC (35 min) caused a minimum increase in SUVA for all leachates. However, this result was not significant, because the mineralisation and the variation on absorbance at 254 were very small under these conditions. When the operating conditions were achieved, the SUVA of stabilised leachate decreased. In contrast, SUVA of old and young leachates increased, being this behaviour more marked for the latter. This change on SUVA for stabilised leachate indicated that wet oxidation caused a remarkable reduction in the amount of unsaturated carbon–carbon bonds in its composition. Although unsaturated carbon–carbon bonds were also reduced in young and old leachates (their absorbance at 254 decreased), the increase in their SUVA suggests that for these samples, the attack of hydroxyl radicals had a higher affinity on saturated compounds than on aromatic compounds, especially for young leachate. In fact, hydroxyl radicals are known to react with organic compounds by addition to a double bond or by abstraction of hydrogen atoms from aliphatic organic molecules (Dwyer et al. 2008).

After 95 min of treatment, the SUVA of all leachates showed a slight decline and subsequently achieved a stable value. Finally, wet oxidation caused a reduction of the aromaticity in old and stabilised leachates (SUVA decrease from 1.67 and 2.54 to 1.45 and 2.04 L mg C^{-1} m^{-1}, respectively), whilst the aromatic character prevailed on the composition of young leachate effluent (SUVA changed from 1.90 to 2.18 L mg C^{-1} m^{-1}). Wang et al. 2017 reported a decline in SUVA from 2.67 to 1.44 L mg C^{-1} m^{-1} after applying a combined process of O_3/H_2O_2 to a leachate concentrated by nanofiltration. However, the mineralisation achieved by that treatment (around 15% TOC removal) was considerably lower than that obtained in this work, although the COD removals (around 50%) were similar. Those results suggest that in the oxidation mechanisms involved during O_3/H_2O_2 process, the partial oxidation of organic matter prevailed to its complete oxidation, whilst in wet oxidation both reactions were equally significant.

3.4 Analysis of wet oxidation of leachates by LC-MS

The evolution of the area of the peaks for the main ions during wet oxidation of leachate samples is showed in Figure 3. In order to avoid an overestimation on the calculated area by presence of other ions in the same peak, the signal of each ion was separated of the other ones before its area was estimated. As it can be seen, the treatment by wet oxidation caused a decrease in the area of almost all ions for each leachate, with the exception of the $[M\ H]^{-}$ ions at *m/z* 112.9, 158.9 and 160.8 (0.8, 0.8 and 1.2 min, respectively), in which the area showed an increase, this last fact being more marked for the second one. In fact, $[M\ H]^{-}$ ion at *m/z* 158.9 initially presented a very low area (60 ± 22) and then increased dramatically (812 ± 74) after operating conditions were achieved. At this point, it is worth noting that those ions were present at short elution times, which indicated that wet oxidation produced compounds with hydrophilic characteristics.

On the other hand, $[M\ H]^{-}$ ion at *m/z* 112.9, which was identified as TFA, showed an increase and subsequently declined in its concentration at the end of the reaction, achieving similar areas to the initial ones, although the reaction time in which this happened was different in function of the type of leachate. TFA is the simplest perfluorinated carboxylic acid, and thus its formation during wet oxidation of leachates makes sense since is well-known that low molecular weight carboxylic acids are produced by this treatment. Furthermore, considering that leachates present a great variety of substances in their composition, TFA could have been formed from complex molecules containing fluorine atoms. As the degradation of low molecular weight carboxylic acids by means of wet oxidation requires severe treatment conditions, the area decline of TFA during the treatment suggests that it could have reacted in some way with other compounds.

Despite 100 ºC did not produce significant treatment effects on leachates in terms of oxidation, marked changes on the area of the ions were observed under this condition. For instance, the area of [M H]$^-$ ions at *m/z* 186 and 171 (2.2 and 3.9 min) decreased around 42 ± 5% for old leachate and 24 ± 4% for young leachate. Furthermore, [M H]$^-$ ions at *m/z* 214.9 and 209.1 (7.8 and 9.0 min) that had the highest area in initial leachate samples, completely disappeared from that treatment time. Likewise, it was observed in both young and old leachates the formation of two new [M H]$^-$ ions at *m/z* 221.1 and 236.1 that eluted at the same time (12.6 min), and which in turn, showed a fast area decrease with the advance of the reaction. The similar theoretical formula calculated for [M H]$^-$ ions at *m/z* 209.1 ($C_{10}H_{13}N_2O_3$) and 236.1 ($C_{11}H_{14}N_3O_3$) seemed to indicate that the second one had been formed from the first one. However, that hypothesis was discarded as [M H]$^-$ ion at *m/z* 236.1 was not detected in stabilised leachate samples after wet oxidation.

Finally, the area of the ions was reduced between 40 and 80% after 375 min of wet oxidation, which indicates that this treatment caused an important effect on the composition of the different leachate samples. In addition, the abundance of the [M H]$^-$ ions corresponding to humic acid (*m/z* 112.9, 153.0, 214.9, 209.0 and 209.1) decreased in all leachate samples during the treatment. This result is consistent with those reported by other works, in which was determined that wet oxidation was effective to degrade humic substances of different samples, such as biological sludge and leachates (Oulego et al. 2016, Urrea et al. 2017).

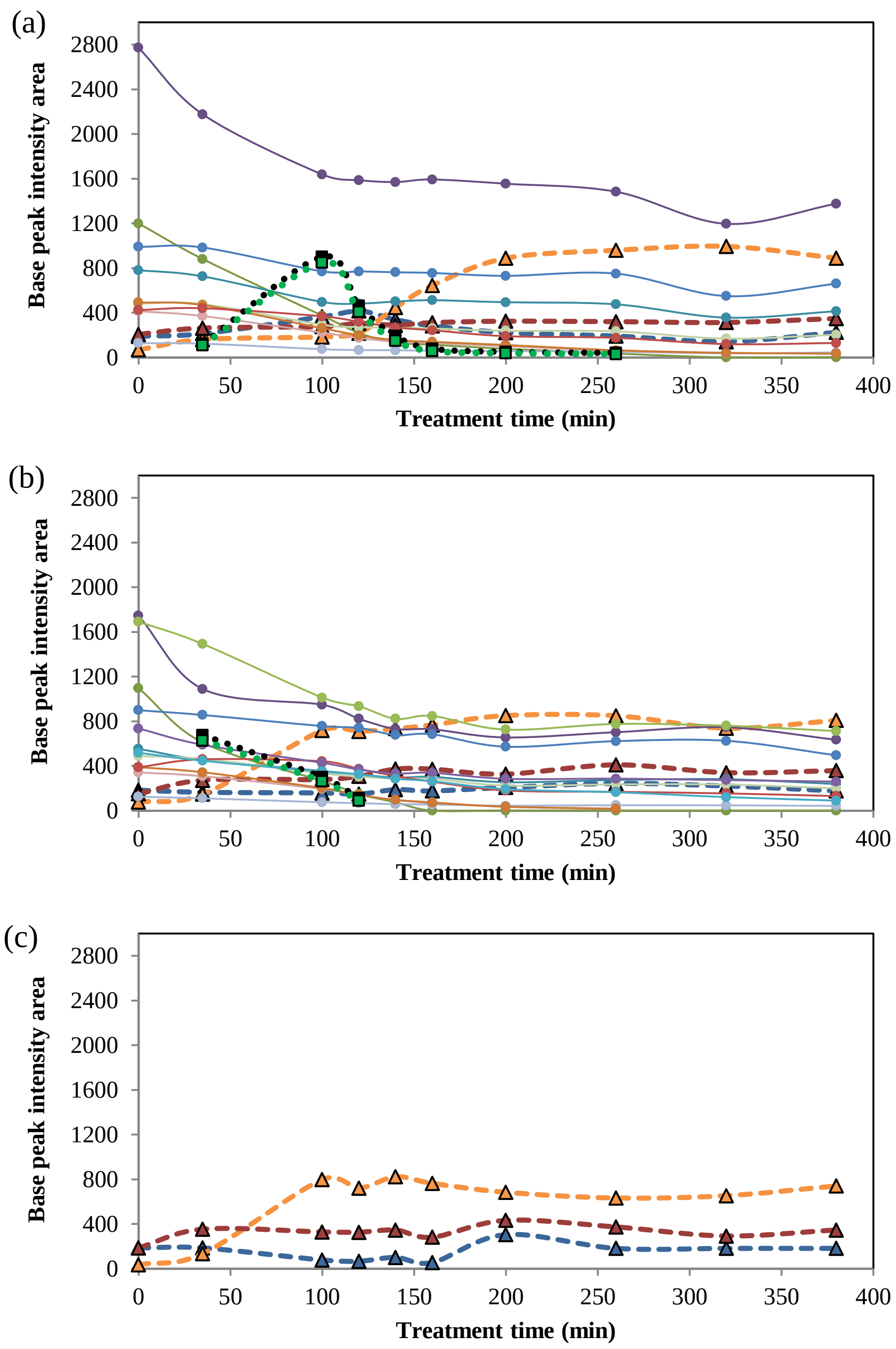

Figure 3. Evolution of the area of the main ions of leachates during wet oxidation. (a) Young, (b) old and (c) stabilised leachates. Formed ions (dotted lines and square symbol) and ions increasing (dashed lines and triangular symbol). [M H]$^{-}$ (▲) 112.9 (0.9 min), (▲) 158.9 (0.9 min), (▲) 160.8 (1.2 min), (●)186 (2.2 min), (●) 171 (3.9 min), (●) 185 (6.6 min), (●) 206.9 (7.3 min), (●) 219.9 (7.3 min), (●) 206.9 (7.8 min), (●) 219.9 (7.8 min), (●) 199 (8.7 min), (●) 477 (9.2 min), (■) 221.1 (12.6 min), (■) 236 (12.6 min), (●) 417.1 (13.4 min), (●) 417.1 (13.7 min) and (●) 456.1 (14.4 min).

4. Conclusions

The highest presence of ions detected by LC-MS was observed in old and young leachates. In addition, a great proportion of those ions were present in both samples, although with some variation in their abundance, thus indicating that old and young leachates presented quite similarity in their composition. While old leachate had the lowest oxidation state for organic substances, stabilised leachate presented the highest aromaticity degree.

The ions observed for humic acid standard were present in each leachate sample, revealing that these substances are a common component of leachates of different age and are resistant to biological degradation. Nevertheless, the abundance of those ions decreased in all leachate samples during wet oxidation, demonstrating that this treatment was effective to degrade persistent compounds from leachates.

Wet oxidation caused a substantial improvement on the leachates characteristics, achieving a mineralisation around 50% of organic matter, a decolourisation of those from a dark brown colour to a pale yellow colour and a decrease in the aromaticity for old and stabilised leachates. Furthermore, the total area of the ions in the chromatogram was reduced between 40 and 80%, whilst some ions of hydrophilic characteristics (molecules more oxidised) increased their concentration.

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5. CONCLUSIONS

- Hydrothermal technologies represent an interesting alternative for improving the dewaterability characteristics of the sludge, thus making its subsequent management easier. The hydrothermal treatments destroyed the floc structure of the sludge releasing bound water and producing a high solubilisation degree of extracellular and cellular components.

- Thermal hydrolysis and wet oxidation showed a high efficiency to reduce the volume of activated sludge. However, the use of an oxidising atmosphere offered a more complete solution of treatment, providing better results of solubilisation, settleability and mineralisation of the sludge than an inert one.

- The temperature has proved to be the most influential parameter for both thermal hydrolysis and wet oxidation, causing the improvement of the rate of solubilisation reactions with its increase. Therefore, less reaction time was necessary to achieve the same results obtained at lower temperatures. Moreover, the changes in pressure did not produce significant effects for thermal hydrolysis, whilst those increased slightly the rates of oxidation during wet oxidation.

- The free radicals initially formed by wet oxidation favoured a higher solubilisation of the sludge instead of its mineralising. Likewise, these radicals showed particularly high affinity to cause the cellular lysis at the beginning of the reaction, rather than the complete hydrolysis of the cellular components such as cellular wall and membrane.

- Short treatment times of the sludge by wet oxidation caused a marked worsening of some important properties, such as settleability and dewaterability. These results were attributed to the increased of electrostatic repulsion forces between the solid particles due to the solubilisation of polymers, as well as to the increase of the bound water content in such solids. Nevertheless, once a high degree of solubilisation and oxidation was reached, the characteristics of settleability and dewaterability were largely improved.

- Although wet oxidation caused a higher degree of solids solubilisation than thermal hydrolysis, the yield of solubilisation of the main component of the sludge, the protein, was similar between both techniques. This result was a factor of the degradation of proteins caused by free radicals formed under an oxidising atmosphere.

- The presence of oxygen during the hydrothermal treatment of sludge favoured a higher solubilisation of low and medium size polymers, as well as a higher hydrophilic character in the composition of the effluent. In addition, an oxidising atmosphere caused the degradation of each solubilised biopolymers from sludge, whilst an inert one only caused the degradation of proteins, demonstrating thus that proteins were more sensitive a thermal decomposition.

- The decrease in the concentration of organic solids by either thermal hydrolysis or wet oxidation affected the interaction between the particles of the sludge, generating a positive effect on the rheological characteristics of the effluent. A reduction in the apparent viscosity of up to two orders of magnitude was achieved, as well as the transformation of the flow behaviour from a Bingham plastic for the initial sludge to a Newtonian flow at the end of the treatment.

- The modelling of the rheological parameters (Herschel–Bulkley model) from sludge during thermal hydrolysis showed an exponential growth of the yield stress (τ_0) with an increase in the concentration of total suspended solids, while the consistency (K) and the flow behaviour index (n) were fitted to a polynomial function of third and second order respectively.

- A kinetic model that accurately described the reactions that occur during the wet oxidation of the sludge in basis to COD evolution has been proposed, achieving a good fit with the experimental data. The model implies an initial solubilisation of the solid COD, followed by the oxidation of the solubilised COD, finally generating a refractory COD.

- According to the fingerprints for initial EPS fractions, it was identified that LB-EPS gathers characteristics of the other EPS fractions in terms of molecular size distribution. This behaviour was attributed to the exchange of polymers that occurs between LB-EPS and the others EPS fractions, due to its intermediate location in the arrangement of the floc.

- Considering the fingerprint peaks present only in the size exclusion stretch, SMP was composed of 14, 13 and 73% polymers of low (>35 kDa), medium (35-150 kDa) and high (>150 kDa) molecular weight, respectively. Likewise, the content of these polymers in LB-EPS and TB-EPS was 44, 10, 46% and 83, 15, 2%, respectively.

- The analysis of the structural components of the sludge during wet oxidation revealed that the inner fractions of the microbial aggregates are more amenable to oxidation than the outer ones. Therefore, the fractions were more refractory to oxidation following this order: SMP > LB-EPS > TB-EPS > naked cells. This behaviour was attributed to two factors: the screening effect of the oxidative environment in the WWTP on SMP and LB-EPS fractions and the role of the LB-EPS as a physical barrier against the attack of the free radicals in the medium, protecting the more internal biopolymers and promoting their stability against oxidation.

- Wet oxidation caused the degradation of larger molecules in the sludge fractions by effect of reactions of thermal hydrolysis and oxidation, leading to a final effluent with a content of almost 90% of low molecular weight solubilised substances (<35 kDa). In addition, an increase in the proportion of readily biodegradable material was observed for all of the fractions at the end of the treatment.

- Wet oxidation of sludge involves an initial fast solubilisation of biopolymers, mainly proteins, followed by their gradual oxidation. Furthermore, the modification of proteins by hydroxylation of phenylalanine generated polymers with a higher absorbance capacity in the UV zone.

- A new kinetic model for sludge solubilisation by wet oxidation has been proposed and proved. The model includes the effects of oxygen concentration and temperature on volatile suspended solids solubilisation rates and on the formation and reaction rates of the main solubilised biopolymers.

- The high degree of solubilisation of biopolymers obtained (231, 147, 83, 9 and 5 mg per gram of initial volatile suspended solids, for proteins, humic acids, carbohydrates, uronic acids and DNA, respectively), suggested the possibility of applying wet oxidation in order to recover products of potentially high added value, thus also improving the economic appeal of the process.

- Wet oxidation caused a substantial improvement on the leachates characteristics, achieving a mineralisation around 50% of organic matter, a decolourisation of those from a dark brown colour to a pale yellow colour and a decrease in the aromaticity for old and stabilised leachates. Furthermore, the total area of the ions in the chromatogram was reduced between 40 and 80%, whilst some ions of hydrophilic characteristics (molecules more oxidised) increased their concentration.

5. CONCLUSIONES

- Las tecnologías hidrotérmicas representan una alternativa interesante para mejorar las características de deshidratabilidad del lodo, y por lo tanto, facilitar su posterior gestión. Los tratamientos hidrotérmicos destruyeron la estructura flocular del lodo, liberando el agua fuertemente retenida y produciendo un alto grado de solubilización de los componentes celulares y extracelulares.

- La hidrolisis térmica y la oxidación húmeda mostraron una alta eficiencia para reducir el volumen del lodo activo. No obstante, el uso de una atmósfera oxidante supuso una solución de tratamiento más completa, aportando mejores resultados en términos de solubilización, sedimentabilidad y mineralización del lodo con respecto a una atmósfera inerte.

- El parámetro operacional más influyente en la hidrólisis térmica y la oxidación húmeda del lodo fue la temperatura, la cual causó una mejora en las velocidades de las reacciones de solubilización con su incremento. Por consiguiente, fueron necesarios menores tiempos de reacción para alcanzar los mismos resultados obtenidos a más bajas temperaturas. Además, los cambios en la presión no produjeron efectos significantes para la hidrólisis térmica, mientras que éstos causaron un suave incremento en las velocidades de oxidación durante la oxidación húmeda.

- Los radicales libres formados inicialmente durante la oxidación húmeda, favorecieron una mayor solubilización del lodo en lugar de su mineralización. Asimismo, estos radicales mostraron particularmente mayor afinidad para causar solo una lisis celular al comienzo de la reacción, en lugar de producir la hidrólisis completa de las células.

- El empleo de oxidación húmeda durante cortos tiempos de reacción causó un marcado empeoramiento de algunas propiedades importantes del lodo, tales como la sedimentabilidad y la deshidratabilidad. Estos resultados fueron asociados al incremento de las fuerzas de repulsión electroestática entre las partículas sólidas por efecto de la solubilización de polímeros, así como al incremento del agua fuertemente retenida en los sólidos. Sin embargo, una vez que un alto grado de solubilización y oxidación fueron alcanzados, las características de sedimentabilidad y deshidratabilidad mejoraron considerablemente.

- Aunque la oxidación húmeda causó un mayor grado de solubilización de sólidos que la hidrólisis térmica, el rendimiento de solubilización del principal componente del lodo, la proteína, fue similar entre ambas técnicas. Este resultado fue debido al ataque de los radicales libres formados bajo una atmósfera oxidante, lo cual causó una degradación más significativa de las proteínas, que la generada por descomposición térmica durante la hidrólisis térmica.

- La presencia de oxígeno durante el tratamiento hidrotérmico del lodo favoreció una mayor solubilización de polímeros de bajo y mediano peso molecular, al igual que un mayor carácter hidrofílico en la composición del efluente. Adicionalmente, una atmósfera oxidante causó la degradación de cada uno de los biopolímeros solubilizados del lodo, mientras que una atmósfera inerte solo causó la degradación de las proteínas, demostrando así que las proteínas fueron los biopolímeros más sensibles a la descomposición térmica.

- La reducción en la concentración de sólidos orgánicos por hidrólisis térmica u oxidación húmeda afectó a la interacción entre las partículas del lodo, generando un efecto positivo en las características reológicas del efluente. Se alcanzó una reducción en la viscosidad aparente del lodo de hasta dos órdenes de magnitud, al igual que un cambio en el comportamiento del flujo, pasando desde un plástico de Bingham del lodo inicial a un fluido newtoniano al final del tratamiento.

- El modelado de los parámetros reológicos (modelo de Herschel–Bulkley) del lodo durante la hidrólisis térmica, mostró un crecimiento exponencial del límite de esfuerzo (τ_0) con el incremento en la concentración de los sólidos suspendidos totales. En cambio, la consistencia (K) y el índice de comportamiento del flujo (n) fueron ajustados a una función polinómica de tercer y segundo orden, respectivamente.

- Se propuso un modelo cinético que describió con precisión las reacciones que ocurren durante la oxidación húmeda del lodo, en base a la evolución de la DQO, siendo obtenido además un buen ajuste con respecto a los datos experimentales. El modelo implica la solubilización inicial de la DQO de la fracción sólida, seguida por la oxidación de la DQO solubilizada y, finalmente, la producción de una DQO refractaria.

- En base a las huellas dactilares de las fracciones iniciales de SPE (sustancias poliméricas extracelulares), se identificó que SPE-DL (SPE débilmente ligadas) reúnen características de las otras fracciones de SPE, en términos de la distribución del tamaño molecular. Este comportamiento fue atribuido al intercambio de polímeros que ocurre entre SPE-DL y las otras fracciones de SPE, debido a su localización intermedia en la distribución del flóculo.

- Considerando los picos de las huellas dactilares presentes solo en la zona de exclusión por tamaño, PMS (productos microbianos solubles) estuvieron compuestos por 14, 13 y 73% de polímeros de bajo (<35 kDa), mediano (35-150 kDa) y alto (>150 kDa) peso molecular, respectivamente. Asimismo, el contenido de este tipo de polímeros en SPE-DL y SPE-FL (SPE fuertemente ligadas) fue de 44, 10, 46% y 83, 15, 2%, respectivamente.

- El análisis de los componentes estructurales del lodo durante la oxidación húmeda, reveló que las fracciones más internas de los agregados microbianos son más sensibles a la oxidación que las dispuestas en la zona exterior. Por lo tanto, las fracciones fueron más refractarias a la oxidación en este orden. PMS > SPE-DL > SPE-FL > células. Este comportamiento fue atribuido a dos factores. En primer lugar, el efecto de cribado del ambiente oxidativo en la EDAR sobre las fracciones de PMS y SPE-DL. En segundo lugar, el papel de barrera física de SPE-DL contra el ataque de los radicales libres presentes en el medio, protegiendo los biopolímeros más internos y promoviendo su estabilidad en contra de la oxidación.

- La oxidación húmeda causó la degradación de las moléculas de mayor tamaño en las fracciones del lodo, por efecto de las reacciones de hidrólisis térmica y oxidación, produciendo un efluente final con un contenido cercano al 90% de sustancias solubilizadas de bajo peso molecular (<35 kDa). Además, se observó un incremento en la proporción del material rápidamente biodegradable para todas las fracciones al finalizar el tratamiento.

- La oxidación húmeda del lodo implica una rápida solubilización de los biopolímeros, principalmente proteínas, seguida por su oxidación gradual. Además, la modificación de las proteínas por efecto de la hidroxilación de la fenilalanina generó polímeros con una capacidad mayor de absorbancia en la zona UV.

- Se propuso y probó un nuevo modelo cinético para la solubilización del lodo durante la oxidación húmeda. El modelo incluye los efectos de la concentración de oxígeno y de la temperatura sobre las velocidades de solubilización de los sólidos suspendidos volátiles, y sobre las velocidades de formación y reacción de los principales biopolímeros solubilizados.

- El alto grado de solubilización de biopolímeros obtenido (231, 147, 83, 9 y 5 mg por gramo inicial de sólidos suspendidos volátiles, para proteínas, ácidos húmicos, carbohidratos, ácidos urónicos y ADN, respectivamente), sugirió la posibilidad de aplicar la oxidación húmeda para la recuperación de productos con potencial valor añadido, contribuyendo así a mejorar el potencial económico del proceso.

- El tratamiento por oxidación húmeda causó una mejora importante de las características de los lixiviados. La mineralización de materia orgánica alcanzada fue cercana al 50 %, mientras que el color se redujo, cambiando desde un marrón oscuro a un amarillo claro. Además, la aromaticidad decreció en lixiviados maduros y en lixiviados estabilizados biológicamente. Finalmente, el área total de los iones en el cromatograma se redujo entre 40 y 80%, mientras que la concentración de algunos iones de características más hidrofílicas incrementó.

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7. APPENDIX

7.1 SUPPORTING INFORMATION

Article.

I. Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review.

Table of contents

1. Compounds generated during thermal hydrolysis of sewage sludge. Conditions and relevant results published (Table S1).
2. Compounds generated during wet oxidation of sewage sludge. Conditions and relevant results published (Table S2).
3. Results of the application of TH and WO to the same sludge in the same experimental device (Table S3).
4. Effect of the level of the oxygen fed into the reactor on the products obtained during hydrothermal treatment of sewage sludge (Table S4).

1. Compounds generated during thermal hydrolysis of sewage sludge. Conditions and relevant results published.

Table S1. Components generated during thermal hydrolysis of sewage sludge. Conditions and relevant results published.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th colspan="11">RESULTS AND/OR COMMENTS</th></tr>
<tr><td rowspan="10">Brooks (1968)</td><td rowspan="10">Activated sludge from Abergavenny sewage works, settled to around 1% TS. It contained 0.04 mg/L DON, 0.25 mg/L NH₄-N, 7.82 % insoluble N, 500 mg/L TDS, 10000 mg/L TSS, 30 mg/L colloidal (col) solids and an amount of phosphorus that, if fully solubilized, would generate 80 mg/L phosphate.</td><td rowspan="10">A lab-scale apparatus at 100 – 216ºC for 0.5 – 24 hour (pre-heating time of 30 min).</td><td rowspan="2">Parameter</td><td colspan="2">100ºC</td><td colspan="2">130ºC</td><td colspan="2">163ºC</td><td colspan="2">197ºC</td><td colspan="2">216ºC</td></tr>
<tr><td>0.5 h</td><td>3 h</td><td>0.5 h</td><td>3 h</td><td>0.5 h</td><td>3 h</td><td>0.5 h</td><td>3 h</td><td>0.5 h</td><td>3 h</td></tr>
<tr><td>DON(mg/L)</td><td>124</td><td>154</td><td>323</td><td>391</td><td>502</td><td>486</td><td>520</td><td>524</td><td>512</td><td>497</td></tr>
<tr><td>Insoluble N (%)</td><td>7.80</td><td>7.77</td><td>7.02</td><td>6.96</td><td>5.51</td><td>4.80</td><td>3.48</td><td>3.84</td><td>2.61</td><td>2.30</td></tr>
<tr><td>NH₄-N (mg/L)</td><td>19.3</td><td>24.8</td><td>52.4</td><td>38.6</td><td>82.5</td><td>94.5</td><td>157</td><td>240</td><td>232</td><td>282</td></tr>
<tr><td>TDS (mg/L)</td><td>594</td><td>1419</td><td>3465</td><td>4092</td><td>4965</td><td>5198</td><td>n.d.*</td><td>5082</td><td>5886</td><td>4925</td></tr>
<tr><td>TSS (mg/L)</td><td>9769</td><td>9089</td><td>8581</td><td>7590</td><td>5959</td><td>4705</td><td>3828</td><td>3663</td><td>3262</td><td>2640</td></tr>
<tr><td>Col solids (mg/L)</td><td>n.d.*</td><td>1419</td><td>1485</td><td>495</td><td>759</td><td>297</td><td>495</td><td>165</td><td>363</td><td>362</td></tr>
<tr><td>Soluble PO₄</td><td>n.d.*</td><td>n.d.*</td><td>47</td><td>low</td><td>n.d.*</td><td>n.d.*</td><td>10</td><td>low</td><td>n.d.*</td><td>n.d.*</td></tr>
<tr><td colspan="11">DON, insoluble N, NH₄-N, TDS and TSS were also measured for 24 h at 163ºC (taking values of 510 mg/L, 3.22%, 259 mg/L, 5069 mg/L and 3336 mg/L, respectively) and at 216ºC (with values of 366 mg/L, 1.99%, 389 mg/L, 4174 mg/L and 2495 mg/L, respectively).</td></tr>
<tr><td>Brooks (1970)</td><td>Primary/secondary sludge with 3% TS and 4.4 wt% N.</td><td>Operation from 151 to 192ºC for 1 h.</td><td colspan="11">NH₄-N increases with temperature , from 45 to 185 mg/L. DON goes through a maximum of 750 mg/L at 177ºC, taking the values of 450 and 680 mg/L at 151 and 192ºC, respectively.</td></tr>
<tr><td rowspan="6">Wang et al. (1988)</td><td rowspan="6">A sludge with 46.98 g/L TCOD, 1.37 g/L SCOD, 17.39 g/L TProt (almost nothing soluble) and 411 mg/L VFA.</td><td rowspan="6">A reactor of 16 mL internal volume at 100 – 200ºC for 15 – 90 min.</td><td colspan="11">SCOD increases with both time and temperature. For 100ºC, it was 10.0 mg/L at 15 min and become 11.2 mg/L at 90 min. For 200ºC, it varies between 24.4 mg/L at 15 min and 27.6 mg/L at 90 min.
TProt decreases with both time and temperature. For 100ºC there are 12.17 mg/L at 15 min and 11.07 mg/L at 90 min. For 200ºC there are 4.12 mg/L at 15 min and 3.22 mg/L at 90 min. Data for sProt are scattered (they vary between 0.04 and 0.98 mg/L) and the trend is not clear, although it seems that, at a given temperature, the concentration decrease with increasing time, but at a given time, it could go through a maximum with temperature.
VFA concentrations vary between 2306 and 3598 mg/L and they are considerably disperse as well. At 100 – 160ºC, they go through a maximum with time at 45 – 60 min, whereas at 180 – 200ºC, they seem to be time independent. At a given time, the effect of temperature is less clear. The amount of each of the acids was only measured at 30 min:</td></tr>
<tr><td></td><td>Feed</td><td>100ºC</td><td>120ºC</td><td>140ºC</td><td>160ºC</td><td>180ºC</td><td colspan="4">200ºC</td></tr>
<tr><td>Acetic acid (mg/L)</td><td>164</td><td>1080</td><td>1063</td><td>1063</td><td>1114</td><td>1564</td><td colspan="4">1616</td></tr>
<tr><td>Propionic acid (mg/L)</td><td>74</td><td>795</td><td>708</td><td>708</td><td>743</td><td>847</td><td colspan="4">778</td></tr>
<tr><td>Butyric acid (mg/L)</td><td>107</td><td>570</td><td>501</td><td>501</td><td>536</td><td>657</td><td colspan="4">674</td></tr>
<tr><td>Valeric acid (mg/L)</td><td>66</td><td>605</td><td>622</td><td>622</td><td>639</td><td>715</td><td colspan="4">743</td></tr>
</table>

*n.d. means not determined. All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th colspan="6">RESULTS AND/OR COMMENTS</th></tr>
<tr><td rowspan="13">Li and Noike (1992)</td><td rowspan="13">Activated sludge from the Minamigamo WWTP in Sendai, Japan.</td><td rowspan="13">A 500 mL reactor at 120, 150 and 175ºC for 30 min, and at 170ºC for 60 min (not clear if pre-heating time is included).</td><td>Parameter</td><td>Feed</td><td>TH 120ºC</td><td>TH 150ºC</td><td>TH 170ºC</td><td>TH 175ºC</td></tr>
<tr><td>pH</td><td>7.50</td><td>7.25</td><td>7.43</td><td>7.35</td><td>7.17</td></tr>
<tr><td>VSS (mg/L)</td><td>9580</td><td>4770</td><td>6100</td><td>4950</td><td>4760</td></tr>
<tr><td>VSS/TSS</td><td>0.820</td><td>0.763</td><td>0.774</td><td>0.763</td><td>0.765</td></tr>
<tr><td>TCOD (mg/L)</td><td>15090</td><td>13030</td><td>15040</td><td>15960</td><td>15930</td></tr>
<tr><td>SCOD (mg/L)</td><td>1192.11</td><td>5694.11</td><td>6166.4</td><td>7932.12</td><td>8793.36</td></tr>
<tr><td>TCarb (mg/L)</td><td>713</td><td>866</td><td>917</td><td>713</td><td>823</td></tr>
<tr><td>sCarb (mg/L)</td><td>42.78</td><td>361.99</td><td>443.83</td><td>332.26</td><td>418.08</td></tr>
<tr><td>TProt (mg/L)</td><td>5880</td><td>5930</td><td>5570</td><td>5570</td><td>5520</td></tr>
<tr><td>sProt (mg/L)</td><td>282.24</td><td>2039.92</td><td>2467.51</td><td>2467.51</td><td>2649.6</td></tr>
<tr><td>TLip (mg/L)</td><td>738</td><td>685</td><td>862</td><td>1080</td><td>1492</td></tr>
<tr><td>sLip (mg/L)</td><td>122.51</td><td>207.55</td><td>247.39</td><td>412.56</td><td>449.09</td></tr>
<tr><td>VFA (mg//L as COD)</td><td>166</td><td>1120</td><td>1230</td><td>2058</td><td>1912</td></tr>
<tr><td>Shier and Purwono (1994)</td><td>A secondary sludge from the Metropolitan Waste Control Commission, Metro Plant, St Paul, MN, USA, concentrated by flotation to 4-5% solids.</td><td>Tubes of 40 mL were charged with 15 mL sludge and sealed under air or nitrogen.
Work at 120 – 295ºC for 30 min
At 120ºC, the effect of pH was studied.</td><td colspan="6">Oxygen has no effect in the results, so, it was a TH treatment and not a WO one.
Hydrolyzed material was subjected to centrifugation, and the supernatant, analyzed.
Gel filtration chromatography was used to determine the molecular weight distribution of the proteinaceous material and nucleic acids obtained at each temperature. The optimum temperature at natural pH (6.6-6.75) was at 150 – 155ºC.
At 120ºC, pH was increased to 12.5 with NaOH. The largest amounts of high molecular weight protein were obtained at the highest pH values examined.</td></tr>
<tr><td rowspan="8">Inoue et al. (1996)</td><td rowspan="8">Dewatered sewage sludge collected from sewage treatment plants in Ibaraki, Japan, with 83.8% moisture.</td><td rowspan="8">A 1 L autoclave at 175ºC for 1 h (excluding a pre-heating time of 30 min) was pressurized to 2 MPa using N_2 (Dote et al.,1993). The effluent was centrifuged, and the liquid and solid phases were also analyzed.</td><td>Parameter</td><td>Feed</td><td>TH</td><td colspan="2">Liquid phase TH</td><td>Solid phase TH</td></tr>
<tr><td>TS (wt %)</td><td>16.2</td><td>15.2</td><td colspan="2">7.5</td><td>23.0</td></tr>
<tr><td>TVS (wt %)</td><td>12.4</td><td>11.4</td><td colspan="2">6.9</td><td>18.0</td></tr>
<tr><td>TProt/ TVS</td><td>0.334</td><td>0.276</td><td colspan="2">0.472</td><td>0.272</td></tr>
<tr><td>TLip/ TVS</td><td>0.066</td><td>0.138</td><td colspan="2">0.155</td><td>0.119</td></tr>
<tr><td>TCarb/ TVS</td><td>0.033</td><td>0.040</td><td colspan="2">0.092</td><td>0.045</td></tr>
<tr><td>NH_4-N (mg/L)</td><td>2574</td><td>n.d.*</td><td colspan="2">3243</td><td>n.d.*</td></tr>
<tr><td>TOC (mg/L)</td><td>16707</td><td>n.d.*</td><td colspan="2">40396</td><td>n.d.*</td></tr>
</table>

*n.d. means not determined. All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th>RESULTS AND/OR COMMENTS</th></tr>
<tr><td>Inoue et al. (1997)</td><td>Dewatered sewage sludge with 84.5 wt% moisture and 15.5 wt% TS (TVS/TS= 0.743). Nitrogen content of 8.2 wt%,on dry basis -.</td><td>40 g of sludge in a 300 mL autoclave, pressurized with N_2 at 2 MPa.
100 – 350ºC for 1 h.
Only the distribution of N was analyzed.</td><td><table>
<tr><th>Parameter</th><th>100ºC</th><th>150ºC</th><th>200ºC</th><th>250ºC</th><th>300ºC</th><th>350ºC</th></tr>
<tr><td>% N in Oil</td><td>0.0</td><td>3.2</td><td>11.2</td><td>16.0</td><td>20.4</td><td>15.6</td></tr>
<tr><td>% Organic N in water</td><td>24.8</td><td>40.8</td><td>27.2</td><td>17.6</td><td>12.0</td><td>8.8</td></tr>
<tr><td>% NH_4-N in water</td><td>19.2</td><td>26.4</td><td>33.6</td><td>39.2</td><td>36.4</td><td>44.8</td></tr>
<tr><td>% N in remaining solids</td><td>49.2</td><td>17.6</td><td>19.6</td><td>12.0</td><td>12.8</td><td>4.6</td></tr>
<tr><td>% Loss of N</td><td>6.8</td><td>12.0</td><td>8.4</td><td>15.2</td><td>18.4</td><td>23.2</td></tr>
</table></td></tr>
<tr><td>Aravinthan et al. (1998)</td><td>Sludge with pH 7 – 7.5 from a sequencing batch fluidized bed bioreactor, containing protein (34%VSS) and carbohydrates (14% VSS). sProt and sCarb were negligible.</td><td>1 L sample at 121ºC for 4 h (including pre-heating time)
Comparison with addition of chemicals (NaOH or HCl) and combination of TH with chemicals.</td><td><table>
<tr><th rowspan="2">Parameter</th><th colspan="2">Chemical</th><th>TH</th><th colspan="2">Thermochemical</th></tr>
<tr><th>pH 11</th><th>pH 3</th><th>pH7</th><th>pH 11</th><th>pH 3</th></tr>
<tr><td>COD solubilization (%)</td><td>22</td><td>11</td><td>35</td><td>66</td><td>45</td></tr>
<tr><td>Carbohydrate solubilization (%)</td><td>29</td><td>74</td><td>44</td><td>52</td><td>80</td></tr>
<tr><td>Protein solubilization (%)</td><td>25</td><td>11</td><td>41</td><td>81</td><td>41</td></tr>
</table></td></tr>
<tr><td>Kim et al. (2003)</td><td>Activated sludge from a treatment facility in Chonan, Korea, with 38 g/L of TS, 26 g/L of TVS and pH 6.7.</td><td>Autoclave at 121°C and 0.152 MPa for 30 min (not clear if the pre-heating time is included).
Comparison with other pretreatments.</td><td><table>
<tr><th>Parameter</th><th>Feed</th><th>HT</th><th>Chemical (7 g/L NaOH)</th><th>Ultrasonic (42 kHz, 120min)</th><th>Thermal followed by chemical</th></tr>
<tr><td>TCOD (mg/L)</td><td>27700</td><td>27841</td><td>28161</td><td>26630</td><td>26347</td></tr>
<tr><td>SCOD (mg/L)</td><td>2250</td><td>4900</td><td>12250</td><td>4900</td><td>22500</td></tr>
<tr><td>sProt (mg/L)</td><td>30.2</td><td>85.7</td><td>1665.4</td><td>61.7</td><td>1983.0</td></tr>
</table></td></tr>
<tr><td>Kim et al. (2005)</td><td>Excess sludge of biological treatment process in a local fishery factory in Japan.</td><td>A 65.9 mL batch reactor at 300ºC and 9 MPa for 27 min, 350ºC and 17 MPa for 7 min, and 400ºC and 30 MPa for 7 min.
The pre-heating time was always lower than 7 min.</td><td><table>
<tr><th>Parameter</th><th>Feed</th><th>300ºC</th><th>350ºC</th><th>400ºC</th></tr>
<tr><td>Acetic acid (mg/L COD)</td><td>n.d.*</td><td>490</td><td>640</td><td>730</td></tr>
<tr><td>Propionic acid (mg/L COD)</td><td>n.d.*</td><td>620</td><td>110</td><td>140</td></tr>
<tr><td>Other VFA (mg/L COD)</td><td>n.d.*</td><td>240</td><td>180</td><td>30</td></tr>
<tr><td>TOC (mg/L)</td><td>10408</td><td>8135</td><td>4500</td><td>2654</td></tr>
<tr><td>DOC (mg/L)</td><td>173</td><td>5135</td><td>2885</td><td>2423</td></tr>
<tr><td>TCOD (mg/L)</td><td>22400</td><td>17600</td><td>11200</td><td>6700</td></tr>
<tr><td>BOD (mg/L)</td><td>1980</td><td>10640</td><td>6640</td><td>6640</td></tr>
</table></td></tr>
</table>

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS			
Graja et al. (2005)	Secondary sludge from a WWTP, previously thickened.	175ºC for 40 min (not clear if pre-heating time included).	Parameter	Feed	TH	
					70	
			TSS (g/L)	83	89	
			TCOD (g/L)	92	17	
			SCOD (g/L)	6.9	3.5	
			Total N (g/L)	3.7	1.6	
			Soluble N (g/L)	0.60	0.35	
			NH_4-N (g/L)	0.14	2.9	
			TP (g/L)	3.70	0.17	
			PO_4-P (g/L)	0.12	1.496	
			VFA (g/L)	n.d.*		
Bougrier et al. (2007)	Flotation-thickened secondary sludge from the municipal WWTP of Toulouse (France) with 17.7 g/L of TCOD, 5.6 g/L of SCOD, 14.9 g/L of TS and 11.7 g/L of TSS.	Samples of 0.5 L in a 1 L autoclave at 135ºC for 30 min and at 190ºC for 15 min (excluding pre-heating times, which were 35 min for reaching the lower temperature and 50 min for reaching the higher).	Parameter	Feed	135ºC	190ºC
			TCOD (g/L)	17.7	17.3	16.4
			SCOD (g/L)	5.6	5.9	7.6
			TS (g/L)	14.9	14	14.5
			TSS (g/L)	11.7	10.2	8.9
			TVS (g/L)	12.1	10.85	11.3
			VSS (g/L)	9.40	8.45	6.90
			VFA (g/L)	3.65	4.20	3.65
			TCarb (g/L)	2.12	1.37	1.45
			sCarb (g/L)	0.32	0.38	0.57
			TProt (g/L)	6.1	6.3	6.3
			sProt (g/L)	1.5	1.6	3.3

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS							
Bougrier et al. (2008)	Activated sludge with 33.7 g/L of TS, TVS/TS= 0.81 and 35.1 g/L of TCOD.	Samples of 0.7 L in an autoclave at 95 – 170ºC for 30 min (excluding pre-heating times, which vary between 25 and 60 min depending on the temperature).	Parameter	Feed	95ºC	130ºC	150ºC	160ºC	170ºC	
			TSS (% TS)	93	93	74	64	n.d.*	57	
			VSS (% TSS)	82	82	79	74	n.d.*	72	
			SCOD (g/L)	1.52	5.76	10.1	12.8	13.6	16.2	
			NH_4-N (g/L)	0.35	0.7	0.7	0.7	0.7	0.7	
			TProt (g/L)	12.2	10.7	11.1	12.0	13.3	13.5	
			sProt (g/L)	0.31	1.41	2.39	3.09	4.47	5.90	
			TCarb (g/L)	2.66	2.09	2.33	1.95	2.21	1.86	
			sCarb (g/L)	0.13	0.54	1.04	0.83	0.83	0.78	
			TLip (g/L)	1.50	1.79	1.87	2.49	3.00	2.57	
			sLip (g/L)	0.02	0.14	0.29	0.46	0.11	0.40	
			VFA (g/L)	0.23	1.10	2.01	n.d.*	1.97	2.40	
	Activated sludge with 15 g/L of TS, TVS/TS= 0.82, 10.5 g/L of TCOD, 4.3 g/L of TProt, 1.34 g/L of TCarb and 0.065 g/L of VFA.	The same, but at 95 – 210ºC for 30 min.	Parameter	Feed	110ºC	130ºC	150ºC	170ºC	190ºC	210ºC
			TSS (% TS)	97	79	72	63	59	46	45
			VSS (% TSS)	83	82	78	71	71	65	61
			SCOD (g/L)	0.04	1.79	2.68	4.97	6.66	8.64	8.62
			sProt (g/L)	0.30	0.86	0.82	1.06	1.78	2.76	3.24
			sCarb (g/L)	0.02	0.21	0.32	0.47	0.46	0.61	0.19
	Activated sludge with 14.8 g/L TS, TVS/TS= 0.76, 11.4 g/L of TCOD, 5.1 g/L of TProt, 1.45 g/L of TCarb and 0.016 g/L of VFA.	The same, but at 95 – 210ºC for 30 min.	Parameter	Feed	110ºC	130ºC	150ºC	170ºC	190ºC	210ºC
			TSS (% TS)	95	87	77	68	58	45	47
			VSS (% TSS)	78	73	72	68	66	68	56
			SCOD (g/L)	0.04	2.11	3.04	4.27	6.19	7.16	7.50
			sProt (g/L)	0.20	0.49	0.20	0.43	1.02	1.91	2.76
			sCarb (g/L)	0.02	0.34	0.38	0.52	0.65	0.49	0.30
Ramirez et al. (2009)	Activated sludge from a highly loaded WWTP in France with 64 g/L of TCOD and 5.6 g/L of SCOD.	110, 165 and 220ºC for 30 min (excluding the pre-heating time) in a 10 L agitated autoclave.	Parameter	110ºC	165ºC	220ºC				
			SCOD (g/L)	7.8	16.0	21.3				
			TCOD (g/L)	64.7	61.5	60.0				
			Carbohydrate solubilization (%)	2.60	15.0	1.20				
			Protein solubilization (%)	8.93	34.6	40.2				
			Lipid solubilization	≈0.0	≈0.0	≈0.0				

*n.d. means not determined. All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th colspan="7">RESULTS AND/OR COMMENTS</th></tr>
<tr><td rowspan="10">Wang et al. (2009)</td><td rowspan="10">Raw sludge was collected from the gravity thickener of a municipal WWTP located in Beijing, China.</td><td rowspan="10">9 L of hydrolyzed sludge in a 10 L autoclave at 170ºC for 30 min
(nothing is said about the pre-heating time).</td><td>Parameter</td><td>Feed</td><td colspan="5">TH</td></tr>
<tr><td>pH</td><td>6.62</td><td colspan="5">6.07</td></tr>
<tr><td>TS (g/L)</td><td>43.42</td><td colspan="5">40.42</td></tr>
<tr><td>VS (g/L)</td><td>27.34</td><td colspan="5">24.20</td></tr>
<tr><td>SS (g/L)</td><td>42.02</td><td colspan="5">34.36</td></tr>
<tr><td>VSS (g/L)</td><td>23.25</td><td colspan="5">19.17</td></tr>
<tr><td>TCOD (g/L)</td><td>56.41</td><td colspan="5">54.20</td></tr>
<tr><td>SCOD (g/L)</td><td>1.82</td><td colspan="5">13.77</td></tr>
<tr><td>TKN (mg/L)</td><td>1513</td><td colspan="5">1445</td></tr>
<tr><td>VFA (mg/L as COD)</td><td>376</td><td colspan="5">2581</td></tr>
<tr><td rowspan="12">Wilson and Novak (2009)</td><td rowspan="6">Primary sludge from the District of Columbia Water and Sewer Authority Blue Plains Advanced WWTP, thickened to around 6% of TS, 73200 mg/L of TCOD and 13400 mg/L of TProt.</td><td rowspan="6">A Parr 4745 reactor at 130ºC (0.28 MPa), 150ºC (0.51 MPa), 190ºC (0.89 MPa), 190 ºC (1.46 MPa) or 220ºC (2.87 MPa) for 2 h (including initial heating time).</td><td>Parameter</td><td>Feed</td><td>130ºC</td><td>150ºC</td><td>170ºC</td><td>190ºC</td><td>220ºC</td></tr>
<tr><td>NH₄-N (mg/L)</td><td>129</td><td>150</td><td>150</td><td>193</td><td>286</td><td>371</td></tr>
<tr><td>SCOD (mg/L)</td><td>6257</td><td>9043</td><td>11214</td><td>12929</td><td>14357</td><td>16857</td></tr>
<tr><td>sProt (mg/L)</td><td>32</td><td>496</td><td>2239</td><td>2347</td><td>2722</td><td>2717</td></tr>
<tr><td>sCarb (mg/L)</td><td>110</td><td>386</td><td>917</td><td>966</td><td>1043</td><td>1287</td></tr>
<tr><td>VFA (mg/L as acetic)</td><td>4720</td><td>4782</td><td>5195</td><td>8023</td><td>8506</td><td>9793</td></tr>
<tr><td rowspan="6">Secondary sludge
65700 mg/L of TCOD and 31200 mg/L of TProt.</td><td rowspan="6">The same.</td><td>Parameter</td><td>Feed</td><td>130ºC</td><td>150ºC</td><td>170ºC</td><td>190ºC</td><td>220ºC</td></tr>
<tr><td>NH₄-N (mg/L)</td><td>100</td><td>168</td><td>236</td><td>264</td><td>668</td><td>975</td></tr>
<tr><td>SCOD (mg/L)</td><td>820</td><td>6714</td><td>10314</td><td>11429</td><td>14107</td><td>16714</td></tr>
<tr><td>sProt (mg/L)</td><td>400</td><td>436</td><td>2315</td><td>2827</td><td>2870</td><td>3178</td></tr>
<tr><td>sCarb (mg/L)</td><td>58</td><td>477</td><td>987</td><td>1124</td><td>1226</td><td>1293</td></tr>
<tr><td>VFA (mg/L as acetic)</td><td>680</td><td>897</td><td>1287</td><td>1701</td><td>1931</td><td>2115</td></tr>
<tr><td>Arakane et al. (2011)</td><td>Excess sludge from a laboratory-scale apparatus with 26000 mg/L of SS.</td><td>A 10 L lab-scale apparatus at 100 – 350ºC for 60 min.</td><td colspan="7">Solubilization of solids increases from 15% at 100ºC to 80% at 225ºC, and then remained unchanged up to 350ºC.
The oxidized stream is filtrated, and the pH of the filtrate is adjusted to 9 with Mg(OH)₂. The phosphorus from the solubilized solids was recovered by precipitation as magnesium ammonium phosphate.
The phosphate recovery is around 25% at 100ºC, 75% at 350ºC and goes through a maximum of 95% between 180 and 240ºC.</td></tr>
</table>

All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS							
			Parameter	Feed	0 min	5 min	10 min	15 min	25 min	30 min
Donoso-Bravo et al. (2011)	A digested mixture of primary and secondary sludge from the WWTP of Valladolid (Spain), with 99.8 g/L of TS, 75.7 g/L of VS, 108.022 g/L of TCOD, 13191of mg/L TCarb and 41950 of mg/L TProt	A lab-scale apparatus of 0.5 L at 170ºC and 8 bar for 0 – 30 min (without taking into account the pre-heating time).	sCarb (mg/L SCOD)	873	9375	8871	11429	10572	7243	8766
			sProt (mg/L SCOD)	3710	31093	34585	39891	45774	43669	44013
			NH_4-N (mg/L)	263	419	428	521	530	568	557
			Acetic (mg/L SCOD)	410	361	504	548	540	530	500
			Propionic (mg/L SCOD)	846	887	833	976	850	843	726
			Isovaleric (mg/L SCOD)	266	341	403	458	456	726	372
			SCOD (mg/L)	7376	37715	46270	54006	56385	55121	55121
			Parameter	Feed	0 min	5 min	10 min	15 min	25 min	30 min
	A digested mixture of primary and secondary sludge from the WWTP of Valladolid (Spain), with 76.8 g/L of TS, 54.0 g/L of VS, 90.168 g/L of TCOD, 10868 mg/L of TCarb and 37807 mg/L of TProt	A pilot-scale apparatus of 20 L at 170ºC and 8 bar for 0 – 30 min.	sCarb (mg/L SCOD)	749	8986	9026	8145	10071	7987	9843
			sProt (mg/L SCOD)	2551	18778	18344	21910	22915	25726	24828
			NH_4-N (mg/L)	134	169	145	110	127	147	136
			Acetic (mg/L SCOD)	117	93	133	178	235	316	383
			Propionic (mg/L SCOD)	281	156	158	154	165	156	162
			Isovaleric (mg/L SCOD)	90	132	152	157	142	115	95
			SCOD (mg/L)	4829	30430	29682	35083	34801	36923	40149

REF.	FEED	EXPERIMENTAL		Fredericia		Naestved	
			Parameter	Feed	TH	Feed	TH
Morgan-Sagastume et al. (2011)	Dewatered sludge from WWTP of Fredericia and Naestved (Denmark).	CAMBI^{TM} process: Steam is fed at 12 bar and the sludge is heated to 160ºC and 6 bar for an unspecified time.	pH	7.0	5.9	6.4	6.5
			TCOD (g/L)	130	89	134	84
			SCOD (g/L)	3.6	33	5.3	34
			VFA (g/L COD)	0.1	2.8	5.7	7.4
			TOC (g/L)	24	23	36	24
			DOC (g/L)	1.3	12	1.8	12
			TS (g/L)	130	83	144	78
			VS (g/L)	93	59	92	49
			TSS (g/L)	124	60	n.d.*	n.d.*
			VSS (g/L)	89	40	n.d.*	n.d.*
			Total N (g/L)	7.2	5.3	7.0	4.3
			NH_4-N (g/L)	0.3	0.8	0.69	0.9
			TP (g/L)	3.7	2.5	4.5	2.6
			PO_4-P (g/L)	1.8	2.1	3.7	2.4

*n.d. means not determined. All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th>RESULTS AND/OR COMMENTS</th></tr>
<tr><td>Braguglia et al. (2015)</td><td>Two activated sludge from the WWTP "Roma-Nord". One was thickened gravitationally (resulting in around 20000 mg/Lof TCOD) and the other was thickened dynamically (resulting in 40353 mg/L of TCOD).</td><td>400 mL samples in a 25 L autoclave at 135ºC and 0.32 MPa for 5 – 20 min (none is said about the pre-heating time).</td><td>
<table>
<tr><th></th><th></th><th>Gravity thickening</th><th></th><th></th><th></th><th>Dynamic thickening</th><th></th></tr>
<tr><th>Parameter</th><th>Feed</th><th>5 min</th><th>10 min</th><th>20 min</th><th>Feed</th><th>10 min</th><th>20 min</th></tr>
<tr><td>TS (g/L)</td><td>19.3</td><td>19.1</td><td>19.2</td><td>20..3</td><td>37.2</td><td>37.1</td><td>39.4</td></tr>
<tr><td>VS/TS (%)</td><td>72</td><td>75</td><td>75</td><td>74</td><td>75</td><td>75</td><td>75</td></tr>
<tr><td>SCOD (mg/L)</td><td>45</td><td>3860</td><td>3990</td><td>4340</td><td>90</td><td>5310</td><td>5500</td></tr>
<tr><td>SCOD + colCOD (mg/L)</td><td>88</td><td>4320</td><td>4050</td><td>4460</td><td>120</td><td>6250</td><td>9780</td></tr>
<tr><td>sProt + colProt (mg/L)</td><td>30</td><td>1687</td><td>1782</td><td>1822</td><td>59</td><td>2942</td><td>3878</td></tr>
<tr><td>sCarb + colCarb (mg/L)</td><td>2.7</td><td>314</td><td>356</td><td>418</td><td>17.2</td><td>632</td><td>847</td></tr>
</table>
</td></tr>
<tr><td>Xue et al. (2015b)</td><td>Dewatered activated sludge from a WWTP in Shanghai, China. 16.7% of TS, TVS/TS= 0.705, 166 g/L of TCOD, 7.55g/L of SCOD, 1.45 g/L of VFA and 1.10 g/L of NH_4-N, 1427.4 mg/L of sProt and 218.15 of mg/L sCarb.</td><td>70 g of sludge in 100 mL Teflon jars at 120 – 180ºC for 15 – 180 min (including the pre-heating time, which varies from 95 min for 120ºC to 115 min for 180ºC).</td><td>Between 15 and 180 min, SCOD increases from 31507 to 50962 mg/L at 120ºC, from 33904 to 57602 mg/L at 140ºC, from 41096 to 70550 mg/L at 160ºC and from 47260 to 88644 mg/L at 180ºC; sCarb increases from 909 to 2448 mg/L at 120ºC, from 910 to 3357 mg/L at 140ºC, from 1399 to 4336 mg/L at 160ºC and from 1678 to 4685 mg/L at 180ºC;
Time and temperature dependence of sProt and NH_4-N are plotted in Figure 2.
VFA were only determined in the feed and in the effluents at 180 min.
<table>
<tr><th></th><th>Feed</th><th>120ºC</th><th>140ºC</th><th>160ºC</th><th>180ºC</th></tr>
<tr><td>Acetic acid (mg/L)</td><td>710</td><td>671</td><td>735</td><td>755</td><td>1284</td></tr>
<tr><td>Propionic acid (mg/L)</td><td>323</td><td>226</td><td>277</td><td>245</td><td>200</td></tr>
<tr><td>Isobutyric acid (mg/L)</td><td>103</td><td>84</td><td>97</td><td>90</td><td>52</td></tr>
<tr><td>Butyric acid (mg/L)</td><td>145</td><td>90</td><td>103</td><td>90</td><td>194</td></tr>
<tr><td>Isovaleric acid (mg/L)</td><td>129</td><td>116</td><td>152</td><td>142</td><td>116</td></tr>
<tr><td>Valeric acid (mg/L)</td><td>40</td><td>23</td><td>38</td><td>71</td><td>90</td></tr>
</table>
</td></tr>
</table>

All references are cited in the main text.

2. Compounds generated during wet oxidation of sewage sludge. Conditions and relevant results published.

Table S2. Components generated during wet oxidation of sewage sludge. Conditions and relevant results published.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS										
Hurwitz and Dundas (1960)	Five sewage sludges from the Metropolitan Sanitary District of Greater Chicago (USA).	WO in a pilot plant of 0.61 m diameter and 9.14 m of high, with three successive heat exchangers for pre-heating. 829 L/h of sludge 1 with 0.22 kg/L air at 8.27 MPa and 254ºC; 923 L/h of sludge 2 with 0.28 kg/L air at 8.33 MPa and 264ºC; 836 L/h of sludge 3 with 0.33 kg/L air at 8.33 MPa and 266ºC; 537 L/h of sludge 4 with 0.19 kg/L air at 10.5 MPa and 273ºC; 488 L/h of sludge 5 with 0.21 kg/L air at 12.6 MPa and 277ºC	Parameter	Sludge 1		Sludge 2		Sludge 3		Sludge 4		Sludge 5	
				Feed	WO	Feed	WO	Feed	WO	Feed	WO	Feed	WO
			Flow (L/h)	829	n.d.*	923	n.d.*	836	n.d.*	537	n.d.*	488	n.d.*
			Air (kg/L)	n.d.*	0.22	n.d.*	0.28	n.d.*	0.33	n.d.*	0.19	n.d.*	0.21
			Pressure (MPa)	n.d.*	8.27	n.d.*	8.33	n.d.*	8.33	n.d.*	10.5	n.d.*	12.6
			Temperature (ºC)	n.d.*	254	n.d.*	264	n.d.*	266	n.d.*	273	n.d.*	277
			TS (%)	4.09	1.46	5.21	1.59	6.42	2.05	3.29	1.14	3.48	0.92
			VS (%)	2.61	0.299	3.43	0.356	4.33	0.436	2.16	0.24	2.31	0.17
			TCOD (g/L)	50.8	10.2	63.4	13.2	75.4	16.6	44.5	9.30	49.8	8.1
			NH_4-N (mg/L)	n.d.*	1370	n.d.*	1625	n.d.*	1640	n.d.*	1410	n.d.*	1625
			Organic N (mg/L)	n.d.*	368	n.d.*	425	n.d.*	548	n.d.*	n.d.*	n.d.*	n.d.*
			VFA (mg/L as acetic)	n.d.*	3200	n.d.*	3480	n.d.*	3980	n.d.*	3930	n.d.*	3830
			BOD (mg/L)	n.d*	5420	n.d.*	7030	n.d.*	8460	n.d.*	n.d.*	n.d.*	n.d.*
Teletzke et al. (1967)	Raw primary sludge from the treatment plant of Wasau, in Wisconsin (USA),	WO with air at 150 – 250ºC in a 3.785 L autoclave for unknown time.	Parameter	Feed	150ºC	175ºC	200ºC	225ºC	250ºC				
			TCOD (g/L)	57.7	57.9	51.9	34.6	16.5	12.9				
			SCOD (g/L)	2.13	13.5	23.8	20.5	14.4	12.3				
			TProt as TON×6.25 (g/L)	6.2	6.1	3.9	2.5	1.2	Traces				
			sProt as DON×6.25 (g/L)	0.18	4.28	3.55	2.09	1.2	Traces				
			TLip (g/L)	10.5	10.7	8.9	3.6	0.6	0.5				
			sLip (g/L)	0.24	1.28	3.16	2.86	0.48	0.5				
			Total sugars (g/L)	0.0	0.6	0.9	0.0	0.0	0.0				
			Soluble sugars (g/L)	0.0	0.6	0.9	0.0	0.0	0.0				
			Total starch (g/L)	3.6	3.0	1.2	0.6	0.1	0.0				
			Soluble starch (g/L)	n.d.*	1.12	0.38	0.18	0.0	0.0				
			Total crude fiber (g/L)	13.5	13.5	8.9	5.8	0.9	0.0				
			Soluble fiber (g/L)	0.0	0.0	0.0	0.0	0.0	0.0				
			Total VFA as acetic acid (g/L)	1.3	3.0	4.9	6.5	8.1	7.7				
			Soluble VFA (g/L)	1.12	2.6	4.5	6.0	6.8	7.0				
			TVS (g/L)	35.5	42.0	31.4	19.0	5.1	3.1				

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS					
			Parameter	Feed	175ºC	200ºC	225ºC	250ºC
Teletzke et al. (1967)	Raw primary sludge from the treatment plant of Wasau, in Wisconsin (USA),, but with another composition	WO with air at 150 – 250ºC in a 3.785 L autoclave for unknown time.	TCOD (g/L)	61.2	52.7	39.4	20.7	11.4
			TON (g/L)	1.15	1.15	1.14	1.07	1.09
			DON (g/L)	0.28	1.04	1.00	0.98	1.02
			Total NH_4-N (g/L)	0.30	0.62	0.83	0.88	1.05
			Soluble NH_4-N (g/L)	0.24	0.59	0.77	0.84	0.99
			Total Free aminoacid N (g/L)	0.042	0.045	0.021	0.022	0.019
			Free soluble aminoacid N (g/L)	0.008	0.043	0.027	0.025	0.016
			Total aminoacid N (g/L)	0.517	0.233	0.070	0.020	0.005
			Total soluble aminoacid N (g/L)	n.d.*	0.211	0.081	0.035	0.028
			% total aspartic acid	6.82	4.30	2.07	2.28	0.00
			% total glutamic acid	17.46	24.50	31.74	19.42	0.00
			% total serine	6.11	4.59	Traces	0.00	0.00
			% total glycine	7.82	10.46	12.99	13.07	15.24
			% total threonine	6.17	4.15	2.56	0.00	0.00
			% total alanine	19.35	19.83	18.95	8.63	0.00
			% total β-alanine	0.00	2.94	11.44	38.07	50.00
			% total arginine	6.32	0.00	0.00	0.00	0.00
			% total valine	5.95	5.65	4.79	2.28	0.00
			% total methionine	10.35	6.40	0.00	0.00	0.00
			% total isoleucine	3.60	5.51	3.90	0.00	0.00
			% total leucine	6.38	5.05	2.88	0.00	0.00
			% total proline	2.85	3.87	Traces	0.00	0.00
			% total γ-amino butyric acid	0.00	1.40	6.94	13.71	34.76
			VFA (g/L)	1.6	4.9	6.4	7.5	7.0
			Lipids (g/L)	10.05	6.67	2.26	0.59	0.25
			Analysis of lipids fraction:					
			% C	76.9	70.4	65.0	59.3	65.2
			% H	11.-6	10.0	8.8	6.8	8.7
			% N	0.4	1.0	1.9	1.7	5.0
			% P	0.3	0.1	0.1	0.4	0.3
			% O	9.8	16.4	24.2	31.8	12.8
			% total non-volatile fatty acids	52.0	50.0	23.8	28.8	12.0
			% free non-volatile fatty acids	31.6	48.6	19.0	28.8	12.0

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS				
			Parameter	Speedway		Terre Haute	
				Feed	WO	Feed	WO
Sommers and Curtiss (1977)	Anaerobically digested sludge from the Terre Haute plant and a mixture of primary and secondary sludge from Speedway, Ind.	Full-scale operation at unknown conditions.	TSS (% TS)	5.6	23.0	3.5	21.3
			TVS (% TS)	47.1	36.7	57.2	36.3
			TP (% TS)	1.156	1.223	1.554	2.325
			Total soluble P (% TS)	0.082	0.004	0.153	0.010
			Inorganic soluble P (mg/L)	22.78	14.98	40.00	29.85
			Organic soluble P (mg/L)	21.64	<0.01	15.48	8.83
			Total N (% TS)	3.476	1.327	4.193	1.775
			Total soluble N (% TS)	1.356	0.471	1.354	0.427
			Total NH_4-N (% TS)	1.342	0.308	1.570	0.309
			Soluble NH_4-N (% TS)	1.059	0.296	1.221	0.255
			TON (% TS)	2.130	1.036	2.621	1.499
			DON (% TS)	0.293	0.173	0.131	0.170
			Total Cu (mg/kg TS)	1090	1011	649	852
			Soluble Cu (mg/L)	0.37	0.67	0.12	0.25
			Total Zn (mg/kg TS)	1996	1974	1814	2497
			Soluble Zn (mg/L)	0.34	0.54	0.06	0.24
			Total Ni (mg/kg TS)	70	70	911	1064
			Soluble Ni (mg/L)	0.19	0.32	2.41	5.11
			Total Cd (mg/kg TS)	11.4	11.3	58.4	77.2
			Soluble Cd (mg/L)	≈0.0	≈0.0	≈0.0	≈0.0
			Total Pb (mg/kg TS)	451	471	686	978
			Soluble Pb (mg/L)	≈0.0	≈0.0	≈0.0	≈0.0

All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th>RESULTS AND/OR COMMENTS</th></tr>
<tr><td rowspan="2">Wu et al. (1987)</td><td>Anaerobically digested sludge,
2.9% of TS.</td><td>A 3.8 L autoclave
Ratio of O_2 to TCOD of 1.2
100 – 275ºC for 1 h
500 rpm.</td><td>
<table>
<tr><th>Parameter</th><th>Feed</th><th>100 ºC</th><th>125ºC</th><th>150ºC</th><th>175ºC</th><th>200ºC</th><th>225ºC</th><th>250ºC</th><th>275ºC</th></tr>
<tr><td>Acetic acid (g/L)</td><td>0.10</td><td>0.76</td><td>0.74</td><td>0.85</td><td>1.18</td><td>1.70</td><td>2.15</td><td>2.13</td><td>1.95</td></tr>
<tr><td>Propionic acid (mg/L)</td><td>20.0</td><td>123.0</td><td>120.0</td><td>121.0</td><td>n.d.*</td><td>117.0</td><td>99.0</td><td>73.5</td><td>60.8</td></tr>
<tr><td>Butyric acid (mg/L)</td><td>2.0</td><td>n.d.*</td><td>n.d.*</td><td>30.0</td><td>n.d.*</td><td>25.7</td><td>20.7</td><td>10.0</td><td>8.0</td></tr>
</table>
</td></tr>
<tr><td>Anaerobically digested sludge, 1.1 - 10% of TS.</td><td>Ratio of O_2 to TCOD of 1.2
275ºC for 1h
500 rpm.</td><td>
<table>
<tr><th>Parameter</th><th>Feed</th><th>1.1% TS</th><th>2.5% TS</th><th>5% TS</th><th>6.4% TS</th><th>7.5% TS</th><th>10% TS</th></tr>
<tr><td>Acetic acid (g/L)</td><td>n.d.*</td><td>0.97</td><td>n.d.*</td><td>2.77</td><td>2.94</td><td>3.22</td><td>3.80</td></tr>
<tr><td>Propionic acid (mg/L)</td><td>n.d.*</td><td>22.8</td><td>57.7</td><td>111.0</td><td>162.0</td><td>119.0</td><td>96.9</td></tr>
<tr><td>Butyric acid (mg/L)</td><td>n.d.*</td><td>0.00</td><td>8.05</td><td>22.8</td><td>46.3</td><td>44.3</td><td>20.1</td></tr>
</table>
</td></tr>
<tr><td rowspan="2">Wu et al. (1987)</td><td>Anaerobically digested sludge, 2.1 – 3.2% of TS.</td><td>Ratio of O_2 to TCOD of 1.2
150 – 235ºC for 1 h
500 rpm.</td><td>
<table>
<tr><th>Parameter</th><th>feed</th><th>150ºC</th><th>195ºC</th><th>235ºC</th></tr>
<tr><td>Organic N (mg/g TVS_{feed})</td><td>n.d.*</td><td>7.59</td><td>2.17</td><td>38.0</td></tr>
<tr><td>NO_2-N (mg/g TVS_{feed})</td><td>n.d.*</td><td>4.88</td><td>10.3</td><td>5.42</td></tr>
<tr><td>NO_3-N (mg/g TVS_{feed})</td><td>n.d.*</td><td>64.5</td><td>51.5</td><td>5.97</td></tr>
<tr><td>NH_3-N (mg/g TVS_{feed})</td><td>n.d.*</td><td>72.1</td><td>99.8</td><td>132</td></tr>
</table>
</td></tr>
<tr><td>Anaerobically digested sludge, 9% of TS and 18 mg/L of PO_4-P.</td><td>Ratio of O_2 to TCOD of 1.2
100 – 300ºC for 1 h
500 rpm.</td><td>Reduction from 39 mg/L at 100ºC to 2 mg/L PO_4-P at 300ºC, which may results from precipitation with calcium and magnesium during WO treatment at high temperatures</td></tr>
<tr><td>Friedman et al (1988)</td><td>Anaerobically digested sludge of the Metropolitan WWTP, Syracuse, New York, with around 22030 mg/L of TS, 12540 mg/L of VSS, 23940 mg/L of TCOD and 390 mg/L of SCOD.</td><td>A 3.78 L batch reactor, charged with 2.5 L of sludge. The headspace was filled with air at 2.07 MPa and at room temperature before heating. WO between 160 and 320ºC for 1 h.</td><td>From 160 to 250ºC, acetic acid concentration increases from 310 to 360 mg/L and VSS destruction from 58 to 64%, whereas TCOD destruction decreases from 27 to 20%. At 270ºC, acetic acid concentration is 755 mg/L, VSS destruction is 80% and TCOD destruction is 42%. Beyond this temperature, the VSS destruction slightly decreases, the TCOD destruction goes through a tiny minimum and the acetic acid concentration becomes higher (1180 mg/L at 320ºC).
An undefined parameter, called SCOD generation, varies between 24 and 30%.
Formic acid is detected between 250 and 290ºC (maximum value of 190 mg/L at 270ºC) and propionic acid between 270 and 320º (maximum value of 90 mg/L at 290ºC).
The oxidized stream at 230ºC was subjected to settling for several hours, giving a solid that occupied 8-12% of the original sludge and contained 36 to 96% of the heavy metals presented in the untreated feed.</td></tr>
</table>

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Shanableh and Gloyna (1991)	Excess activated sludge derived from an industrial wastewater treatment plant, 5% of TS, 46.5 g/L of TCOD 280 – 590 mg/L of NH_4-N and 2010 – 2690 of mg/L acetic acid.	WO at 300 – 450ºC for 1-30 min. The 20 mL capacity reaction vessel contained 5 mL of sludge and the initial oxygen pressure was 8.2 MPa.	The higher the residence time, the higher the TCOD removal. At a constant time, the higher the temperature, the higher the removal, e.g., at 20 min, removals increased from about 84% at 300 °C, to more than 99.8% at 425ºC. At 300ºC, NH_4-N increases as the reaction progresses, reaching a plateau of 1.7 times the feed value beyond 6 min. At 343ºC, the increase is lower and the plateau is not permanent, since the concentration start to decrease after 20 min. The higher the temperature, the lower the increase and the lower the duration of the plateau. For 300 and 343ºC, acetic acid concentration goes through a maximum with time at 6 min, whereas for higher temperatures, the concentrations decreased monotonically with the duration of the oxidation, as can be seen in Figure 3a.
Luck et al. (1998) Djafer et al. (2000)	Mixture of raw primary and excess activated sludge, with 4 wt% of TS, TVS/TS=0.68, 68 g/L of COD, 0.62 g/L of NH_4-N, 1.90 g/L of TKN and 0 g/Lof NO_3-N.	Bench-scale and - pilot plant experiments. The bench scale works at 1500 rpm, 235ºC and 6 MPa for 1 hour, with pure oxygen in excess of 50% with respect to TCOD. The operating conditions in the pilot plant were not clearly specified.	In the lab tests, the final COD and TVS/TS were 16 g/L and 0.15, respectively. The contribution of the VFA to this COD was of 8.82 mg/L (5.6 mg/L acetic acid, 1.0 mg/L propionic acid, 0.25 mg/L butyric acid, 0.75 mg/L isobutyric acid, 0.087 mg/L valeric acid, 0.2 mg/L isovaleric acid, 0.1 mg/L methyl-4-valeric acid, 0.2 mg/L hexanoic acid and 0.1 mg/L heptanoic acid). Concentrations of NH_4-N, TKN and NO_3-N were - 1.41, 1.49 and 0.03 g/L, respectively. Experiments at other temperatures and pressures, both with and without copper catalyst, were performed, but it is only said that, even at 285º C in the presence of copper, acetic acid remained refractory to oxidation, limiting the COD removal to about 85%. On the other hand, catalyst enhances ammonia oxidation to molecular nitrogen. The residual solid from wet oxidation of sludge (from both the lab and the pilot plant) were subjected to Fourier infrared spectroscopy, granulometric analyses, weight loss method at 500ºC, differential thermal analyses and leaching of the heavy metals. Except for mercury, which is partially solubilised in the water phase, all the initial metal concentrations are completed transferred to the solid by-product, which consists of a fine powder mainly comprising aluminium phosphates, kaolin, quartz, calcite and an amorphous fraction which immobilizes heavy metals in the form of hydroxides, carbonates and insoluble metal phosphates.
Khan et al. (1999)	Gravity-thickened activated sludge, collected from Newton Aycliffe WWTP. TVS varied from 1206 to 38600 mg/L.	WO between 200 and 300ºC for 60 min in a 3.78 L autoclave with a partial pressure of 2.5 MPa O_2.	The TVS was correlated with the SCOD, NH_4-N and acetic acid concentrations after WO at the four tested temperatures: $TVS=0.7(SCOD)^{0.91}(NH_4\text{-}N)^{0.54}(acetic)^{-0.22}$ at 200ºC $TVS=46.7(SCOD)^{2.62}(NH_4\text{-}N)^{-2.6}(acetic)^{-0.03}$ at 260ºC $TVS=6.6(SCOD)^{0.54}(NH_4\text{-}N)^{0.07}(acetic)^{0.37}$ at 280ºC $TVS=0.8(SCOD)^{0.82}(NH_4\text{-}N)^{-0.73}(acetic)^{1.02}$ at 300ºC The level of acetic acid production was found to be temperature dependent, being about 20% of the VFAs at 200ºC compared to 90% at 300ºC. Some formaldehyde is formed. VFA and formaldehyde represent 85 – 99% of the SCOD.

All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th>RESULTS AND/OR COMMENTS</th></tr>
<tr><td>Shanableh (2000)
Shanableh and Shimizu (2000)</td><td>Thickened secondary activated sludge with 3% of TS, 1.8% of TVS, 30-300-31-900 mg/L of TCOD, 1250-1950 mg/L of SCOD, 510 – 650 mg/L and almost null acetic acid content.</td><td>Continuous flow reactor with a reaction zone of 1.5 L, at 280 – 455ºC and sludge flows of 50 and 100 g/min Reactor pressure at 28 MPa, with O_2 injected.</td><td>At 50 g/min (30 min residence time) and between 280 and 455ºC, TCOD decreases from 9028 to 1085 mg/L, SCOD from 7000 to 1048 mg/L, acetic acid from 3000 to 652 mg/L and propionic acid from 205 to 14 mg/L, whereas NH_4-N increases from 335 to 500 mg/L.
At 100 g/min (15 min residence time) and between 300 and 455ºC, TCOD decreases from 10000 to 3210 mg/L, SCOD from 8070 to 2940 mg/L, acetic acid from 2705 to 1650 mg/L and propionic acid from 210 to 60 mg/L, whereas NH_4-N increases from 320 to 465 mg/L.</td></tr>
<tr><td>Shanableh (2000)</td><td>Thickened secondary activated sludge with 3% of TS, 1.8% of TVS, 30300-31900 mg/L of TCOD, 1250-1950 mg/L of SCOD, 510 – 650 mg/L and almost null acetic acid content.</td><td>A batch reactor of 20 mL
O_2 was injected
250 – 460ºC
0.5 – 60 min.</td><td>Instead of plotting TCOD, SCOD and acetic acid as a function of reaction time for several temperatures, it was represented SCOD and acetic acid vs. TCOD removal (an abscise which roughly comprises both the time and the temperature).
The production of acetic acid reaches a maximum of approximately 3000 mg/L between 30 and 75% TCOD removal. SCOD also goes through a maximum (9000 – 10000 mg/L), but at 30% TCOD removal.</td></tr>
<tr><td>Lendormi et al. (2001)</td><td>Sludge A (45 g/L of TCOD, from extended aeration + dissolved air flotation),
sludge B (60 g/L of TCOD from biological treatment + anaerobic digestion)
sludge C (28 g/L of TCOD from physicochemical treatment + static thickening).</td><td>A batch reactor of 1.3 L.
No data about the oxidant.
220, 260 and 300ºC
30, 60 and 75 min for sludge A, 60 min for sludge B and C.</td><td>
<table>
<tr><th>Parameter</th><th>T(ºC)</th><th colspan="3">Sludge A</th><th>Sludge B</th><th>Sludge C</th></tr>
<tr><th></th><th></th><th>30 min</th><th>60 min</th><th>75 min</th><th>60 min</th><th>60 min</th></tr>
<tr><td>TCOD reduction (%)</td><td>220</td><td>n.d.*</td><td>60</td><td>67</td><td>72</td><td>30</td></tr>
<tr><td></td><td>260</td><td>n.d.*</td><td>80</td><td>n.d</td><td>76</td><td>62</td></tr>
<tr><td></td><td>300</td><td>82</td><td>88</td><td>n.d.*</td><td>85</td><td>83</td></tr>
<tr><td>VFA+solvents (% COD)</td><td>220</td><td>n.d.*</td><td>49</td><td>62</td><td>38</td><td>32</td></tr>
<tr><td></td><td>260</td><td>n.d.*</td><td>67</td><td>n.d*</td><td>54</td><td>50</td></tr>
<tr><td></td><td>300</td><td>60</td><td>82</td><td>n.d*</td><td>61</td><td>99</td></tr>
</table>
</td></tr>
<tr><td>Lendormi et al. (2001)</td><td>Sludge A and B of Lendormi et al. (2001)</td><td>Operation in bubble columns: one of 350 L for sludge A and other of 1.6 L for sludge B at 240 – 300ºC and 30 – 75 min.
O_2/TCOD=1.0</td><td>
<table>
<tr><th>Parameter</th><th colspan="3">Sludge A</th><th colspan="3">Sludge B</th></tr>
<tr><td>Temperature (ºC)</td><td>300</td><td>280</td><td>280</td><td>300</td><td>240</td><td>240</td></tr>
<tr><td>Residence time (min)</td><td>36</td><td>32</td><td>30</td><td>30</td><td>76</td><td>75</td></tr>
<tr><td>Catalyst (w/w COD)</td><td>No</td><td>No</td><td>No</td><td>No</td><td>No</td><td>1% Cu</td></tr>
<tr><td>TCOD reduction (%)</td><td>80</td><td>76</td><td>72</td><td>83</td><td>71</td><td>86</td></tr>
<tr><td>VFA+solvents (% COD)</td><td>36</td><td>34</td><td>25</td><td>89</td><td>51</td><td>52</td></tr>
</table>
</td></tr>
</table>

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Shanableh and Jomaa (2001)	Mixed primary (75 wt%) and secondary (25 wt%) sludge from the WWTP of Loganholme (Queensland, Australia), with 19900 mg/L of TS, 1298 mg/L of TVS, and 21000 mg/L of TCOD, 2680 mg/L of SCOD, 1030 mg/L of VFA as COD (570 mg/L acetic and 460 mg/L propionic	A 30 mL stainless steel tube 10 mL sample oxidized with 4 g H_2O_2 (50% strength) Oxygen to TCOD ratio of 1.0 150 – 450ºC 0 - 60 min.	At 150ºC, acetic acid concentration increases from 570 mg/L at 0 min to 2960 mg/L at 60 min. At 200ºC, it goes through two maxima of 2550 mg/L, one at 20 min and other at 60 min. At 250ºC, the maximum is located at 30 min (2770 mg/L), whereas at 350ºC, it appears at 10 min (2070 mg/L). At 400 and 450ºC, the maxima are of 1860 and 1850 mg/L, respectively, and both are placed at 5 min. Propionic acid takes values between 300 and 500 mg/L for every time at 150 – 350ºC. At 400ºC, it seems always in the range 200 - 300 mg/L, and at 450ºC, it becomes 0.0 beyond 20 min. Other VFAs are only significant at 200 – 350ºC.The effect of the residence time in them is not clear. Concentrations of acids are expressed as COD.
Shanableh and Jomaa (2001, 2005, 2006)	Primary, secondary and mixed sludge from the WWTP of Loganholme (Queensland, Australia). The primary one contained 24800 mg/L of TCOD, 1810 mg/L of SCOD, 20960 mg/L of TS, 13280 mg/L of TVS, 510 mg/L of VFAs as COD (being 210 mg/L of acetic acid). The secondary one contained 22700 mg/L of TCOD, 2150 mg/L of SCOD, 21200 mg/L of TVS and 910 mg/L of VFA (180 mg/L acetic acid). The mixture is the mentioned in the previous row.	A 30 mL stainless steel tube 10 mL sample oxidized with 4 g H_2O_2 (50% strength) Oxygen to TCOD ratio of 1.0 150 – 450ºC 0 - 60 min.	Instead of plotting TCOD, SCOD, total VFA and acetic acid as a function of reaction time for several temperatures, it was represented SCOD, VFA and acetic acid vs. TCOD removal. All these curves show maxima. SCOD reached the highest values at 50% TCOD removal, whereas VFAs and acetic acid reached their maxima at 60% TCOD removal. These maximum concentrations were -6500 mg/L of COD, 2700 mg/L of VFAs and 2100 mg/L of acetic acid for primary sludge, 7500 mg/L of COD, 3200 mg/L of VFAs and 2700 mg/L of acetic acid for secondary sludge and 6500 mg/L of COD, 3000 mg/L of VFAs and 2400 mg/L of acetic acid for the mixed sludge. In the mixed sludge, the evolution of PO_4-P and NH_4-N was also determined. Phosphorus decreases from 90 mg/L at 0% TCOD removal to <10 mg/L at 60% and remained at this concentration until 90%, whereas ammonia increases from 109 mg/L in the feed to 950 mg/L at 70-80% TCOD removal, and then, decreases to 900 mg/L at 90%.

All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Stendahl and Jafverstrom (2003, 2004)	Dewatered digested sludge (from the Bromma sewage treatment plant in Stockholm and from Karlskoga sewage treatment plant, Sweeden) at 15-17% of TS, with 125000 mg/L of TCOD and a NH_4-N content of 1400 mg/kg TS.	Aqua Reci^{TM} process at pilot scale. Temperature around 550ºC for 60 s. O_2 to TCOD ratio of 1.05.	The oxidized effluent contained a TCOD around 40 mg/L and 6.0 – 7.0% of TS. If the working temperature was above 550ºC, there were 10 mg/L ammonia; but if it was below 550ºC, there were 150 mg/L ammonia. This effluent was filtered, obtaining a cake of 50% TS. Phosphorus is extracted by leaching, acid (HCl, H_2SO_4) or caustic (NaOH). Leaching with 1% NaOH solution at 80-90ºC during 90 minutes, extract 90% of the phosphorus in Stockholm, but only 65% in Karlskoga, because the high calcium content in the last one. This dissolved phosphorus, in the form of Na_3PO_4, can be precipitated with $Ca(OH)_2$ as hydroxyl apatite and separated from the process via a filter. Acidic extraction efficiencies were around 100% for both WWTPs, but faster for HCl than for H_2SO_4 at a given temperature. In the case of H_2SO_4 at 150ºC for 1 h, the resulting liquor contains phosphorus and heavy metals, which are separated by a 3-step process with magnesium oxide. In the first step, the pH is adjusted to 2 in order to precipitate $FePO_4$; in the second step, pH is fitted to 4.5 to precipitate aluminum as alunite and residual iron (if present) as jarosite. The final step is to further elevate the pH to 7–8 in order to precipitate all the heavy metals. $FePO_4$ can be treated later with NaOH, to obtain $Fe(OH)_3 + Na_3PO_4$ or with HCl, to produce $FeCl_3 + H_3PO_4$.
Sardelli and Valle (2006)	870 L/h secondary sludge from a plant that treats the effluents of a chemical factory and various aqueous wastes of external origin. 28000 mg/L of TSS VSS/TSS= 0.85 36000 mg/L of COD.	Reactor of 0.6 m diameter and 5 m height, operated at 240ºC, 4.5 MPa and O_2/TCOD=1.0 The feed is preheated with 280 kg/h of steam, saturated at 5.5 MPa.	The oxidized effluent, 940 L/h, contains 4300 mg/L of TSS, VSS/TSS= 0.102 and 11600 mg/L of COD. It is fed to a settling tank and separated in a supernatant (900 L/h) and slurry (40 L/h, 10.2% TSS). This slurry, after scrubbing and drying, can be used in devising ceramic (refractory) products, since it is mainly comprised by apatite, $Ca_5(PO_4)_3$.

All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Chung et al. (2009)	Sewage sludge with 7500 mg/L of TCOD, 0 mg/L of SCOD, 6500 mg/L of TSS, 11000 mg/L of VSS, pH 6.9 – 7.3, 450 mg/L of total N and 215 mg/L of TP and 0 mg/L of VFA.	1L stirred tank followed by a 1L plug flow reactor.	In all the cases, TCOD and TSS decreases with reaction time, organic acid concentrations increases and SCOD goes through a maximum at 10-20 min (except at 180ºC, where it monotonically increases with time)
		180 – 240ºC at 5.07 MPa for 10 – 80 min and 1 L/min air.	At 1 L/min air, 5.07 MPa, 240ºC and between 10 and 80 min, TCOD falls from 4890 to 2350 mg/L, SCOD from 3210 to 2080 mg/L and TSS from 1545 to 1046 mg/L, whereas organic acids increase from 460 to 690 mg/L. At 180ºC, TCOD falls from 7120 to 5825 mg/L and TSS from 2915 to 1495 mg/L, whereas SCOD increases from 2830 to 3590 mg/L and acids from 220 to 465 mg/L. At 40 min of reaction, the authors indicated the amount of acetic acid and propionic acid at each temperature (105 mg/L acetic acid and 0 mg/L propionic acid at 180ºC, 307 mg/L acetic acid and 3.6 mg/L propionic acid at 200ºC, 447 mg/L acetic acid and 12.6 mg/L propionic acid at 220ºC and 603 mg/L acetic acid and 13.5 mg/L propionic acid at 240ºC).
		4.05 – 6.08 MPa at 240ºC for 10 – 80 min and 1 L/min air.	At 1 L/min air, 240ºC, 4.05 MPa and between 10 and 80 min, SCOD falls from 3110 to 2060 mg/L and organic acids increase from 329 to 550 mg/L. At 6.08 MPa, SCOD falls from 4240 to 2950 mg/L and organic acids increase from 590 to 960 mg/L. TCOD and TSS seem pressure independent.
		0.5 – 3 L/min air at 240ºC and 5.07 MPa for 10 – 80 min.	At 240ºC, 5.07 MPa, 0.5 L/min air and between 10 and 80 min, TCOD falls from 6220 to 3560 mg/L and SCOD from 3210 to 2350 mg/L, whereas acid concentration increases from 95 to 320 mg/L. At 3 L/min air, TOC falls from 5080 to 2480 mg/L and SCOD from 3430 to 1840 mg/L, whereas acids increase from 260 to 640 mg/L. TSS are quite independent of the air flux.
Chung et al. (2009)	Sewage sludge with 28000 mg/L of TS, 24500 mg/L of SS, 17440mg/L of TCOD, 768 mg/L of SCOD, 288 mg/L of TP and 1238 mg/L of total N.	1 – 10 h at 220ºC, 3.04 MPa and 50% of the stoichiometric oxygen requirement.	The plant required three hours after loading to reach the working temperature. Between 1 and 5 hours, TS rises from 4160 to 7960, SCOD from 4900 to 10000 mg/L, TCOD from 5300 to 9766 mg/L, total N from 835 to 1100 and percentage of acetic acid from 0.025 to 0.065%, whereas the percentage of butyric acid decreased from 0.007 to 0.002%. Beyond 5 hours, the values remain more or less constant. TSS, TP and percentage of propionic acid are almost constant, being 490 mg/L, 23 mg/L and 0.017%, respectively. The authors do not explain why TS, TCOD and total N fall from 0 to 1 hour, and then, increase with time.

All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th>RESULTS AND/OR COMMENTS</th></tr>
<tr><td>Strong et al. (2011a)</td><td>A mixture of belt-pressed waste activated sludge and primary solids (2:1) from a local municipal wastewater, 57.8 g/L of TSS.</td><td>50 mL of sludge into a 190 mL stainless steel reactor pressurized to 2 MPa with pure air, at 220ºC for 2 h.</td><td>
<table>
<tr><th>Parameter</th><th>Feed</th><th>WO</th></tr>
<tr><td>pH</td><td>6.86</td><td>5.51</td></tr>
<tr><td>DOC (g/L)</td><td>1.1</td><td>8.6</td></tr>
<tr><td>SCOD (g/L)</td><td>2.0</td><td>15.4</td></tr>
<tr><td>TSS (g/L)</td><td>57.8</td><td>15.7</td></tr>
<tr><td>VSS (g/L)</td><td>47.6</td><td>9.4</td></tr>
<tr><td>Acetic acid (mg/L)</td><td>811</td><td>6250</td></tr>
<tr><td>Formic acid (mg/L)</td><td>11</td><td>800</td></tr>
<tr><td>Propionic acid (mg/L)</td><td>46</td><td>325</td></tr>
<tr><td>Butyric acid (mg/L)</td><td>29</td><td>300</td></tr>
<tr><td>Methanol (mg/L)</td><td>6</td><td>128</td></tr>
</table>
</td></tr>
<tr><td>Gapes et al. (2013)</td><td>Non-thickened (feed 1, 3% TS) and thickened (feed 2, 6% TS) activated sludge from a WWTP.</td><td>Three Parr reactors of 600 mL of total volume, 2 MPa overpressure of O_2 at 220ºC for 2 h.
200 mL Feed 1 are oxidized to WO1. 100 mL of WO1 are mixed with 100 mL Feed 2 and oxidized in the second stage, giving WO2. Finally, 100 mL of WO2 are mixed with 100 mL of Feed2 and oxidized in a third stage, giving WO3.</td><td>
<table>
<tr><th>Parameter</th><th>Feed 1</th><th>Feed 2</th><th>WO1</th><th>WO2</th><th>WO3</th></tr>
<tr><td>TSS (mg/L)</td><td>24657</td><td>39514</td><td>3466</td><td>4616</td><td>5269</td></tr>
<tr><td>VSS (mg/L)</td><td>21565</td><td>33711</td><td>855</td><td>773</td><td>707</td></tr>
<tr><td>Ash (mg/L)</td><td>3092</td><td>5803</td><td>261</td><td>3843</td><td>4562</td></tr>
<tr><td>DOC (mg/L)</td><td>615</td><td>1864</td><td>4450</td><td>6031</td><td>6533</td></tr>
<tr><td>TCOD (mg/L)</td><td>38800</td><td>51695</td><td>11780</td><td>14320</td><td>13420</td></tr>
<tr><td>Acetic acid (mg/L)</td><td>0</td><td>0</td><td>3877</td><td>5170</td><td>5782</td></tr>
<tr><td>Propionic acid (mg/L)</td><td>0</td><td>0</td><td>116</td><td>124</td><td>115</td></tr>
<tr><td>Methanol (mg/L)</td><td>0</td><td>0</td><td>0</td><td>42</td><td>44</td></tr>
</table>
</td></tr>
<tr><td>Gapes et al. (2013)</td><td>Raw and fermented Sludge from a WWTP
1-3% solids by weight
10 – 25 g/L</td><td>A 200 L total reactor volume pilot plant
20 bar overpressure of O_2 at 220ºC for 4 h (4 – 5 MPa total pressure)</td><td>After 4 h, the VFA yield is roughly the same for fermented and for raw sludge (20 – 50%, scattered data). Nonetheless, the yield of acetic acid is higher for the fermented sample (90%) than for the unfermented one (60%).
VFA purity is higher (45%) for the digested sludge than for the raw one (25%). Nonetheless, acetic acid purity is higher between 1 and 3.5 h, but beyond 4 h oxidation, both raw and digested samples give the same purity (around 27%).</td></tr>
</table>

All references are cited in the main text.

<table>
<tr><th>REF.</th><th>FEED</th><th>EXPERIMENTAL</th><th>RESULTS AND/OR COMMENTS</th></tr>
<tr><td>Andrews et al. (2015)</td><td>A mixture of primary and secondary sludge, which has been anaerobically digested.</td><td>A 200 L reactor, filled to a level of 30% at 220ºC and a total pressure of 4.3 MPa, employing O_2 for 1 – 5 h (without the pre-heating time of 2 h).</td><td>
<table>
<tr><th>Parameter</th><th>Feed</th><th>1.5 h</th><th>2 h</th><th>2.5 h</th><th>3 h</th><th>3.5 h</th><th>4 h</th><th>4.5 h</th><th>5 h</th></tr>
<tr><td>VSS (mg/L)</td><td>13735</td><td>6426</td><td>2892</td><td>1606</td><td>1205</td><td>482</td><td>450</td><td>418</td><td>402</td></tr>
<tr><td>Acetic (mg/L)</td><td>117</td><td>324</td><td>1257</td><td>1802</td><td>2152</td><td>2372</td><td>2644</td><td>2722</td><td>2813</td></tr>
<tr><td>Propionic (mg/L)</td><td>1102</td><td>1024</td><td>972</td><td>856</td><td>791</td><td>739</td><td>687</td><td>648</td><td>609</td></tr>
<tr><td>Other VFA (mg/L)</td><td>480</td><td>415</td><td>363</td><td>298</td><td>285</td><td>259</td><td>220</td><td>181</td><td>169</td></tr>
</table>
</td></tr>
<tr><td>Baroutian et al. (2015)</td><td>A mixture of 40% primary and 60% secondary sludge, anaerobically digested from the Rotorua Lakes Council WWTP (New Zealand), with 10340 mg/kg of TSS, 8400 mg/kg of VSS, 19590 mg/L of TCOD, 1990 mg/L of DOC, 1360 mg/L of acetic acid, 670 mg/L of propionic acid, 540 mg/L of butyric acid, 140 mg/L of isobutyric acid, 470 mg/L NH_4-N and 560 mg/L DKN</td><td>A lab-scale Parr reactor, initially charged with 250 mL water, then, pressurized with O_2, heated to the desired temperature and finally, loaded with the sludge.
Operation from 220 to 240ºC for 60 min, at an oxygen to biomass ratio of 1:1 – 2:1 (2 to 4 MPa O_2) and an unspecified stirring speed.</td><td>
<table>
<tr><th></th><th colspan="3">220ºC</th><th colspan="3">230ºC</th><th colspan="3">240ºC</th></tr>
<tr><th>Parameter</th><th>1:1</th><th>1.5:1</th><th>2:1</th><th>1:1</th><th>1.5:1</th><th>2:1</th><th>1:1</th><th>1.5:1</th><th>2:1</th></tr>
<tr><td>TSS (mg/kg)</td><td>2727</td><td>2690</td><td>2157</td><td>2331</td><td>2095</td><td>2008</td><td>1599</td><td>1488</td><td>1463</td></tr>
<tr><td>VSS (mg/kg)</td><td>1329</td><td>1236</td><td>873</td><td>860</td><td>688</td><td>635</td><td>410</td><td>304</td><td>304</td></tr>
<tr><td>TCOD (mg/L)</td><td>11455</td><td>7785</td><td>10960</td><td>10910</td><td>8479</td><td>10116</td><td>9322</td><td>7240</td><td>7884</td></tr>
<tr><td>DOC (mg/L)</td><td>1822</td><td>2690</td><td>2415</td><td>3153</td><td>2603</td><td>2979</td><td>2893</td><td>2140</td><td>1938</td></tr>
<tr><td>Acetic (mg/L)</td><td>2754</td><td>2670</td><td>2372</td><td>2973</td><td>2750</td><td>3363</td><td>3186</td><td>2478</td><td>2124</td></tr>
<tr><td>Propionic (mg/L)</td><td>448</td><td>390</td><td>357</td><td>390</td><td>315</td><td>390</td><td>343</td><td>238</td><td>223</td></tr>
<tr><td>Butyric (mg/L)</td><td>180</td><td>171</td><td>188</td><td>154</td><td>121</td><td>151</td><td>130</td><td>74</td><td>102</td></tr>
<tr><td>Isobutyric (mg/L)</td><td>69</td><td>71</td><td>76</td><td>63</td><td>54</td><td>69</td><td>56</td><td>36</td><td>50</td></tr>
<tr><td>NH_4-N (mg/L)</td><td>700</td><td>658</td><td>538</td><td>596</td><td>590</td><td>606</td><td>564</td><td>546</td><td>436</td></tr>
<tr><td>DNK (mg/L)</td><td>769</td><td>731</td><td>854</td><td>689</td><td>718</td><td>689</td><td>786</td><td>689</td><td>672</td></tr>
</table>
</td></tr>
<tr><td>Prince-Pike et al. (2015)</td><td>The same as Baroutian et al (2015), but it is revealed that the concentration of other VFA than acetic acid in the feed was around 1500 mg/L of COD.</td><td>The same as Baroutian et al (2015), but with stirring speeds of 300, 400 and 500 rpm and residence times from 0 to 60 min (not only 60 min).</td><td>The main objective was to develop a kinetic model, and contrast these results with those obtained in a pilot plant.
Twelve combinations of temperature, amount of oxygen and stirring speed were done, but only results for four ones were reported: 240ºC, 300 rpm and 20 bar O_2; 220ºC, 300 rpm and 40 bar O_2; 220ºC, 500 rpm and 20 bar O_2 and 230ºC, 400 rpm and 30 bar O_2.
In these cases, particulate COD (PCOD), TCOD and other VFA decreased with time, whereas acetic acid concentration increased (reaching values around 0.1, 1.1, 1100 and 3100 mg/L at 60 min, respectively). SCOD initially increased, went through a maximum of 0.8 – 1.0 mg/L around 5 min, and then, decreased to 0.4 – 0.6 mg/L at 60 min.
VFA and acetic acid concentrations were expressed as mg/L COD.</td></tr>
</table>

All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Prince-Pike et al. (2015)	The same as Baroutian et al (2015) but with 3150 mg/L of PCOD, 5950 mg/L of sCOD, 10940 mg/L of TCOD, 950 mg/L of acetic acid as COD and 1120 mg/L of other VFA as COD. TCOD is not equal to PCOD + SCOD	A pilot plant (300 L bubble column), loaded with 150 kg sludge (1.5% of TS), initially pressurized to 2 MPa with air, and later, with pure O_2 to maintain an oxidant concentration of 20 – 25% Reaction time from 0 to 260 min. Unspecified temperature.	Given that the pilot plant did not have a stirrer, an average value of 400 rpm was selected. Within the experimental error, PCOD, sCOD, TCOD and other VFA decreased with time, being 160, 1670, 5380 and 430 mg/L at 260 min, respectively. Intermediate values (e.g., at 100 min) were 250, 2520, 5280 and 490 mg/L, respectively. The concentration of acetic acid increased with time, being around 2120 mg/L at 100 min and 2330 mg/L at 260 min. The kinetic model developed for the lab-scale Parr reactor gave reasonably good results.
Yin et al. (2015)	Dewatered sludge from a sewage disposal plant in Chongquin, China, with 7.47% of TS, 330 mg/kg of DOC, 243 mg/kg of NH_4-N, 0 mg/kg of acetic acid 100 mg/kg of sProt and 70 mg/kg of sCarb. Concentrations are expressed as "**mg substance / kg wet feed**", because experimental problems to determine the volume.	Two tubular reactors of 5 mm diameter and 8.2 mL volume, loaded with 7.64 – 6.12 g of sludge. Operation at 180 – 300ºC for 0 – 90 min. Nothing is said about the oxidant.	At 180ºC, between 20 and 90 min, NH_4-N increases from 255 to 792 mg/kg. sCarb also increases, but reach a plateau of 2680 mg/kg beyond 60 min, whereas sProt goes through a maximum of 3700 mg/kg at 40 min and then decreases to 3020 mg/kg. At 220ºC, sCarb goes through a maximum at 50 min (2030 mg/kg) and then, decreases to 1180 mg/kg sCarb at 90 min. sProt reached the maximum (2850 mg/kg) at 20 min, being its concentration at 90 min of 1040 mg/kg. NH_4-N seems to monotonically increase with time and - tend to a constant concentration of 960 mg/kg of NH_4-N beyond 70 min. At 260ºC, sProt goes through a maximum at 10 min (2420 mg/kg) and sCarb at 20 min (1625 mg/kg). NH_4-N increases with time according to an S-shape curve. After 90 min of reaction, sProt, sCarb and ammonia concentrations are of 273, 580 and 1295 mg/kg, respectively. At 300ºC, sCarb and sProt go through maxima at 10 min (2110 mg/kg and 360 mg/kg, respectively), decreasing to values of 310 mg/kg of polysaccharide and 190 mg/kg of protein at 80 min. NH4-N increases with time according to an S-shape curve, which value at 80 min is 1650 mg/kg. Temperature and time dependence of acetic acid and DOC can be seen in Figures 3c and 4b, respectively.
Baroutian et al. (2016)	The same as Baroutian et al (2015), but is revealed that it also contained 186 mg/L pentanoic and 4 mg/L methanol.	The same as Baroutian et al (2015), but only at 1:1 oxygen to biomass ratio (2 MPa O_2) Residence time from 0 to 60 min (not only at 60 min).	The acetic acid concentration increases with reaction time and temperature, whereas the amount of the other acids decreases. At 60 min, data for acetic and propionic acid are the same as those of Baroutian et al (2015), but for butyric and isobutyric acid, they were slightly different. Pentanoic acid falls from 42 mg/L at 220ºC to 18 mg/L at 240ºC. Methanol concentrations go through a maximum at 20 min (124 mg/L for 220ºC, 139 mg/L for 230ºC and 145 mg/L for 240ºC) and at 60 min reach values of 70, 80 and 118 mg/L for 220, 230 and 240ºC, respectively.

All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Urrea et al. (2016)	Activated sludge from a WWTP of Asturias (Spain), with 21.6 g/L of TSS, 15.6 g/L of VSS, 22963 mg/L of TCOD , 1034 mg/L of SCOD and pH 6.9	A PARR series 4520 reactor of 1 L, at 65 bar and 500 rpm under 1.2 L/min of O_2 for 210 min. It lasted 90 min to reach the selected temperature (190ºC).	Size exclusion chromatography was used to determine the molecular weight distribution of the products generated during the WO between 0 and 210 min, although there were considerable interactions between the substances and the filling material of the chromatographic column. From 0 to 90 min, both the absorbance and the range of molecular weights increased. Between 100 and 110 min, there is a drop in absorbance for all the molecular weights, and beyond 110 min, there is almost no change in the chromatograms, indicating that oxidation occurred quickly, and finished approximately 20 min after reaching the reactions conditions (190 ºC and 65 bar). For a better understanding of the transformations that took place, the sludge was fractionated in naked cells and three types of extracellular polymeric substances (loosely bound to the cells, tightly bound to the cells and soluble microbial products), and then, each one was subjected to WO separately.

All references are cited in the main text.

3. Results of the application of TH and WO to the same sludge in the same experimental device

Table S3. Results of the application of TH and WO to the same sludge in the same experimental device.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS Parameter	Feed	100°C		130°C		163°C		197°C	
					TH	WO	TH	WO	TH	WO	TH	WO
Brooks (1968)	Abergavenny sewage works, settled to around 1% of TS. It contained an amount of phosphorus that, if fully solubilized, would generate 80 mg/L phosphate.	Lab-scale device 100 – 197°C for 1 hour (pre-heating time of 30 min). Air (6200 mg/L of O_2) or argon in the atmosphere.	SCOD (mg/L)	n.d.*	3040	3450	3660	3990	5100	5150	5450	4950
			DON (mg/L)	0.04	247	282	389	375	462	413	452	328
			NH_4-N (mg/L)	0.25	21	31	41	66	103	158	172	293
			N in solids (% VSS)	7.82	7.13	6.88	6.24	6.28	4.4	4.21	3.93	3.48
			SS (mg/L)	10000	6735	7085	5300	5110	4430	3980	3205	3575
			Col solids (mg/L)	30	3170	2790	1260	740	700	590	360	310
			Total N (mg/L)	782	748	800	761	762	760	739	750	745
			TS (mg/L)	10530	n.d.*	n.d.*	10754	10054	9812	9174	7913	7395
			VFA (mg/L)	0.0	n.d.*	n.d.*	196	246	389	219	511	835
			pH	7.5	7.0	6.75	6.5	5.9	5.9	5.05	5.6	5.9
			Soluble PO_4 (mg/L)	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	31.6	18.6	9.8	4.9

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS
Marshall and Gillespie (1974)	Activated sludge from the treatment of an integrated bleached kraft mill wastewater, with 1% solids.	A 2 L autoclave at 1750 rpm TH at 149 – 250°C for 20 – 45 min. WO at 149 – 315°C with O_2 for 30 – 45 min Pressures between 0.7 and 20.7 MPa.	The working conditions are not well described in all the experiments. At a fixed temperature, TS reduction increases with reaction time until reaching a plateau. The higher the temperature, the higher the value of this plateau. For the same temperature and time, TH gives lower solid destruction than the WO. The sludge filtrate contained more nitrogen and more phosphate when oxidized than when hydrolyzed thermally.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS Parameter	250°C		300°C		350°C	
				TH	WO	TH	WO	TH	WO
Quitain et al. (2002)	Dewatered sludge from a university sewage plant (56 wt.% moisture, 16 wt% dry basis protein)	A 6 mL reactor made of SUS-316 250 - 350ºC, at saturation 0.1 g sample and 5 g deionized water 35 wt% H_2O_2 added in equal proportion of the sample Unspecified time.	Succinic acid (mg/g TS_{feed})	n.d.*	n.d.*	n.d.*	n.d.*	2.36	2.79
			Lactic acid (mg/g TS_{feed})	11.3	13.9	12.0	12.1	1.57	8.27
			Propionic acid (mg/g TS_{feed})	n.d.*	n.d.*	n.d.*	n.d.*	9.06	8.66
			Acetic acid (mg/g TS_{feed})	50.0	60.9	55.4	72.4	81.1	90.1
			Formic acid (mg/g TS_{feed})	16.4	25.4	2.27	12.3	1.60	6.20

*n.d. means not determined. All references are cited in the main text.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS					
Yang et al. (2010)	Waste activated sludge from a WWTP that treated the wastewater from a crude oil refinery by the aeration process.	5 L/h sludge treated at 1 MPa for a retention time of 30 min. WO with air at 100, 200 and 300ºC TH at 200ºC	Parameter	Feed	WO 100ºC	WO 150ºC	WO 200ºC	TH 200ºC
			TCOD (mg/L)	30789	25478	18165	12315	17311
			SCOD/TCOD	0.02	0.03	0.58	0.90	0.71
			BOD/TCOD	0.05	0.15	0.33	0.52	0.31
			VFA (mg/L)	21	1243	2531	3843	3756
			Acetic acid (mg/L)	7.00	355	797	1230	1211
Strong et al. (2011b)	A mixture of belt-pressed waste activated sludge and primary one (2:1) from a WWTP of Rotoura, New Zealand.	200 mL sample in a 600 mL Parr reactor at 20 bar, 300 rpm for 2 h. TH at 140 and 165ºC under an inert atmosphere of N_2 WO at 220ºC under O_2.	Parameter	feed	TH 140ºC	TH 165ºC	WO 220ºC	
			TSS (g/L)	56.5	53.2	45.8	9.5	
			VSS (g/L)	47.8	43.6	37.5	3.	
			Carbon (%)	45	47	48	14	
			Nitrogen (%)	5	4	3	4	
			pH	7.2	5.4	5.2	5.5	
			DOC (mg/g TS)	50	140	170	140	
			SCOD (mg/g TS)	120	510	580	400	
			Acetic acid (mg/g TS)	2	7	17	146	
			Formic acid (mg/g TS)	0	2	5	17	
			Propionic acid (mg/g TS)	1	2	4	5	
			Butyric acid (mg/g TS)	1	0	0	6	
			Methanol (mg/g TS)	0	2	3	4	
			VFA (mg/g TS)	3	11	26	174	
Aggrey et al. (2011)	Mixed primary and secondary sludge from the WWTP of Rotoura, New Zealand, diluted to 2.5 – 3% of TS.	200 mL sample in a 600 mL Parr reactor at 355 rpm. TH at 140ºC and 1 MPa (N_2) for 120 min. WO at 220ºC and 1 MPa (O_2) for 120 min A two-stage process: The first one with N_2 and the second one with O_2, each one for 120 min and at 1 MPa overpressure.	Parameter	feed	TH	WO	2-stage	
			SCOD/TCOD (%)	12	50	80	81	
			TCOD removal (%)	0	44	69	61	
			SCOD/DOC	n.d.*	2.7	2.6	3.5	
			Galactosamine (mg/g TS_{feed})	0.56	1.72	0	0	
			Glucosamine (mg/g TS_{feed})	0.08	2.36	0	0	
			Arabinose (mg/g TS_{feed})	0.24	4.96	0.24	0	
			Galactose (mg/g TS_{feed})	0.96	2.92	0	0	
			Glucose (mg/g TS_{feed})	0.40	9.20	0.48	0.64	
			Xylose (mg/g TS_{feed})	0.08	1.56	0	0	
			Mannose (mg/g TS_{feed})	0.16	3.24	0	0	
			Formic acid (mg/g TS_{feed})	0.05	0.24	2.90	5.92	
			Acetic acid (mg/g TS_{feed})	0.82	1.80	58.0	36.0	
			Propionic acid (mg/g TS_{feed})	0.47	0.26	2.37	3.05	
			Butyric acid (mg/g TS_{feed})	0.05	2.03	0.63	1.45	
			Pentanoic acid (mg/g TS_{feed})	0	0	0.21	0.25	
			Unidentified DOC (mg/g TS_{feed})	10.08	112.9	88.71	155.24	
			Acetic acid yield (%)	0	0.4	12.0	8.0	
			Acetic acid purity (%)	0	1.0	38.0	25.0	

*n.d. means not determined. All references are cited in the main text.

4. Effect of the level of the oxygen fed into the reactor on the products obtained during hydrothermal treatment of sewage sludge.

Table S4. Effect of the level of oxygen fed into the reactor on the products obtained during hydrothermal treatment of sewage sludge.

REF.	FEED	EXPERIMENTAL	RESULTS AND/OR COMMENTS												
Goto et al. (1998)	Municipal excess sludge with 9720 mg/L of TOC	A stainless steel tube (3.18 mm × 1.7 m) at 385°C and 30 MPa for 30 min, oxidation with H_2O_2. O_2/TCOD from 0 to 1.	Parameter	Oxygen to TCOD ratio											
				0.0	0.2	0.4	0.5	0.6	0.8	1.0					
			TOC (mg/L)	3400	3415	3080	2624	2340	1138	0.0					
			NH_4-N (mg/L)	2372	2846	3083	3320	3261	3360	3478					
			Formic acid (mg/L)	0.0	47.5	77.1	94.9	47.5	18.9	0.0					
			Acetic acid (mg/L)	878	1685	2480	2693	2835	2412	0.0					
			Propionic acid (mg/L)	202	190	190	225	154	78.3	0.0					
			Butyric acid (mg/L)	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
			Isovaleric acid (mg/L)	107	71.2	94.99	0.0	0.0	0.0	0.0					
			Succinic acid (mg/L)	47.5	107	249	297	213	87.8	0.0					
Shanableh and Jomaa (2001)	The primary and secondary sludge from the WWTP of Loganholme (Queensland, Australia) described in Table S2.	A 30 mL stainless steel tube where 10 mL sludge are placed. Oxidation with H_2O_2 at 300°C for 30 min O_2/TCOD from 0 to 2.	Parameter	Primary sludge						Secondary sludge					
				feed	0.0	0.4	1.0	1.6	2.0	feed	0.0	0.4	1.0	1.6	2.0
			Total VFA (mg/L)	510	3015	3104	2905	3470	1377	910	3376	4135	4900	3966	2827
			Acetic acid (mg/L)	210	1695	1833	2225	3100	1186	180	1603	2089	4000	3502	2658
			Propionic acid (mg/L)	n.d.*	660	593	340	270	148	n.d.*	591	865	480	295	127
			Other VFA(mg/L)	n.d.*	660	678	340	100	42	n.d.*	1181	1181	420	169	42
			All the concentrations expressed as mg/L COD												
Qian et al. (2015)	Sewage sludge from the WWTP of Beishiqiao in Shaanxi, China, with 87% wt moisture and 128 g/L TCOD	Batch reactor at 450 or 550°C and 25 MPa for 20 min Oxidation with H_2O_2 (30 wt%) O_2/TCOD of 0, 0.5 and 4.0	Parameter	feed	450°C O_2/TCOD=0.0	450°C O_2/TCOD=0.5	550°C O_2/TCOD=4.0								
			Acids, hydrocarbons and esters (%)	55.035	0.879	0.0	0.0								
			Amides (%)	6.378	7.529	8.335	0.0								
			Phenol, cresols and aromatic acids (%)	24.979	40.75	41.076	64.314								
			Pyridine, pyrazine and related (%)	5.144	39.546	43.293	35.688								
			Cyclic ketonic compounds (%)	8.465	11.293	7.294	0.0								
			Total average relative molecular weight	299.158	158.710	104.599	100.654								
			TOC (mg/L)	n.d.*	8912	7298	2736								
			NH_4-N (mg/L)	n.d.*	6842	7105	3526								
			Percentage of remaining TS (%)	100.00	47.56	44.92	28.36								

*n.d. means not determined. All references are cited in the main text.

Article.

IV. Sludge hydrothermal treatments. Oxidising atmosphere effects on biopolymers and physical properties.

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1. UV-VIS absorption spectrum of supernatants during wet oxidation or thermal hydrolysis of sludge.

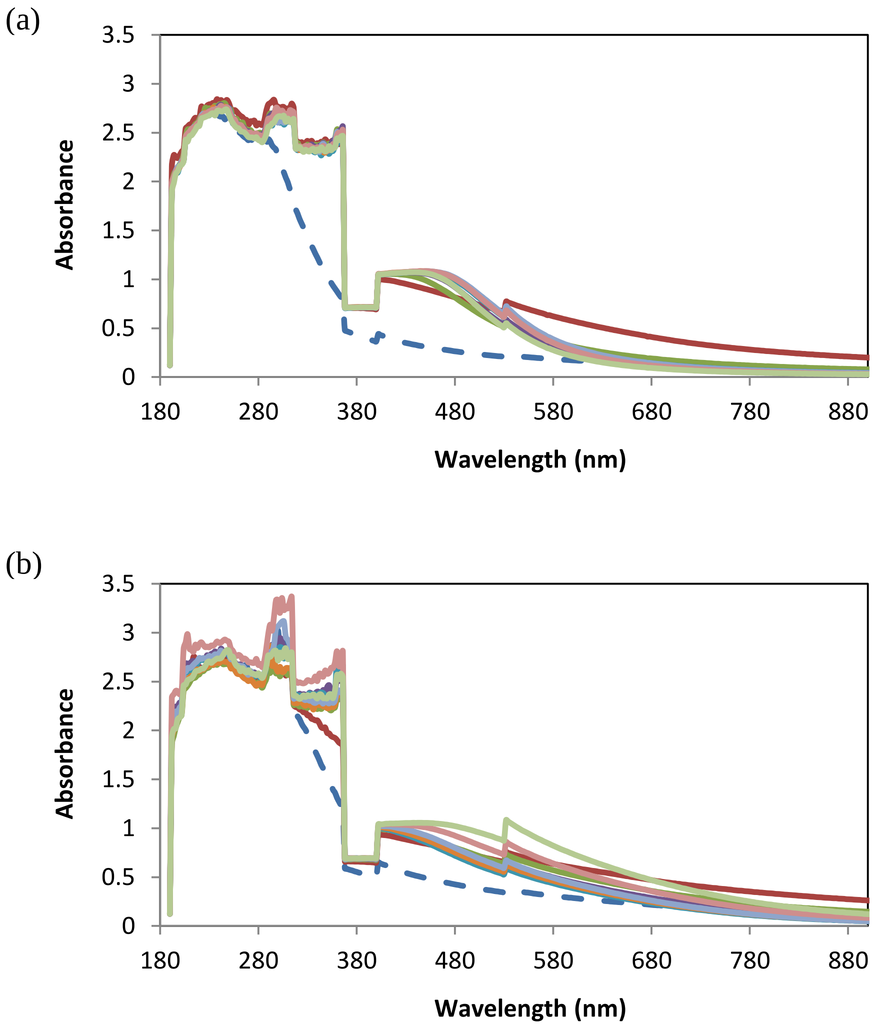

Figure S1. Evolution of UV-VIS spectrum of supernatants from sludge during wet oxidation (a) or thermal hydrolysis (b) at 160 ºC and 40 bar. Time sampling: - - initial, — 45 min, — 67 min, — 77 min, — 87 min, — 97 min, — 117 min, — 147 min, — 187 min.

Article.

V. Wet oxidation of the structural sludge fractions.

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1. Experimental procedure for EPS extractions and the analysis of readily biodegradable COD (RBCOD).

EPS extractions

A thermal extraction was employed to separate the EPS fractions from sludge. Briefly, 450 mL of sludge was centrifuged at 4000 g (4 ºC, 5 min) and the supernatant, corresponding to SMP fraction, was extracted and stored. Subsequently, the pellet was re-suspended in distilled water to its initial volume and subjected to centrifugation (6000g, 4 ºC, 15 min), and then, the supernatant, corresponding to LB-EPS fraction, was collected and stored. Next, the pellet was again re-suspended to its initial volume, this time with distilled water at 65 ºC and was placed in a thermostatically controlled water bath (at 65 ºC) for 30 min. Afterwards, the sample was centrifuged at 8000g (4 ºC, 15 min) and the supernatant, containing the TB-EPS fraction, was separated and stored. Finally, the pellet composed by "naked cells" was once more re-suspended and stored.

Analysis of readily biodegradable COD (RBCOD)

The method is based on determining the effect of introducing a liquid sample on the oxygen uptake rate (OUR) of an activated sludge. The analysis of RBCOD was carried out in a 250 mL jacketed vessel, which was maintained at 20 ºC by means of a thermostatically controlled water bath. The experimental protocol consisted in charging 170 mL of activated sludge previously aerated until to achieve endogenous conditions. The system was stirred to 250 rpm and five drops of nitrification inhibitor were added. The aeration was carried out in feeding cycles (1 minute feeding and 6 minutes turned off). An YSI Professional Optical Dissolved Oxygen instrument was employed to measure the variation in the oxygen concentration with the time. Subsequently, 50 mL of sample, which was previously centrifuged (10000g, 10 min) and filtered (0.45 µm

PVDF filters) was added. The ratio of initial substrate concentration to initial biomass concentration (So/Xo) was established in 0.05-0.10 g COD g VSS^{-1}. The OUR measurements were maintained until the sludge reached the endogenous conditions again. The RBCOD of the sample was estimated from Equation S1 (Orhon and Çokgör 1997), being necessary to subtract the endogenous cumulative oxygen uptake of the total OUR.

$$\mathrm{RBCOD} = \frac{1}{(1-\mathrm{Y_H})}\int_{t_0}^{t_1} \mathrm{OUR}\ \partial t \qquad \text{(S1)}$$

Where Y_H is the heterotrophic yield coefficient

As Y_H depends of the sludge characteristics, it was necessary to carry out a previous test using sodium acetate as an organic standard. The value of Y_H was calculated from Equation S2, obtaining a value of 0.74 mg COD mg COD^{-1}.

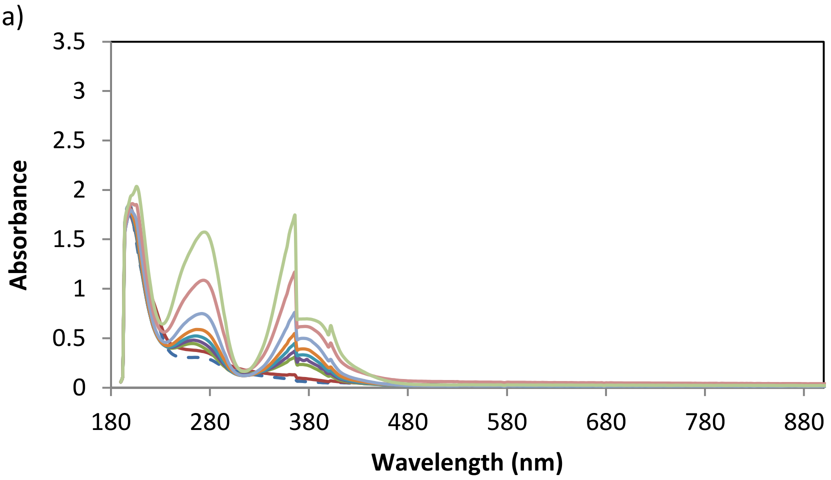
$$\mathrm{Y_H} = 1 - \frac{\int \mathrm{OUR}}{\mathrm{COD\ degradation}} \qquad \text{(S2)}$$

Reference:

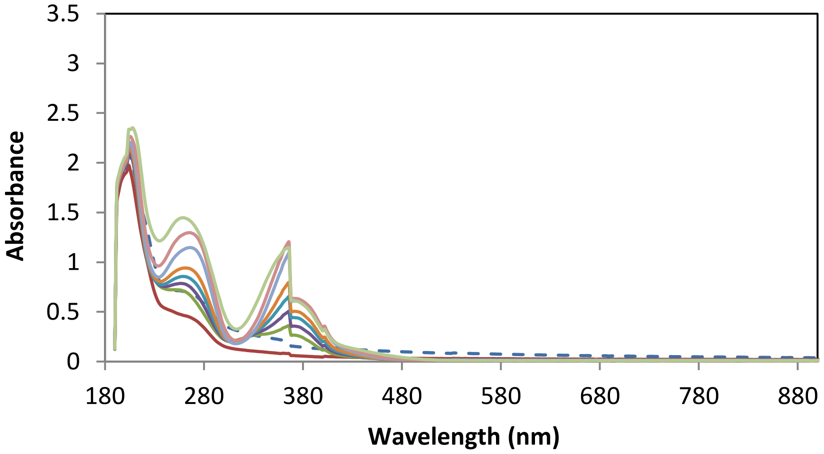
Orhon, D. and Çokgör, E.U. (1997) COD Fractionation in Wastewater Characterization—The State of the Art. Journal of Chemical Technology & Biotechnology 68(3), 283-293.

2. UV-VIS absorption spectra of supernatants during wet oxidation treatment of sludge components and raw sludge.

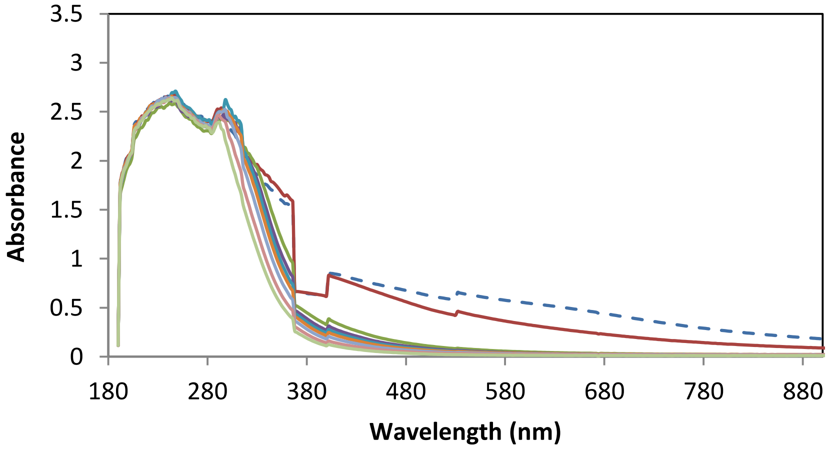
(a)



(b)



(c)



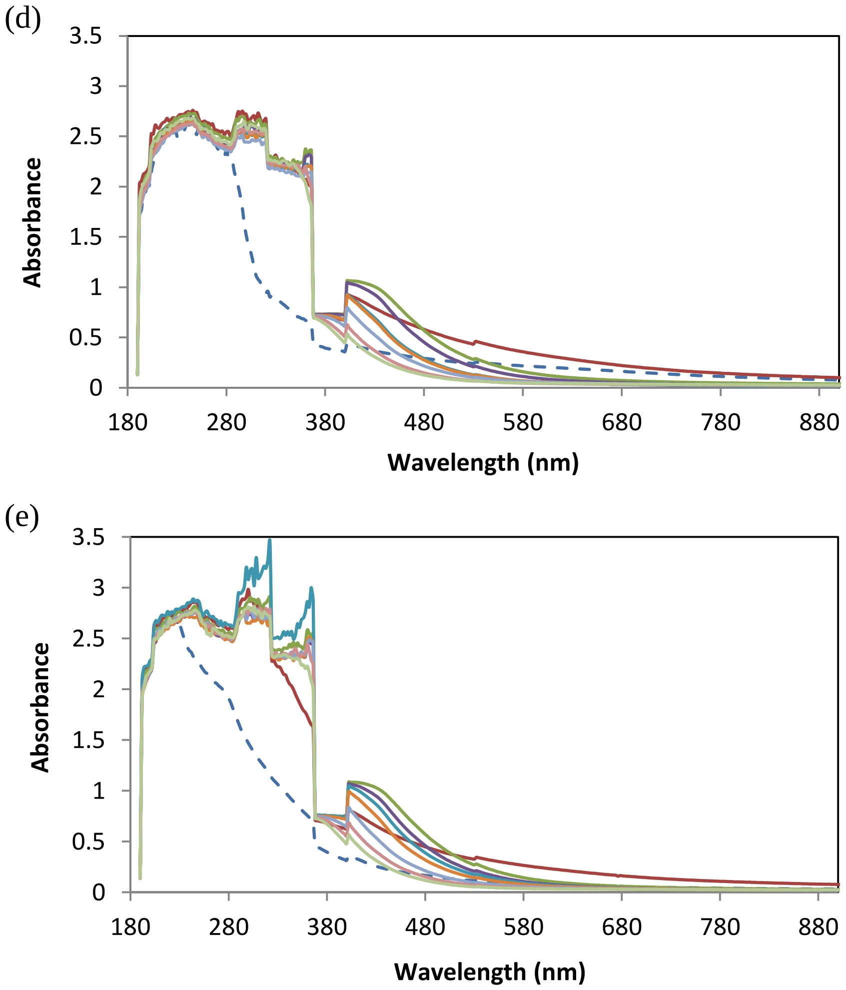


Figure S1. Evolution of UV-VIS spectra of supernatants during wet oxidation treatment at 190 ºC and 65 bar. (a) SMP fraction, (b) LB-EPS fraction, (c) TB-EPS fraction, (d) naked cells fraction, (e) raw sludge. – – initial, —— 40 min, —— 90 min, —— 100 min, —— 110 min, —— 120 min, —— 140 min, —— 170 min, —— 210 min.

Article.

VI. Effect of wet oxidation on the fingerprints of polymeric substances from an activated sludge.

Table of contents

1. ATR-FTIR measurements of the initial and final fractions of SMP, LB-EPS and TB-EPS (Figures S1 to S4).
2. Analysis of hydrophobic interactions during SEC analysis of samples (Figure S5).
3. Effect of the EPS hydrophilicity/hydrophobicity on the MOC (Table S1).

1. ATR-FTIR measurements of the initial and final fractions of SMP, LB-EPS and TB-EPS.

ATR-FTIR measurements were carried out to both initial fractions and final ones (after treatment). The main results obtained related to the initial fractions are explained as follows (see Figure S1):

- The FTIR spectra for initial SMP and LB-EPS samples are quite similar, which is in accordance with the similar compositions in biopolymers observed for both fractions.
- An intense broad band was observed at ~3300 cm^{-1} (amide A) in SMP and LB-EPS fractions, which is characteristic of N-H stretching vibration in proteins.
- A weak band has been detected for ~2900 cm^{-1} in the LB-EPS fraction, confirming that carbohydrates are present in a low concentration. This band was no detected in SMP, since its concentration is lower than that achieved for LB-EPS fraction, as expected considering the data from Table 1.
- In the case of TB-EPS, the characteristic bands for proteins at ~3300 and at ~3100 cm^{-1} were overlapped with those from carbohydrates at ~3350 and ~2900 cm^{-1}, making difficult to obtain conclusions from these bands.
- Both SMP and LB-EPS showed amide I band at 1637 cm^{-1}, which corresponded to proteins. This band is due to ~80% C-O stretching, ~10% C-N stretching, and 10% N-H bending vibrations). Nevertheless, this band was not reported in the case of TB-EPS. On the contrary, TB-EPS showed band at 1577 cm^{-1}, characteristic of amide II (~ 60% N-H bending vibration and ~40% C-N stretching).
- SMP, LB-EPS and TB-EPS showed a band at 1400 cm^{-1}, this being more intense for TB-EPS, which is characteristic of carbohydrates (C-H and OH deformation

vibrations). This band is overlapped with that from proteins, specifically, amide III (between 1400 and 1200 cm^{-1}), thus, being difficult to obtain valuable information.

- A strong band was observed at 1090 cm^{-1} for both SMP and LB-EPS, whereas, this band was shifted to lower wavenumbers for TB-EPS (1042 cm^{-1}), all of them are characteristic of carbohydrates (C-O stretching vibration).

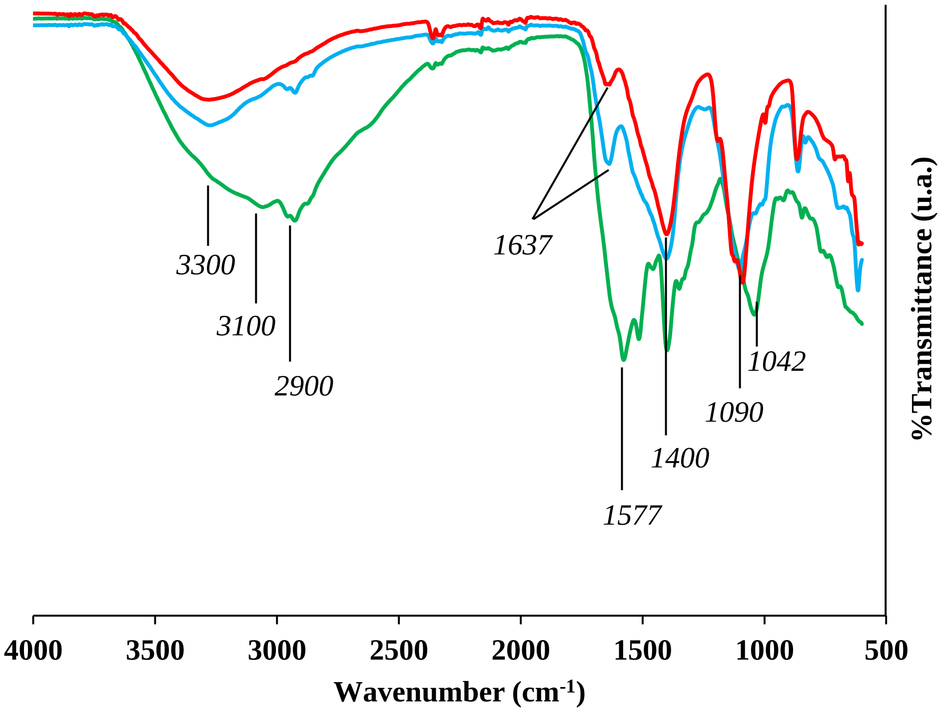


Figure S1. FTIR spectra of the initial fractions of SMP (▪), LB-EPS (▪) and TB-EPS (▪).

The differences between the SMP, LB-EPS and TB-EPS fractions before (initial) and after WO treatment (final) were also studied. Regarding SMP (see Figure S2), both the initial and final fractions showed an Amide A broad band at 3278 and 3327 cm^{-1}, respectively. This can be explained considering that this vibration is exclusively localised on the NH group and is therefore in proteins insensitive to the conformation of the polypeptide backbone. Its frequency depends on the strength of the hydrogen bond. The initial SMP fraction showed a band at 1637 cm^{-1} which is characteristic of amide I vibration of proteins. This band can be assigned to the presence of proteins in which the secondary structure is β-sheet. However, such band was not detected after WO treatment. This can be due to the denaturation of the proteins by the oxidation treatment. Besides, it should be noted that the amide I vibration is hardly affected by the nature of the side chain. After oxidation, a band of amide II vibration of proteins at 1558 cm^{-1} was distinguished. This band can also be related to the flexibility of the proteins and the effect is more marked for smaller molecules. This is due to the oxidation provoked the breakage of proteins into peptides. The initial and final SMP fractions also showed a band at 1400 cm^{-1}, which is characteristic of carbohydrates. However, this band is overlapped with that from proteins, specifically, amide III (between 1400 and 1200 cm^{-1}), thus, indicating the existence of both type of compounds.

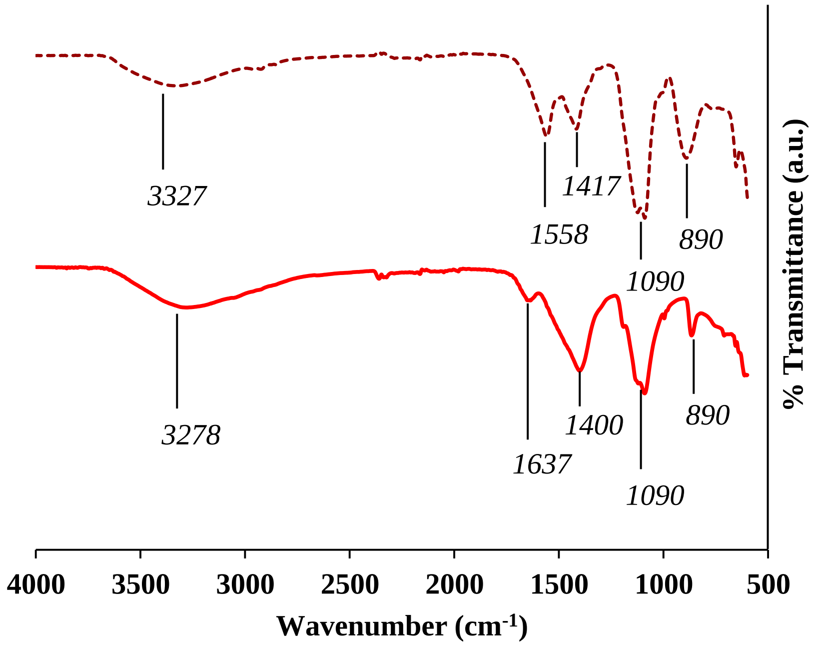


Figure S2. FTIR spectra of the initial (▪) and final (▪▪) fractions of SMP.

A strong band was observed at 1090 cm^{-1} both in the initial and final SMP fractions, which corresponded to carbohydrates (C-O stretching vibration). It can also be identified a band at ~890 cm^{-1}, which is characteristic of carbohydrates (C-H deformation), however it is overlapped with that from proteins, specifically, symmetric C-N-C stretching. Consequently, it is difficult to obtain significant information.

Related to LB-EPS, the spectra obtained and the bands identified were very similar to those obtained for SMP (see Figure S3). This is due to the composition of both fractions SMP and LB-EPS is almost the same. The only difference observed is the existence of a weak band, in the final LB-EPS spectrum, at 1194 cm^{-1}, which is also characteristic of amide III. Such mode is the combination of the NH bending and the CN stretching vibration with small contributions from the CO in plane bending and the CC stretching vibration. In proteins, the composition of this mode is very complex, since it depends on side chain structure and since NH bending contributes to several modes in the 1400 to 1200 cm^{-1} region. Therefore, this can be the reason why this band was not detected on SMP.

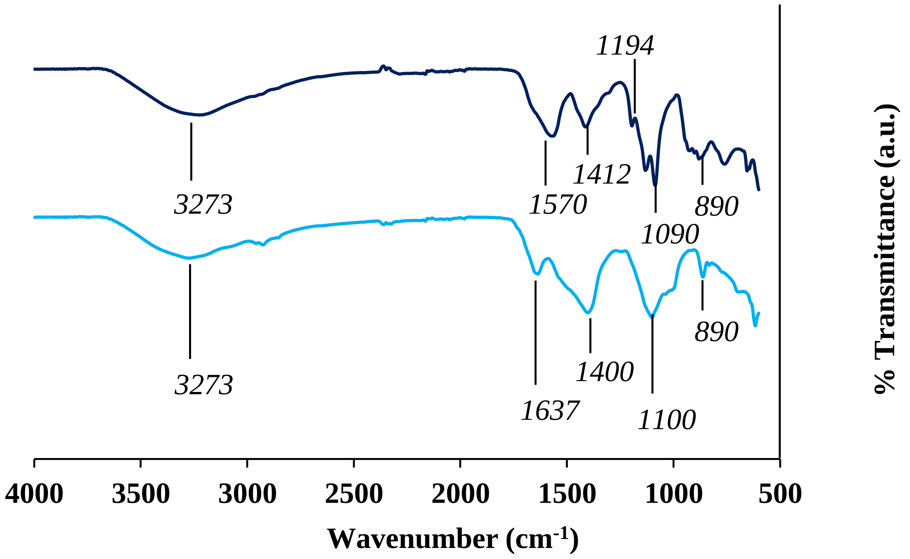


Figure S3. FTIR spectra of the initial (▪) and final (▪) fractions of LB-EPS.

Considering TB-EPS, the initial fraction showed a broad band at ~3300 cm^{-1} (amide A), whereas, in the final fraction, both bands amide A and B was identified at ~3300 and ~3100 cm^{-1}, respectively (see Figure S4). The amide A band is usually part of a Fermi resonance doublet with the second component absorbing weakly between 3100 and 3030 cm^{-1} (amide B). In polypeptide chains, the NH stretching vibration is resonant with an overtone of the amide II vibration, in β-sheets with an amide II combination mode. Besides, in the initial fraction a weak band at 2900 cm^{-1} was observed. This band is typical in carbohydrates. Taking into account the complexity of this fraction is complicated to achieve more significant conclusions. The initial TB-EPS fraction did not showed the characteristic band of amide I vibration of proteins at 1650 cm^{-1}, however such band was identified in the final fraction. The absence of it in the initial fraction can be due to the polymeric matrix and the fact that the band can be included into the band centered at ~1600 cm^{-1}. Both initial and final fractions exhibited a band of amide II vibration of proteins at 1560 cm^{-1}, although it was more intense in the final one. As it was previously commented, this band can be related to the flexibility of the proteins, such effect being more marked for smaller molecules. Therefore, this result is reasonable since oxidation produces the breakage of the proteins. The initial TB-EPS fraction showed a medium and a weak band at ~1400 and 1350 cm^{-1} which can be due to amide III vibration of proteins. It is noteworthy that these bands are overlapped with those from carbohydrates (C-H and OH deformation vibrations). After the oxidation, two more weak bands positioned at 1440 and 1301 cm^{-1} were identified (together with the bands at ~1400 and 1350 cm^{-1}) in the final fraction. Both bands can also be due to carbohydrates or proteins. Since the oxidation treatment degraded these compounds, this can facilitate the identification of more characteristic bands. In the initial TB-EPS fraction was also identified a band which corresponded to carbohydrates, exactly at

1040 cm^{-1} that can be assigned to C-O stretching vibration. This band was also found at the same wavenumber in the final fraction, however, its intensity was lower. This is because of the oxidation treatment degraded the carbohydrates present in such fraction.

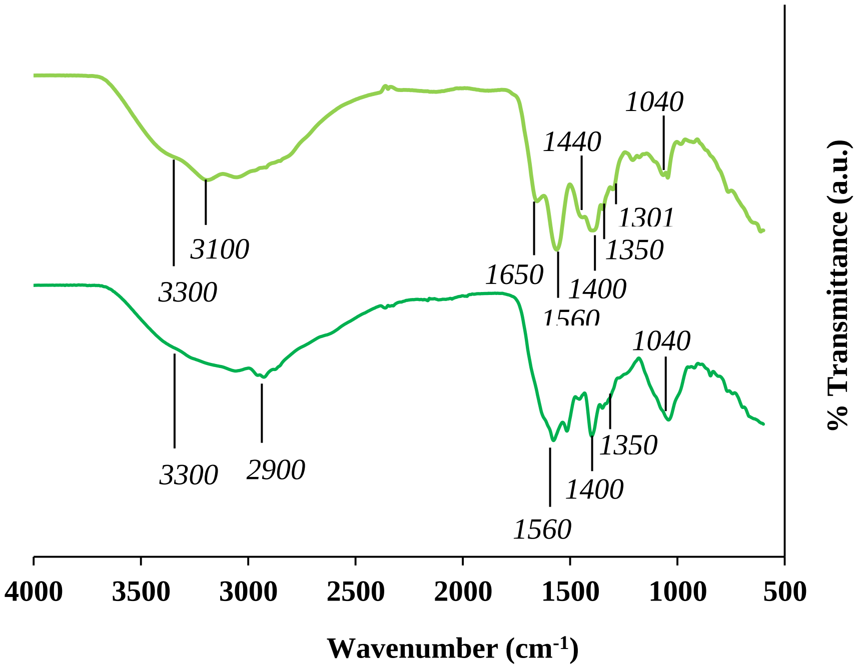


Figure S4. FTIR spectra of the initial (▪) and final (▪) fractions of TB-EPS.

References:

Barth, A. (2007) Infrared spectroscopy of proteins. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1767(9), 1073-1101.

Socrates, G. (2001) Infrared and Raman Characteristic Group Frequencies. Tables and Charts. 3rd edition. John Wiley & Sons, LDT. New York.

2. Analysis of hydrophobic interactions during SEC analysis of samples

SEC analysis was repeated in order to check the presence of hydrophobic interactions between EPS polymers and the column filling material, on this occasion using a mobile phase containing 5% of methanol. When the initial EPS fractions were analysed, the fingerprints maintained the same amount of peaks and their shapes did not change much with respect to the fingerprints obtained without methanol (results not shown). However, the samples collected during WO presented a different behaviour. Firstly, a slight delay in elution time was identified on peaks inside column volume, in particular on the first two and four groups of molecules for EPS and naked cell fractions, respectively. By contrast, the peaks outside of column volume were slightly displaced to lower elution times without these having achieved the size exclusion zone. The figure below (Figure S5) shows a typical result of the mobile phase polarity effect on fingerprints after WO.

Similar results were found by Simon et al. 2009 when they analysed the effect of mobile phase polarity on fingerprints of EPS. In their work, it can be observed that an important displacement of hydrophobic peaks was not reached until 30% of acetonitrile in the mobile phase was used. For the peaks which showed an increase in the elution time, they considered that this occurred due to an increase of electrostatic interactions.

Based on these results, it can be considered that peaks appearing at times longer than the total volume of the column were affected by the higher polarity of mobile phase, which revealed the presence of polymers with hydrophobic characteristics in these peaks.

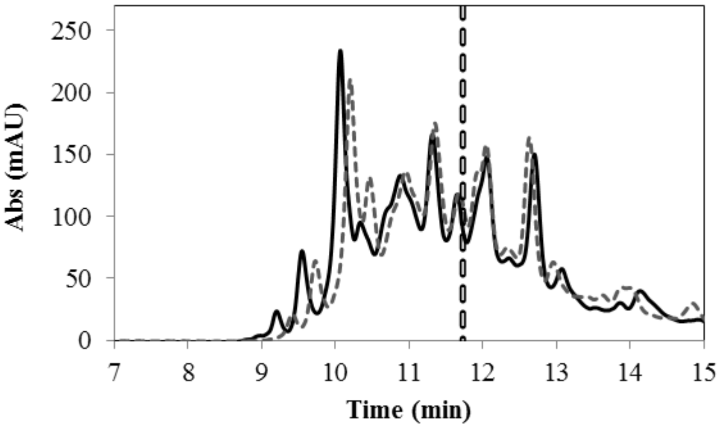


Figure S5. Polarity effect of mobile phase on fingerprints of activated sludge fractions. Result for naked cell after 210 min of WO at 190 ºC and 65 bar. ▬ Mobile phase buffer, ╍╍╍ mobile phase buffer with 5% methanol, ▭▭▭ total column volume. UV absorbance at 260 nm.

3. Effect of the EPS hydrophilicity/hydrophobicity on the MOC

According to the results of the fingerprints of initial EPS fractions, it can be observed that the composition of EPS had a more hydrophobic character when its position was closer to the cells, contrary to what happened with the MOC, which decreased. This fact suggests that the more hydrophobic EPS, the lower MOC, which makes sense and can be demonstrated theoretically through some simple calculations.

It is generally accepted that the higher the number of oxygen atoms in the composition of a molecule, the higher its solubility in polar solvents (acids R-COOH are more hydrophilic than alcohols R-OH; alcohols R-OH are more hydrophilic than alkenes R-H and so on). At the same, the higher the oxygen content in the compound, the lower the COD, by definition of this parameter. Considering two compounds with the same number of carbon atoms, that is, the same TOC, it can be easily deduced that the higher the number of oxygen atoms, the lower the COD and the higher the hydrophobicity. If

COD decreases while the TOC remains constant, then the MOC increases (see formula). So, a high hydrophobicity can be related to low MOC and viceversa.

$$\mathrm{MOC} = 4 - 1.5\,\frac{\mathrm{COD}}{\mathrm{TOC}}$$

For example, if two substances as butanol and butanediol ,which have the same amount of C, but different number of OH groups, are considered, it can be easily verified that more hydrophobic compound (butanol), has a lower MOC. This is due to the presence of an additional molecule of oxygen in the structure of butanediol, which has a direct impact on its COD, decreasing it (see the Table S1).

Table S1. MOC number of butanol and butanediol.

Butanol	**Butanediol**
$C_4H_{10}O$	$C_4H_{10}O_2$
COD = 2091 g O_2/L	COD = 1930 g O_2/L
TOC = 522.8 g C/L	TOC = 526.4 g C/L
MOC = -2	MOC= -1.5

Article.

VII. Formation and degradation of soluble biopolymers during wet oxidation of sludge.

Table of contents

1. Evolution of soluble TOC from sludge fractions during WO at 190ºC and 65 atm (Figure S1).
2. Evolution of volatile suspended solids in naked cells fraction and raw sludge sample during WO at 190 ºC and 65 atm (Figure S2).
3. Evolution of biopolymers solubilised in the raw sludge compared to the sum of all sludge fractions during WO at 190 ºC and 65 atm (Figure S3).

1. Evolution of soluble TOC from sludge fractions during WO at 190 ºC and 65 atm.

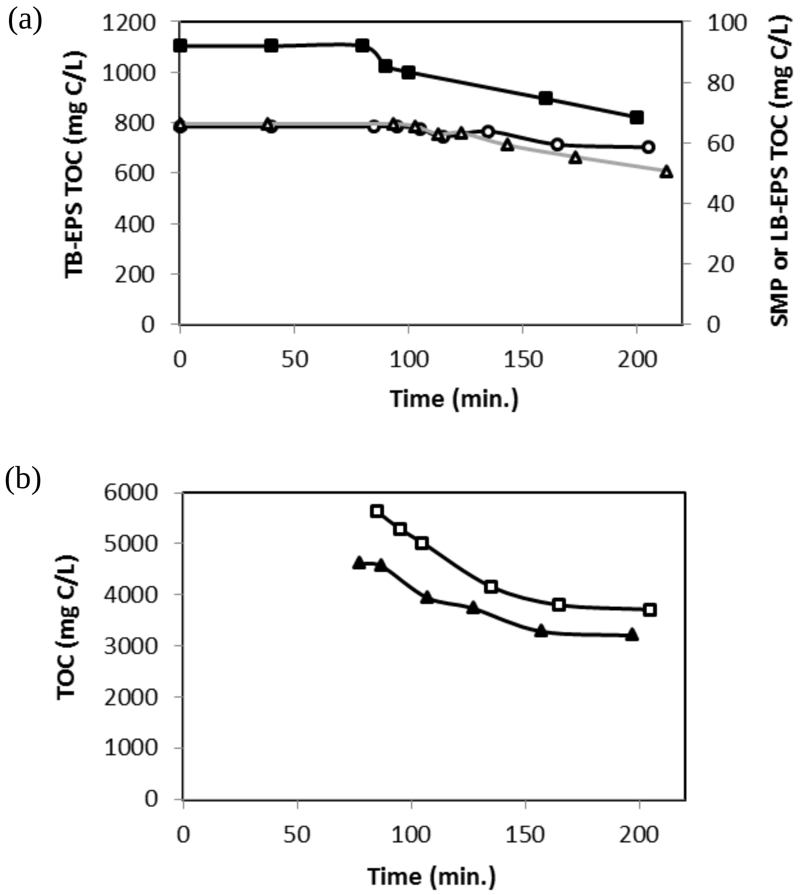


Figure S1. Evolution of soluble TOC from sludge fractions during WO at 190 ºC and 65 atm. (a) -○- SMP, -△- LB-EPS and -■- TB-EPS, (b) -▲- naked cells and -□- raw sludge (results of naked cells and raw sludge after reaching operating conditions).

2. Evolution of volatile suspended solids in naked cells fraction and raw sludge sample during WO at 190 ºC and 65 atm.

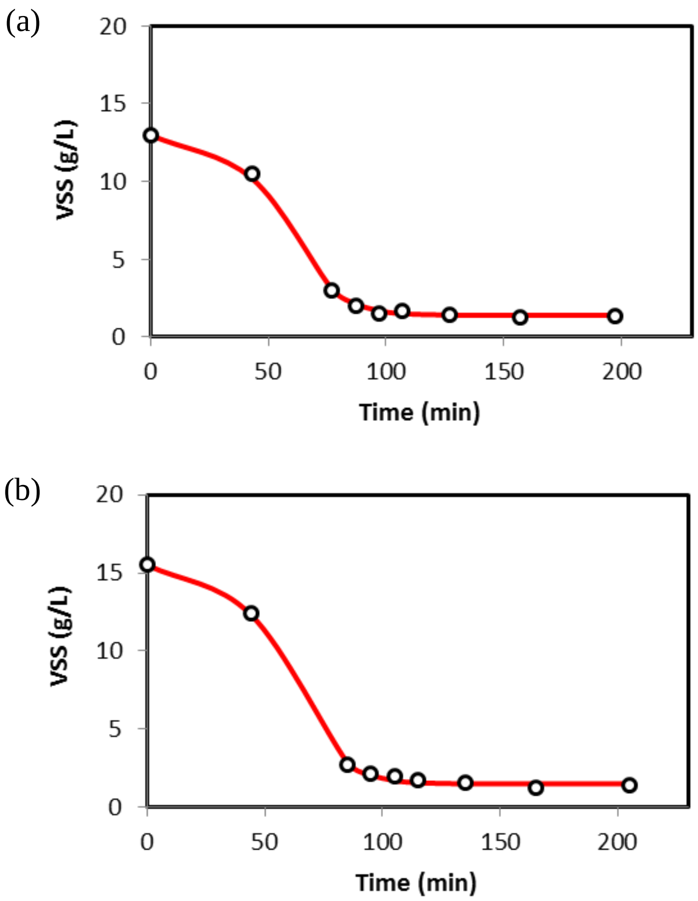


Figure S2. Evolution of volatile suspended solids from (a) naked cells and (b) raw sludge during WO at 190 ºC and 65 atm. Experimental data (symbols) and kinetic model (red line) according to equation 1.

The following fitting parameters were obtained when the kinetic model for the VSS solubilisation (equation 1) was fitted to experimental data of either naked cells or raw sludge: 1.87 $*10^8$ L mg O_2^{-1} min^{-1}, 56 kJ/mol and 0.999 for $k_{0_{VSS}}$, Ea_{VSS} and R^2, respectively.

The value obtained for the activation energy of both naked cells and raw sludge is 56 kJ/mol which is in quite good agreement with values previously published by other authors, who report activation energies for sludge solubilisation ranging from 25 to 80 kJ/mol (Menoni and Bertanza, (2016), Prince-Pike et al. (2015) and Urrea et al. (2014).

References:

Menoni, L.; Bertanza, G. Wet Oxidation of sewage sludge: a mathematical model for estimating the performance based on the VSS/TSS ratio. *Chem. Eng. J.* **2016**, 306, 685-692.

Prince-Pike, A.; Wilson, D. I.; Baroutian, S.; Andrews, J.; Gapes, D. J. A kinetic model of municipal sludge degradation during non-catalytic wet oxidation. *Water Res.* **2015**, 87, 225-236.

Urrea, J. L.; Collado, S.; Laca, A.; Díaz, M. Wet oxidation of activated sludge: Transformations and mechanisms. *J. Environ. Manage.* **2014,** *146*, (0), 251-259.

3. Evolution of biopolymers solubilised in the raw sludge compared to the sum of all sludge fractions during WO at 190 ºC and 65 atm.

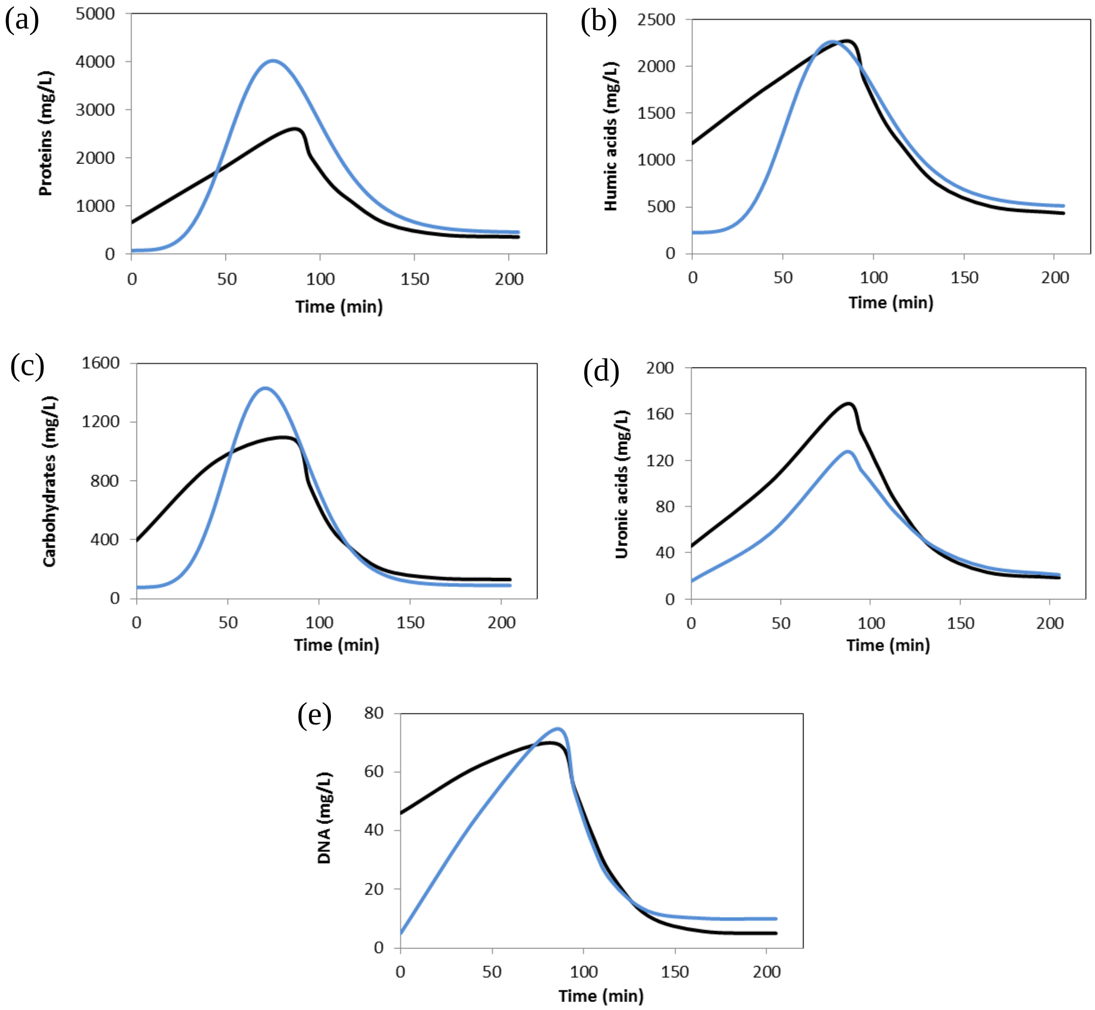


Figure S3. Evolution of biopolymers solubilised in the raw sludge (blue line) compared to the sum of all sludge fractions (black line) during WO at 190 ºC and 65 atm. (a) Proteins, (b) humic acids, (c) carbohydrates, (d) uronic acids and (e) DNA. (The data presented were obtained from equations 1 and 2).

7.2. DIFFUSION OF THE THESIS

List of publications

The results obtained in this Doctoral thesis, corresponding to the hydrothermal treatments of sludge, were distributed in the following seven published articles:

Urrea, J.L., García, M., Collado, S., Oulego, P. and Díaz, M. (2018) Sludge hydrothermal treatments. Oxidising atmosphere effects on biopolymers and physical properties. Journal of Environmental Management 206, 284-290. **Impact factor:** 4.010 (year 2016)

Urrea, J.L., Collado, S., Oulego, P. and Díaz, M. (2017a) Formation and Degradation of Soluble Biopolymers during Wet Oxidation of Sludge. ACS Sustainable Chemistry & Engineering 5(4), 3011-3018. **Impact factor:** 5.951 (year 2016)

Urrea, J.L., Collado, S., Oulego, P. and Díaz, M. (2017b) Wet oxidation of the structural sludge fractions. Journal of Cleaner Production 168(Supplement C), 1163-1170. **Impact factor:** 5.715 (year 2016)

Suárez-Iglesias, O., Urrea, J.L., Oulego, P., Collado, S. and Díaz, M. (2017) Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review. Science of the Total Environment 584–585, 921-934. **Impact factor:** 4.900 (year 2016)

Urrea, J.L., Collado, S., Oulego, P. and Díaz, M. (2016) Effect of wet oxidation on the fingerprints of polymeric substances from an activated sludge. Water Research 105, 282-290. **Impact factor:** 6.942

Urrea, J.L., Collado, S., Laca, A. and Díaz, M. (2015) Rheological behaviour of activated sludge treated by thermal hydrolysis. Journal of Water Process Engineering 5(0), 153-159. **SCImago Journal Rank:** 0.615

Urrea, J.L., Collado, S., Laca, A. and Díaz, M. (2014) Wet oxidation of activated sludge: Transformations and mechanisms. Journal of Environmental Management 146(0), 251-259. **Impact factor:** 2.723

Communications to congress

J.L. Urrea, M. García, S. Collado, P. Oulego, M. Díaz. Solubilised biopolymers from sludge by hydrothermal treatments. Effects of an oxidising atmosphere. 10th World Congress of Chemical Engineering 2017. Barcelona (Spain). 2017. (Oral comunication)

M. García, J.L. Urrea, P. Oulego, S. Collado, M. Díaz. Recovery of soluble biopolymers from hydrothermal treatment of sludge. 10th World Congress of Chemical Engineering 2017. Barcelona (Spain). 2017. (Poster)

J.L. Urrea, S. Collado, P. Oulego, M. Díaz. Oxidación húmeda de lodos: Un análisis a través de los componentes poliméricos. XII reunión de la Mesa Española de Tratamiento de Aguas. (Meta 2016 Madrid). Madrid (Spain). 2016. (Oral comunication)

J.L. Urrea, P. Oulego, S. Collado, M. Díaz. Sludge as new resource for proteins, carbohydrates and humic acids. The 13th IWA Leading Edge Conference on Water and Wastewater Technologies. Jerez (Spain). 2016. (Poster)

J.L. Urrea, S. Collado, A. Laca, M. Díaz. Wet oxidation of sludge: Role of structural components of the floc. XXXV Biannual Congress of the Spanish Royal Society of Chemistry. Coruña (Spain). 2015. (Oral comunication)

J.L. Urrea, S. Collado, A. Laca, M. Díaz. Comportamiento reológico durante la hidrólisis térmica y la oxidación húmeda de lodos. XI reunión de la Mesa Española de Tratamiento de Aguas. (Meta 2014 Alicante). Alicante (Spain). 2014. (Poster)