

**A PROPOSAL FOR THE CLASSIFICATION OF SLUDGE PRODUCTS
THROUGHOUT HYDROTHERMAL TREATMENT**

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Abstract

As the wide variety of compounds that are solubilised and generated during hydrothermal treatments of sewage sludge cannot be defined individually, this study proposes a classification based on chemical characteristics and biodegradability. Moreover, it has deepened understanding of the role of oxygen in the reaction mechanisms and the evolution of chemical oxygen demand (COD) fractions and their components during hydrothermal treatments (180 °C, 80 bar). This information is necessary before separating specific compounds or using the hydrolysates directly as fertiliser or fermentation media. Results revealed the existence of an initial stage of sludge solubilisation (30 – 60 min), mainly produced by hydrolysis reactions and without mineralisation, followed by a second stage where oxygen promoted the conversion of solubilised compounds, mineralisation and the minimisation of the solid fraction. Total COD and total suspended solids (TSS) were reduced by 48% and 75% after 210 min of wet oxidation, compared to 28% and 50% after thermal hydrolysis. Oxygen led to a final liquid hydrolysate with fewer easily assimilable compounds (38% of the initial total COD converted into readily biodegradable), inorganic elements (17 g/kg TSSo) and refractory, inhibitory and less assimilable compounds (1.1 g O₂/L of slowly and non-biodegradable COD) compared to that obtained in its absence (61%, 24 g/kg TSSo and 2.3 g O₂/L, respectively). Oxidation mechanisms favoured the conversion of non-volatile hydroxy acids (NVHA) and intermediate compounds to volatile fatty acids (VFA), with VFA concentration being almost 3 times higher under an oxidising atmosphere, but with NHVFA 57% lower.

Keywords

Fractionation, hydrothermal treatments, sludge management, respirometry, inhibitors.

1. Introduction

Sewage sludge is considered to be the main waste product of biological treatment in wastewater treatment plants (WWTP), in terms of generation and management costs [1–4]. Traditionally, sludge treatments have been focused on minimising its volume, obtaining at the same time energy and/or low-value products [3,5–7]. Nevertheless, due to the growing global interest in replacing non-renewable energy and raw materials, sludge is currently beginning to be considered as a renewable resource for obtaining value-added products [1,3,6,7].

Frequently, the recovery strategies require an initial stage of solubilisation to release specific components, obtain a stable non-soluble solid residue and/or improve the handling of the stream [3,8]. In this regard, hydrothermal treatments, which are widely employed on an industrial scale [9–12], are based on the reactions that take place in an aqueous phase at high temperatures (150 – 320 °C) and pressures (20 – 150 bar) during short residence times (15 – 120 min) [9]. They have been successfully tested for the initial solubilisation of intra and extracellular compounds from sewage sludge [2,3,10,12,13], resulting in the formation of a heterogeneous hydrolysate. This consists of a complex liquid phase, which is a mixture of the solubilised compounds and the products formed, as well as a solid residue, composed of the non-soluble fraction of the sludge and the precipitated materials. Not all these compounds can be defined individually as that would involve considerable analytical effort and time, making it necessary to employ the so-called sum parameters, such as chemical oxygen demand (COD), total organic carbon (TOC) or volatile suspended solids (VSS) (see [9] for typical effects of hydrothermal treatments on these parameters). In some cases, different molecules are encompassed in families such as proteins, carbohydrates or humic acids, according to their common reactivity towards specific chemicals. And, in rare studies, the concentrations of a few specific compounds (heavy metals, organic acids,

phosphorus, among others) were also measured [14–19]. In the field of wastewaters, COD fractionation has also been proposed to determine the biodegradability of the waste stream in more detail and improve its treatment in the WWTP [20–22].

Among the operational variables to be considered during the hydrothermal treatments, the type of atmosphere is probably one of the most important, but also one of the factors that has been least studied. In fact, hydrothermal treatments are classified, depending on the atmosphere employed, into wet oxidation and thermal hydrolysis. The former is carried out in presence of oxygen, while the latter needs an inert atmosphere [2]. With an oxidising atmosphere, oxidation reactions lead to partial oxidation of compounds and higher mineralisation of the sludge [18,19]. Furthermore, aspects such as the changes in the concentration of inhibitory compounds or even the COD fractionation of the hydrolysates have not been studied.

Therefore, the aim of this work is to propose a classification for sludge compounds, based on their chemical characteristics and biodegradability, and to expand the characterisation of the composition of hydrolysates obtained by hydrothermal treatments of sludge under inert and oxidising atmospheres, in order to discuss the role of oxidation reactions in the mechanisms involved. This knowledge constitutes a first stage towards the separation and purification of some specific compounds of industrial interest from this thermally treated sludge and/or for its direct use as a fermentation media for supporting the growth of specific microorganisms and the bioproduction of different metabolites.

2. Materials and methods

2.1. Materials

Secondary sewage sludge, thickened by flotation, was provided by a municipal wastewater treatment plant in Baiña (Asturias, Spain). Once collected, the sludge was stored at 4 °C for no more than 10 days until its treatment. Liquid and solid fractions of sludge were separated by centrifugation at 10,000 g for 20 min. The physicochemical properties measured in the raw sewage sludge are detailed in table 1. The main characteristics of the liquid fraction are in Table S.1. in the Supplementary Material.

Table 1. Physicochemical properties of sewage sludge.

Parameters	Mean values
Total chemical oxygen demand (tCOD) (g O ₂ /L sludge)	35 ± 3
Total suspended solids (g TSSo/L sludge)	33 ± 3
Volatile suspended solids (g VSSo/L sludge)	26 ± 1
Total carbon (g C/kg TSSo)	437 ± 2
Total nitrogen (g N/kg TSSo)	79.1 ± 0.1
Total calcium (g Ca/kg TSSo)	22.1 ± 0.6
Total phosphorus (g P/kg TSSo)	16.5 ± 0.4
Total iron (g Fe/kg TSSo)	12.6 ± 0.2
Total potassium (g K/kg TSSo)	6.3 ± 0.1
Total magnesium (g Mg/kg TSSo)	4.1 ± 0.1
Total sodium (g Na/kg TSSo)	2.06 ± 0.03
Total zinc (mg Zn/kg TSSo)	630 ± 10
Total manganese (mg Mn/kg TSSo)	385 ± 6
Total copper (mg Cu/kg TSSo)	281 ± 6
Total chromium (mg Cr/kg TSSo)	30.5 ± 0.5
Total cobalt (mg Co/kg TSSo)	4.7 ± 0.3

Total mercury (mg Hg/kg TSSo)	2.8 ± 0.3
Total cadmium (mg Cd/kg TSSo)	1.3 ± 0.1
pH	7.1 ± 0.3

2.2. Experimental setup

Hydrothermal experiments were carried out using a 1 L 316 SS semi-batch reactor (Parr Instrument Co., Moline, IL.), equipped with two six-bladed magnetically driven turbine stirrers, controllers and indicators for temperature and pressure, bursting discs, a sampling system and a heating jacket. The experimental setup also consisted of two compressed cylinders to provide the gas flow (oxygen for wet oxidation or nitrogen for thermal hydrolysis) and a 2 L humidifier for saturating the gas before entering the reactor. In addition, in order to ensure a safe operation, the humidifier and the reactor were only loaded up to 70% of their maximum capacities.

The operational conditions are obviously of the utmost importance. Optimal temperatures reported for treating sewage sludge by hydrothermal treatments are between 160 and 180 °C [11] and the range of pressures tested is between 20 and 150 bar [9], allowing the appropriate degrees of solubilisation to be obtained [23]. Therefore, experiments were carried out at 180 °C and 80 bar under an inert (N₂) or an oxidising (O₂) atmosphere, which had already been shown to be appropriate in previous research [24]. The stirrer speed was adjusted to 150 rpm and the gas flow rate, to 1,800 mL/min, regardless of the gas employed.

2.3. Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) were measured following the Standard Methods [25].

Soluble P, K, Mg, Ca, Fe, Na, Cu, Mn, Zn, Hg, Co, Cr and Cd were determined by means of an ICP-MS (Agilent 7700x, Agilent Technologies, CA, USA), equipped with an integrated I-AS autosampler. The collision/reaction cell used 4.3 mL/min of He to eliminate the interferences and the standard used was Sc, with the exception of Hg and Cd, for which it was Ir or Rh, respectively. The same method was used to determine the total content of these elements in the raw sludge but, before this analysis, a sample of raw sludge was previously dried at 105 °C for 24 h and subsequently digested using the microwave acid digestion method (6 mL of HNO₃ and 2 mL of a 30% H₂O₂ solution and microwave equipment (Ethos One, Milestone Systems, Denmark)).

Soluble species of nitrogen were measured using a Segmented Flow Self-Analyser SKALAR SAN PLUS (Skalar Analytical B.V., Netherlands), equipped with a dialyser, and ultraviolet digester, photometric detectors and cadmium columns for nitrate reduction. Soluble species of carbon were obtained by means of a TOC-V-CSH analyser (Shimadzu, Japan). Total carbon and nitrogen content in the raw sewage sludge were also measured by an Elemental Analyser CHNS Elemental Vario EL (Elementar Analysensysteme GmbH, Germany).

The variation in readily biodegradable chemical oxygen demand due to carbon oxidation (rbcCOD) was obtained by respirometry using a BM-EVO analyser (SURCIS S.L., Spain), with the air diffuser set at 55% and at a constant temperature of 20 °C. The respirometer vessel was filled with 1 L of activated sludge (2.7 g VSS/L) from the same municipal wastewater treatment plant. The heterotrophic biomass yield coefficient (Y_H) for this sludge was 0.62 g/g, which was determined with sodium acetate [26]. In order

to avoid nitrification interferences and foam formation, a few drops of allyl thiourea (ATU) inhibitor and antifoam were added, respectively. For each respirometric analysis, 30 mL of the liquid fraction from the corresponding sludge hydrolysate was employed.

Soluble non-volatile hydroxy acids (NVHA) (malic and lactic acid) and volatile fatty acids (VFA) (formic and acetic acid) concentrations were measured by High Performance Liquid Chromatography (HPLC) (Agilent Technologies, CA, USA), coupled to a Coregel ION 300 column (Concise Separations, USA) and a refractive index detector (RID). The temperature of the column was set at 75 °C and the mobile phase was 0.3 mL/min of a 0.45 mM sulphuric acid solution [27]. Levulinic and propionic acid, among other carboxylic acids that could also be detected and measured by this procedure, were not present.

Total soluble carbohydrates were measured in the liquid fraction at 492 nm using the Dubois phenol-sulphuric acid method, whereas reducing sugars were obtained at 540 nm applying the DNS method [18,27].

Soluble protein and humic acid concentrations were analysed in the liquid fraction using the modified Lowry method [18].

Hydroxymethylfurfural (HMF) and furfural were measured by an HPLC Agilent system as well, but coupled to a Gemini-NX 5 μ m C18 110A column (Phenomenex, USA) and a diode-array detection (DAD) set at 285 nm, using a mobile phase of 10:90 methanol/water [27].

Phenolic compounds were analysed at 725 nm using the Folin-Ciocalteu phenol method [28] and uronic acids were determined at 520 nm following the Blumenkrantz and Asboe-Hansen method [14].

Focussing now on physical properties, pH was measured at 25 °C using a Basic 20 Crison pH-meter (Crison Instruments, Spain) and the density of the liquid fraction samples was determined at 25 °C using pycnometers.

Rheological behaviour was analysed with a HAAKE MARS II rheometer (Thermo Scientific, USA), equipped with flat plates of titanium of 60 mm of diameter with a gap of 1 mm [29]. Flow curves were obtained by a rotational test, with the shear rate linearly increased from 0.1 to 100 s⁻¹ in 180 s, at a temperature of 25 °C. In order to determine whether sludge hydrolysates had a time-dependant flow behaviour, the change in apparent viscosity under a constant shear rate of 25 s⁻¹ was measured for 600 s for each sample. The parameters analysed were those involved in the Herschel-Bulkley model [29]:

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

Where τ is the shear stress; τ_0 , the yield stress; K, the flow consistency index; $\dot{\gamma}$, the shear rate and n, the flow behaviour index.

3. Results and discussion

First of all, it is important to note that raw sewage sludge mainly consists of two distinct fractions, as previously mentioned. One is a liquid fraction, mainly composed of water, soluble molecules and ions. The other is a solid one, which comprises the flocs formed by the union between microbial and extracellular polymeric substances (EPS) by means of cations, such as K⁺, Ca²⁺, Mg²⁺, Fe³⁺ and Na⁺ [4,30–32]. At the same time, other organic and inorganic compounds, like phosphorus, heavy metals, fibres and other pollutants, are also present in this network, either outside or inside the cells [8,32]. Both the amount and the composition of the two fractions changed during the hydrothermal treatments due to solubilisation, reaction and/or precipitation processes.

With this in mind, the discussion of the composition of either the raw sludge or the different hydrolysates was initially based on dividing the components into organic (tCOD) or inorganic ones, and these two, into soluble or solid matter (section 3.1). In turn, soluble organic matter (sCOD) was classified according to its biodegradability by heterotrophic microorganisms into readily, slowly or non-biodegradable compounds (section 3.2) (Figure 1).

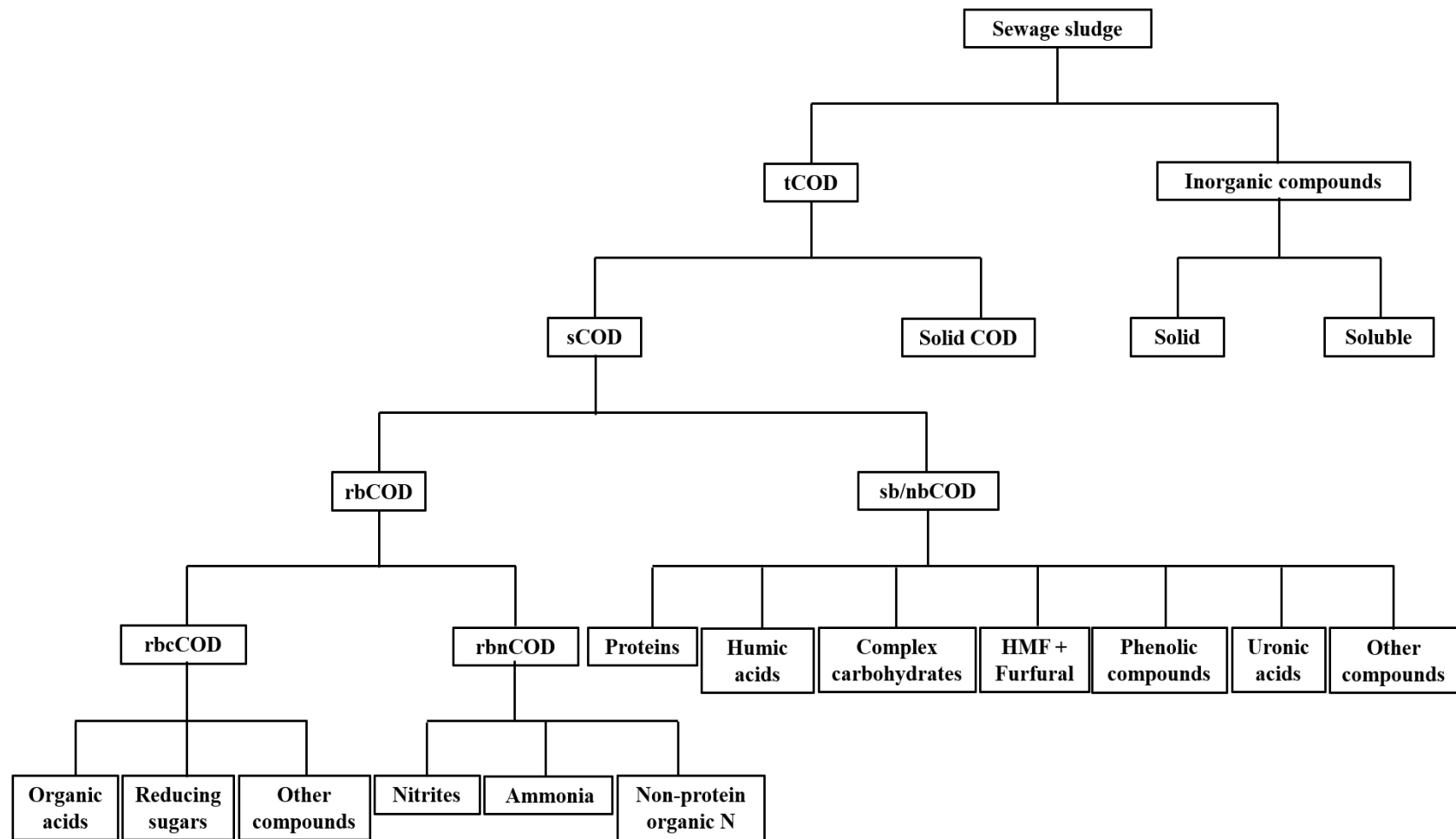


Fig. 1. Scheme followed to classify the compounds of sewage sludge.

3.1. Solubilisation of inorganic matter and mineralisation

Taking into account the classification scheme shown above, this section is focused on explaining how the type of atmosphere affects the organic and inorganic fractions (Figure 2) and, in more detail, the distribution of inorganic compounds in the solid and liquid fractions (Figure 3).

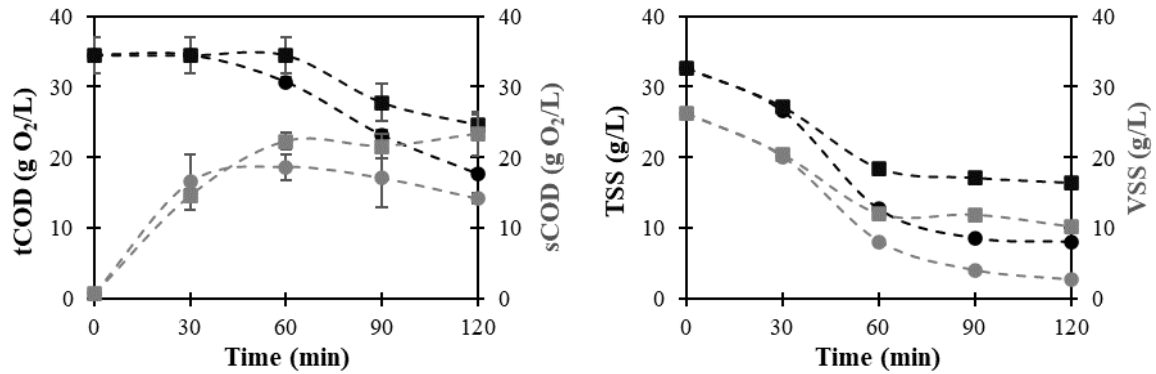


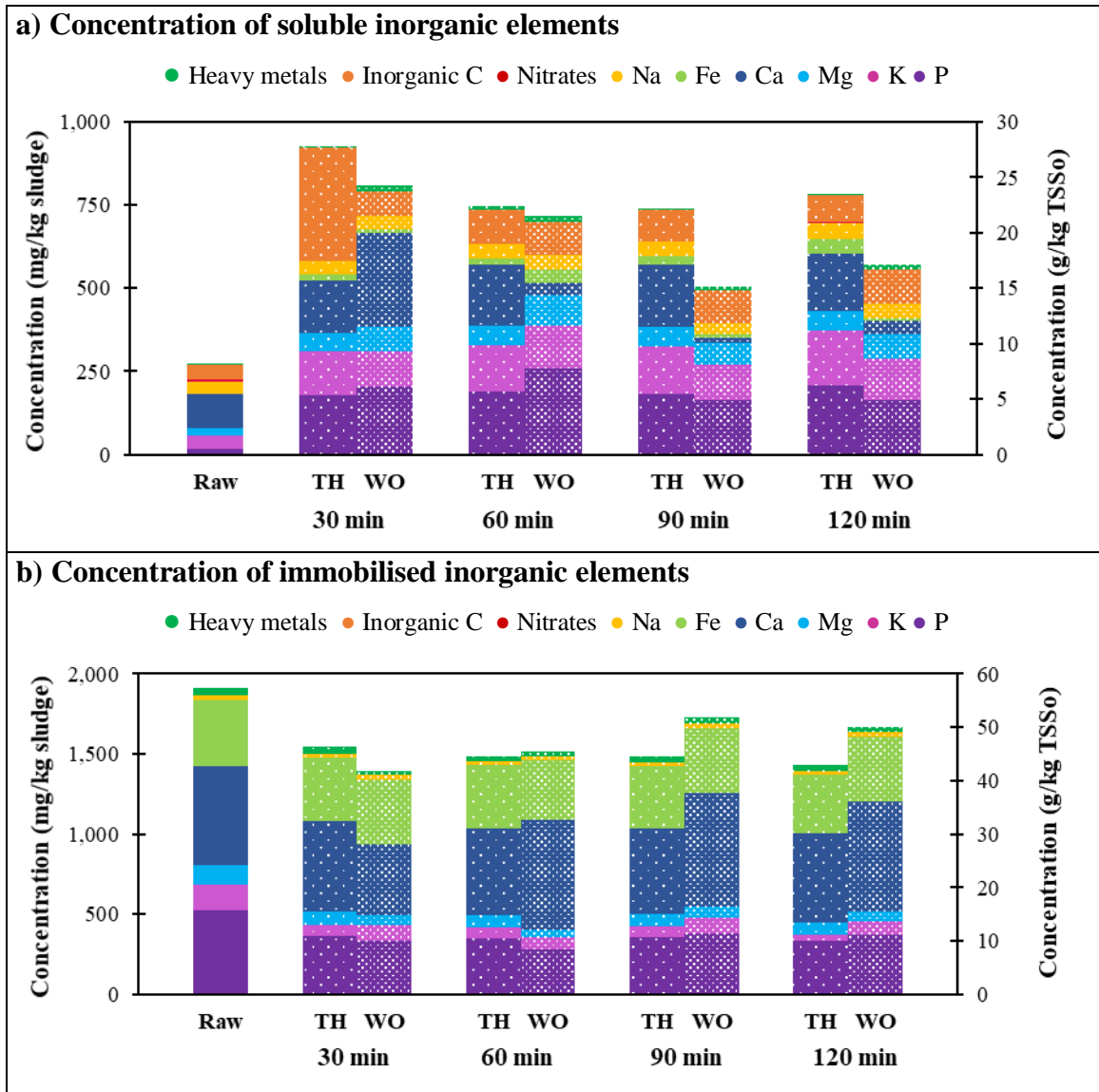
Fig. 2. Changes in tCOD, sCOD, TSS and VSS during wet oxidation (●) and thermal hydrolysis (■) of sewage sludge at 180 °C and 80 bar.

Examining first the changes in tCOD and VSS, as representative parameters of the presence of organic matter in sludge, the cell lysis and the breakage of the bonds between EPS and microorganisms took place mainly during the first 60 min of treatment, by means of a hydrolytic process, releasing intra- and extracellular compounds into the liquid fraction [4,14,29]. This meant that, as this first stage is mainly produced by hydrolysis, no mineralisation and similar solubilisation processes were observed, regardless of the atmosphere. Thus, around 50% of the VSS were solubilised, while only 10% of the tCOD was reduced in the first hour. For longer reaction times, the minimisation of the solid volume was slight, but the mineralisation of the soluble organic matter turned out to be significant (see sCOD changes in Figure 2).

During this period, the solubilised biopolymers were simultaneously degraded into shorter compounds, such as simple sugars, amino acids and volatile fatty acids [9,18]. The combination of hydrolysis and oxidation reactions under an oxidising atmosphere accelerated this conversion, explaining why once the first hour of reaction had elapsed, the type of atmosphere became a key factor. The presence of oxygen resulted in part of these intermediates being subsequently decomposed into carbon dioxide and water [9], which produced continuous mineralisation (reduction of sCOD), with this outweighing the solubilisation rate. On the contrary, under an inert atmosphere, these shorter compounds were only affected by reactions other than oxidation, such as Maillard or condensation reactions, so sCOD continued to increase and the degree of mineralisation was less marked [33]. As expected, as the presence of oxygen caused both hydrolysis and oxidation mechanisms to occur, the final tCOD was 20% lower after wet oxidation than after thermal hydrolysis. In addition, since the difference between tCOD and sCOD represents the COD due to the solid fraction, results revealed that only a very small fraction of the organic compounds remained as solids at the end of treatments (less than 10% of the initial amount after 120 min of treatment) due to the release of most of the solid organic compounds into the liquid fraction.

Similar findings were obtained after observing the changes in the volatile suspended solids, with a fast disintegration of these, especially during the first hour. Moreover, this reduction was more marked in presence of oxygen because it accelerated the process and the subsequent partial oxidation. Thus, after 120 min of treatment in the presence and absence of oxygen, 90% and 61% of the VSS were solubilised, respectively.

Moving on now to inorganic matter, this was also categorised into “soluble” or “solid”, as previously explained, and the composition of these fractions during the hydrothermal treatments is shown in Figure 3.



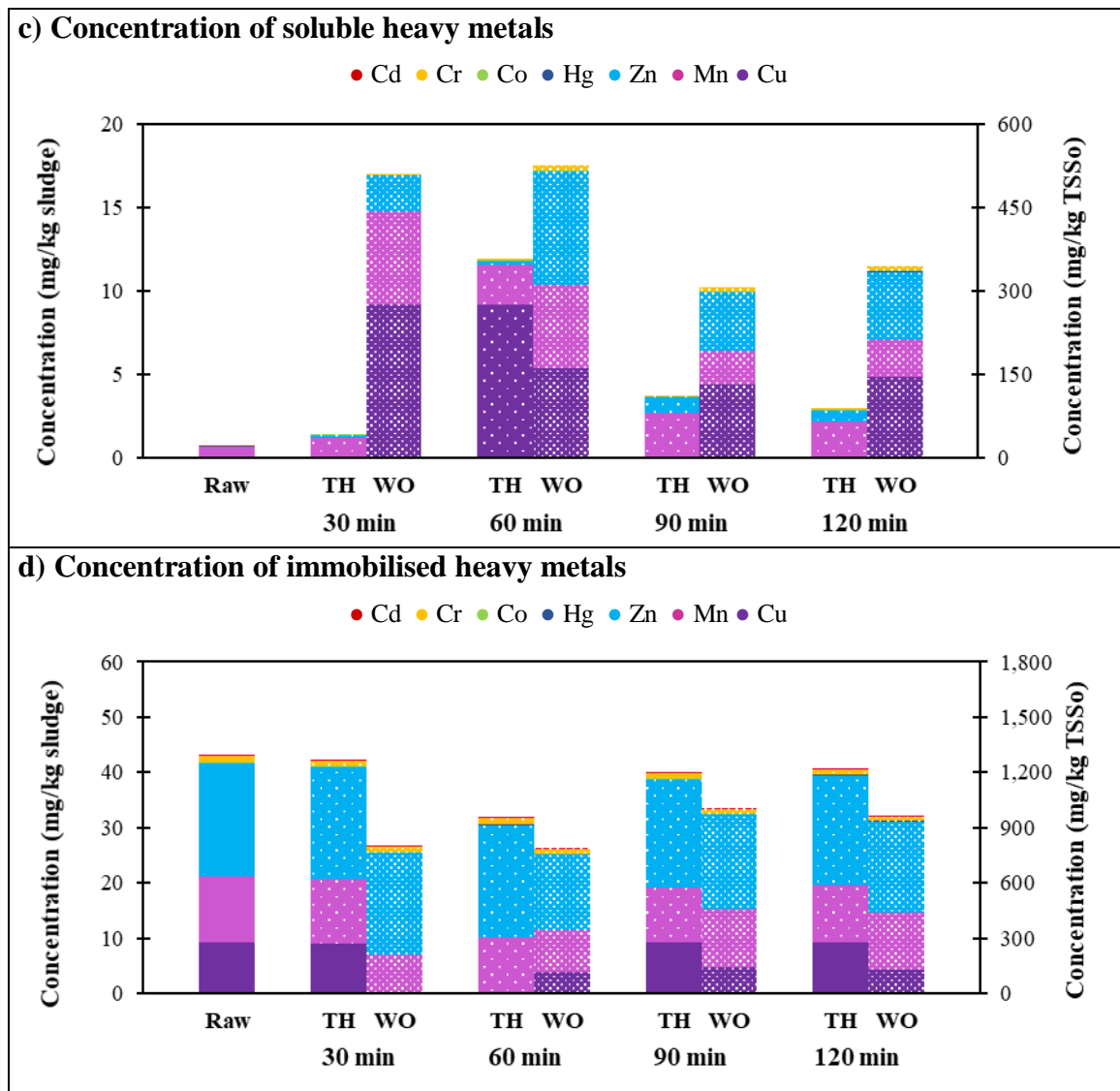


Fig. 3. Changes in soluble (a) and immobilised (b) inorganic elements and, in more detail, soluble (c) and immobilised (d) heavy metals during wet oxidation (WO) and thermal hydrolysis (TH) of sewage sludge at 180 °C and 80 bar.

Both fractions were mainly composed of Ca (22.1 g/kg TSSo) and P (16.5 g/kg TSSo), being predominantly present in the solid fraction in the raw sewage sludge (86% and 97%, respectively). They underwent significant solubilisation during the first 60 min, regardless of the atmosphere and coinciding with the initially fast breakage of the flocs. This explains why the soluble inorganic fraction increased by around 20 g/kg TSSo during the first part of the treatment for both atmospheres. For longer reaction

times, when the greater part of solubilisation had already taken place, the presence of an oxidising atmosphere had an effect on the evolution of many inorganic elements. Thus, under an inert atmosphere, almost all the inorganic compounds had an upward trend in their soluble concentrations. On the contrary, in presence of an oxidising atmosphere, the soluble concentrations of most of them decreased after reaching a maximum, because of the formation of insoluble complexes. This behaviour was closely related to the changes in pH during the corresponding treatment (Figure S.1. in the Supplementary Material), where the acidification of the medium caused by the formation of acidic compounds favoured the solubilisation, whereas the alkalinisation due to the decomposition of part of these intermediates promoted their precipitation by complexation with phosphates [34,35].

There are, however, a few exceptions. The type of atmosphere had no effect on the changes in either K or Na, suggesting that they are mainly associated with the bound EPS in the raw sludge, and also no effect on Cd solubility, which was negligible for all the times and atmospheres tested. Soluble Cu also showed faster initial solubilisation in presence of oxygen, being completely dissolved after 30 min of reaction, in comparison to 60 min in its absence. After this, Cu completely precipitated again after 90 min of thermal hydrolysis, while 53% remained dissolved during wet oxidation. As for nitrates, their concentrations were very low during the treatments, with values lower than 0.2 g/kg TSS₀ and negligible in absence of oxygen.

Furthermore, it is interesting to note that dissolved inorganic C was present in low concentrations in comparison to total dissolved carbon during both treatments. A slight increase in its concentration was observed during the first 30 min, higher in absence of oxygen, although similar values were then reached under both atmospheres and the soluble concentration remained constant in the liquid phase until the end of the

treatments. This behaviour was attributed to the stripping of the CO₂ due to the continuous gas flow.

Finally, the evolution of the inorganic solid fraction during the hydrothermal treatments can be followed by analysing the fixed suspended solids (FSS), which were calculated as the difference between TSS and VSS. FSS values remained similar throughout the reactions (around 0.18 g/kg TSSo), which indicates that the solubilisation of the inorganic compounds was much lower than that of the organic ones. It is also interesting to notice that the percentage of inorganics that were dissolved was very small, so changes in FSS would not be noticeable even if dissolved compounds precipitated in large quantities. However, the percentage of FSS in TSS did increase due to the progressive solubilisation of organic compounds, promoted by the presence of oxygen. Therefore, TSS went from being composed of 20% FSS to 67% or 38% after 120 min of wet oxidation or thermal hydrolysis, respectively.

3.2. Bioassimilation of organic matter

The solubilisation, mineralisation and inorganic matter having been discussed, the effect of the atmosphere on the “sum parameters” and, mainly, on the specific composition of the soluble organic fraction of the hydrolysates will now be analysed. This fraction can be quantified as sCOD (Figure 1) and this is usually subdivided into non-biodegradable (nbCOD), slowly biodegradable (sbCOD) and readily biodegradable COD (rbCOD). The former is the part of the soluble organic fraction that cannot be degraded by heterotrophic microorganisms, the second encompasses the larger molecules which require hydrolysis before their biodegradation by the biomass, such as proteins or complex carbohydrates, and the last one includes the compounds that are directly available for their bioassimilation by the microorganisms, like volatile fatty

acids, simple sugars or amino acids [20–22]. The changes observed in these COD fractions for the different hydrolysates are shown in Figure 4.

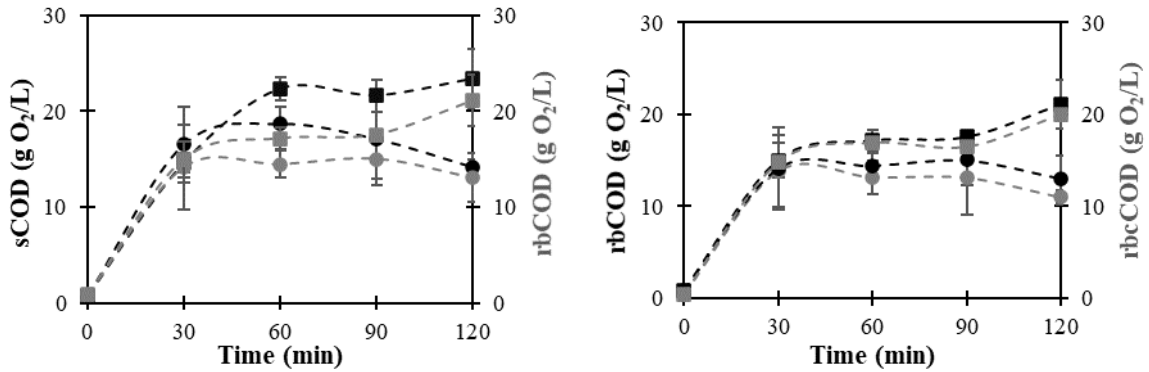


Fig. 4. Changes in sCOD, rbCOD and rbcCOD during wet oxidation (●) and thermal hydrolysis (■) of sewage sludge at 180 °C and 80 bar.

Results revealed that, before the treatment, the concentration of soluble organic matter in the raw sludge was low but easily assimilated by the microorganisms, the values of sCOD and rbCOD almost coinciding. Specifically, 41% of sCOD corresponded to rbCOD due to easily assimilable carbon compounds and the rest was attributed to rbcCOD due to nitrification (this latter value was obtained taking into account the explanations given below in section 3.2.1). As the solubilisation took place, especially during the first 30 min, COD corresponding to readily biodegradable compounds underwent a remarkable increase and to a lesser extent, that corresponding to slowly and non-biodegradable compounds also went up. In fact, the presence of an oxidising atmosphere led to faster solubilisation of sb/nbCOD compounds, due to the simultaneous presence of oxidation and hydrolysis reactions. Thus, in presence of oxygen, after only 30 min, 15% of the total sCOD was attributed to slowly and non-biodegradable compounds, while almost all the total was rbCOD in its absence. After 60 min, when the solubilisation rate was low, rbCOD increased under an inert atmosphere, but slightly decreased under an oxidising one and the values were lower, suggesting that

the readily biodegradable compounds were degraded by oxidation reactions, but not by hydrolysis reactions. This was also confirmed by the higher degree of mineralisation for wet oxidation than for thermal hydrolysis, as previously explained. Moreover, although sb/nbCOD was always significantly lower than rbCOD for all the times and atmospheres tested, it was observed after the first hour of treatment that this fraction was higher under an inert atmosphere. Despite the fact that both atmospheres decomposed the complex molecules into simple substrates, these were partially oxidised in presence of oxygen, whereas other more refractory and less assimilable compounds, such as Maillard products, were produced in its absence [33]. Thus, final sb/nbCOD values of 1.1 and 2.3 g O₂/L (0.04 and 0.07 g sb/nbCOD/g TSSo) after 120 min of wet oxidation and thermal hydrolysis, respectively, were obtained. In conclusion, after 2 h of treatments, 38% and 61% of the initial tCOD were turned into rbCOD in presence and absence of oxygen, which corresponded to yields of 0.4 and 0.6 g rbCOD/g TSSo, respectively.

In order to go deeper into the topic, the effects of the time and type of atmosphere during the hydrothermal treatments on the behaviour of the main compounds responsible for the rbCOD or sb/nbCOD fractions are discussed in more detail in the following subsections.

3.2.1. Readily biodegradable COD

As mentioned above, rbCOD is formed by organic compounds which are directly available to the microorganisms. These can be categorised into two fractions, rbCOD due to carbon (rbcCOD) and nitrogen (rbcnCOD) oxidations. The former is composed of compounds that are readily biodegradable by the heterotrophic mass but are not affected by nitrifying microorganisms. On the other hand, the latter includes simple and easily assimilable nitrogen compounds present in the hydrolysates. It is

important to take into account that the nitrates and the organic nitrogen contained in proteins were not included in this category. This is because nitrates do not undergo nitrification, so they were considered soluble inorganic matter (Figure 3). Regarding organic nitrogen, as proteins are composed of 16% nitrogen on average and are poorly biodegradable, only non-protein organic nitrogen was included in this fraction, while proteins were categorised as part of sb/nbCOD [36,37]. Bearing this in mind, the changes in the main soluble components of rbCOD during the hydrothermal treatments are shown in Figure 5.

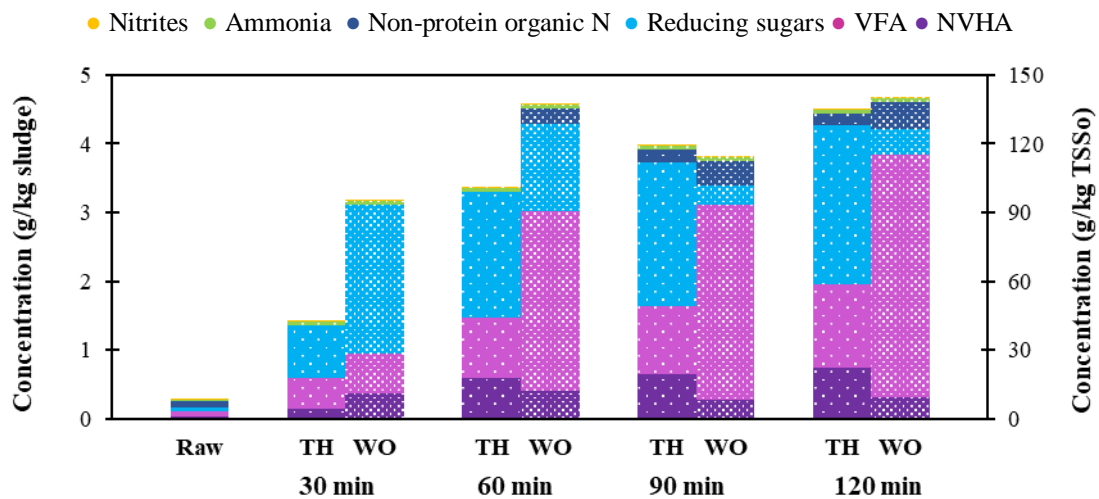


Fig. 5. Changes in soluble components of rbCOD during wet oxidation (WO) and thermal hydrolysis (TH) of sewage sludge at 180 °C and 80 bar.

3.2.1.1. *rbCOD*

When it comes to the liquid fraction, more than 95% of total soluble carbon was organic during the hydrothermal treatments, regardless of the reaction time and the atmosphere employed. This organic carbon includes either readily or slowly and non-biodegradable compounds. Focusing on rbCOD, that is to say, carbon-based compounds with a high uptake rate by microorganisms, this usually comprises simple molecules such as organic acids and reducing sugars, among others like amino acids and peptides [5,38–40]. In this sense, the progressive changes in both carboxylic acids

and reducing sugars were determined and related to the rbcCOD values previously reported.

Turning to organic acids, these also underwent an initial fast solubilisation, although later their behaviour depended on the type of acid and atmosphere. Under an inert atmosphere, both NHVA and VFA soluble concentrations rose throughout the whole treatment due to the absence of oxidative reactions. Under an oxidising atmosphere, the former were partially degraded due to their oxidation to shorter compounds or carbon dioxide, while the concentration of the latter continued to increase, mainly due to the formation of acetic acid by the oxidation of more complex organic matter [41], as expected from Figure 4. More specifically, NHVA reached higher values during thermal hydrolysis: from an initial value of 1 g/kg TSSo (2.6% rbCOD) to 9.6 g/kg TSSo (2.1% rbCOD) and 22.5 g/kg TSSo (3.1% rbCOD) in presence and absence of oxygen, respectively, after 120 min. On the contrary, VFA production was higher during wet oxidation than during thermal hydrolysis, varying from 2.5 g/kg TSSo (3.2% rbCOD) initially to 107.5 g/kg TSSo (27.3% rbCOD) and 37.4 g/kg TSSo (5.4% rbCOD) in presence and absence of oxygen at the end of the treatment.

Moving on to reducing sugars, their evolution was closely related to the progressive changes in total carbohydrates concentrations, as will be seen in section 3.2.2. Initially, while the solubilisation was taking place, the solubilised complex carbohydrates were degraded into shorter compounds (simple sugars), with this process being accelerated in presence of oxygen [9]. For this reason, after 30 min of treatment, the concentration of reducing sugars in the liquid fraction increased from 1.7 g/kg TSSo in the raw sludge (69% of total carbohydrates) to 66.2 g/kg TSSo (47%) in presence of oxygen and 23.2 g/kg TSSo (24%) in its absence. Once this time had elapsed, when

solubilisation slowed down, the previously solubilised carbohydrates were transformed into reducing sugars by either hydrolysis or oxidation mechanisms. Nevertheless, oxidation also caused a further degradation of these sugars towards their complete mineralisation. In this way, at the end of the treatments (120 min), the generation of soluble reducing sugars was 11.9 g/kg TSSo or 70.5 g/kg TSSo in presence or absence of oxygen, representing 88% and 77% of the total carbohydrates available in the liquid medium, respectively. Linking these concentrations with COD values by using glucose as the reference model, reducing sugars represented 6.9%, 3.2% and 11.7% of the total rbCOD for the initial sludge or the hydrolysates after 120 min of wet oxidation and thermal hydrolysis treatment, respectively (see Figure S.2. in the Supplementary Material).

3.2.1.2. *rbnCOD*

With regard to rbnCOD, it is interesting to focus first on how nitrogen was solubilised and transformed during the hydrothermal treatments. Initially, 95% of the total nitrogen was in the solid fraction, representing 7.5% of the TSSo; and the rest was dissolved in the liquid fraction, of which 75% was organic nitrogen. After 30 min of reaction, the concentration of dissolved nitrogen increased rapidly, from 4 g/kg TSSo to 15.4 g/kg TSSo under an oxidising atmosphere and 12.2 g/kg TSSo under an inert one, 87% of this being organic nitrogen. This organic to total nitrogen ratio was maintained until the end of the treatments, regardless of the type of atmosphere. After 60 min of treatment, when the solubilisation stage was low, the trends described for total solubilised nitrogen were similar to those seen for the majority of compounds, decreasing in presence of oxygen (14.3 g/kg TSSo) and increasing in its absence (13.3 g/kg TSSo).

Turning to the different nitrogen species, for the raw sludge, 3 g/kg TSSo of organic nitrogen was dissolved and, in turn, around 14% of this corresponded to soluble proteins, the other 76% probably being attributed to urea, nucleic acids, free amino acids, creatine, creatinine and uric acids, among others [37,42]. Once the treatment started, soluble organic nitrogen concentration quickly increased, as previously explained. Simultaneously, during this initial stage of fast solubilisation, the soluble protein concentration rose considerably as well (see Figure 6), indicating that soluble organic nitrogen was mainly due to proteins. For longer reaction times, as the proteins started to be degraded (see section 3.2.2 and Figure 6), their breakdown products, such as free amino acids or polypeptides, started to be formed, this reaction being accelerated by dissolved oxygen [43]. In such a way, non-protein organic nitrogen increased until it represented 96 or 48% of the total soluble organic nitrogen after 120 min of treatment in presence or absence of oxygen, respectively. In turn, the concentration of soluble nitrites was almost negligible (< 40 mg/kg TSSo), and thus their contribution to nitrification was discarded.

Finally, the generation of ammonia was low but favoured by the oxidising atmosphere, with values of 0.8 g/kg TSSo in raw sewage sludge, and 1.9 g/kg TSSo and 1.7 g/kg TSSo after 120 min of treatment with or without oxygen. This increase was due to the degradation of proteins as well, which formed ammonia as a final product [2]. However, it must be noted that ammonia could have been partially stripped by the gas stream bubbled into the reactor, which would explain why the soluble concentration of this compound remained almost constant after the first stage of solubilisation.

As for rbnCOD, knowing that 4.6 kg O₂/kg N are stoichiometrically consumed during nitrification, the theoretical rbnCOD values were obtained. Thus, despite the fact that 59% of rbCOD was due to nitrogen oxidation in raw sewage sludge, after the first

stage of solubilisation (30-60 min), the bio-oxidation of carbon became the predominant fraction and rbnCOD represented only 2% of rbCOD (see Figure 4). Afterwards, due to the increase in nitrifying species in the liquid phase, the percentage represented by rbnCOD increased until the end of the treatment, it being 16% under an oxidising atmosphere or 5% under an inert one (2.1 and 1.1 g O₂/L, respectively).

3.2.2. Slowly and non-biodegradable COD

As previously explained, some compounds cannot be directly degraded by microorganisms because of their complexity or inhibitory character. The former reason corresponds to the main constituents of EPS (proteins, complex carbohydrates, humic acids and uronic acids), whereas HMF, furfural and phenolic compounds are representative of the latter [4,14,40,44]. All of them were categorised as slowly or non-biodegradable COD (sb/nbCOD) and the changes in the concentrations of the main soluble components of this fraction during the hydrothermal treatments are shown in Figure 6.

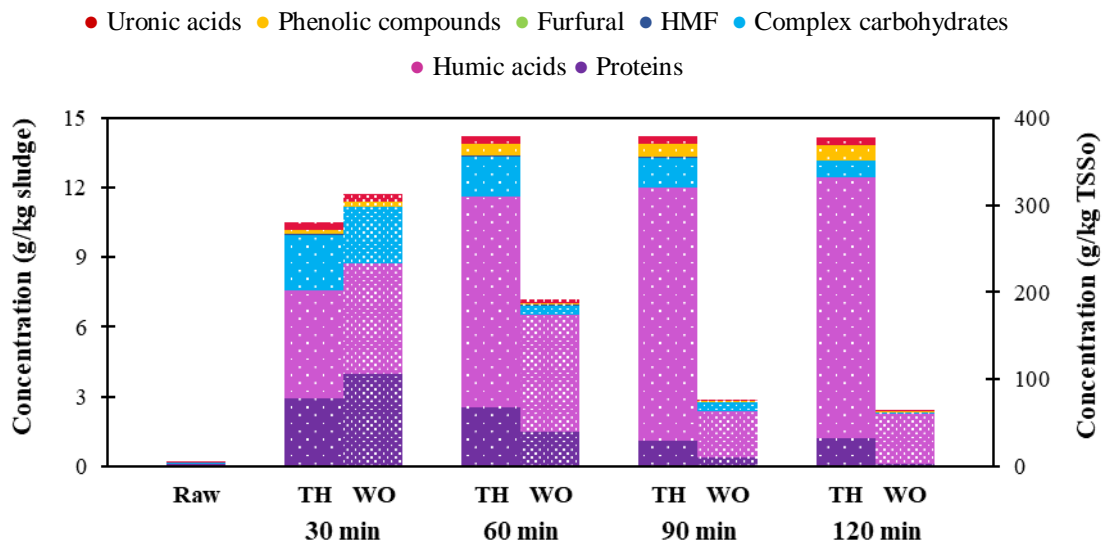


Fig. 6. Changes in soluble components of sb/nbCOD during wet oxidation (WO) and thermal hydrolysis (TH) of sewage sludge at 180 °C and 80 bar.

Focusing firstly on the main biopolymers studied (proteins and humic acids), results confirmed the initial fast solubilisation during the first 30 min, due to the rupture of the bonds between EPS and microorganisms. In presence of oxygen, this solubilisation was accelerated by the simultaneous presence of hydrolysis and oxidation reactions [9]. At this time (30 min), the soluble protein concentration increased from 2.5 g/kg TSSo to 121.7 g/kg TSSo in presence of oxygen and to 89.1 g/kg TSSo in its absence; while the humic acid concentration rose from 1 g/kg TSSo to 145.1 and 142.4 g/kg TSSo, respectively. Once this time had elapsed, the atmosphere effect was more marked and depended on the type of biopolymer. Thus, humic acids were stable and continued to solubilise under an inert atmosphere (342.5 g/kg TSSo after 120 min), but they were degraded in presence of oxygen (66.4 g/kg TSSo after 120 min). In contrast, solubilised proteins were unstable during both hydrothermal treatments, especially in presence of oxygen, where they were almost completely removed after 120 min, transformed into carbon dioxide, ammonia and water by oxidation reactions [9]. This instability of soluble proteins under an inert atmosphere was attributed to Maillard reactions, which converted them into melanoidins and Amadori compounds [33], which, in turn, can act as inhibitors. Thus, at the end of the treatment (120 min), the concentration of proteins in the liquid fraction was 3.1 g/kg TSSo (wet oxidation) and 37.7 g/kg TSSo (thermal hydrolysis). With respect to the trends observed in complex carbohydrates, it is necessary to take into account the explanations given above for reducing sugars (see section 3.2.1.1). During the first 30 min of reaction, both reducing sugars and complex carbohydrates were solubilised from the solid fraction of sewage sludge, the latter increasing from 0.8 g/kg TSSo to 73.7 g/kg TSSo regardless of the atmosphere. The complex carbohydrates were subsequently degraded into reducing sugars or, in presence of oxygen, even into carbon dioxide. For that reason, their

concentration followed a downward trend, more pronounced under an oxidising atmosphere. So, at the end of the treatment (120 min), the soluble complex carbohydrate concentration was 1.6 g/kg TSSo after wet oxidation and 21.5 g/kg TSSo after thermal hydrolysis. In terms of COD, owing to the heterogeneity of these biopolymers, the theoretical sb/nbCOD could not be obtained, so they would be part of the contribution of other compounds to sb/nbCOD in Figure S.3 in the Supplementary Material.

As for uronic acids, their trend was similar to that observed for humic acids. Firstly, a remarkable increase in their soluble concentration was produced during the first 30 min of reaction, regardless of the atmosphere (from 0.1 g/kg TSSo to 9.1 g/kg TSSo), which is mainly caused by their release from EPS and inside the cells by lysis [14]. Afterwards, they were stable under an inert atmosphere (10.0 g/kg TSSo after 120 min) but degraded in presence of oxygen (2.4 g/kg TSSo after 120 min). So, in terms of the percentage of sb/nbCOD, calculated as glucuronic acid equivalent, these final values represented 5.6% of sb/nbCOD after wet oxidation and 11.9% after thermal hydrolysis (Figure S.3. in the Supplementary Material).

Moving on now to inhibitory molecules, three compounds were selected as the most representative of this category: HMF, furfural and phenolic compounds, as they are typical fermentation inhibitors [40,44].

Starting with HMF and furfural, the type of atmosphere played a key role in their progression. Under an inert atmosphere, significant production of both compounds by hydrolysis reactions [40] was observed during the first 60 min of thermal hydrolysis (in the case of HMF, from 0.05 g/kg TSSo to 1.3 g/kg TSSo and in the case of furfural, from 0.07 g/kg TSSo to 0.5 g/kg TSSo), with their concentrations remaining almost constant for furfural or slightly decreasing for HMF, as they are products of Maillard reactions as well [40]. Therefore, the final concentrations in the liquid phase were 0.9 g

HMF/kg TSSo and 0.6 g furfural/kg TSSo, representing 2.1% and 1.4% of sb/nbDQO, respectively (Figure S.3. in Supplementary Material). On the other hand, under an oxidising atmosphere, the trends were similar, but the concentrations of both compounds were significantly lower than under an inert one, possibly due to their oxidation to other derivative compounds such as shorter furans, 2,5-furandicarboxylic acid, 2-furoic acid or other carboxylic acids [40,45]. Thus, the maximum concentrations of furfural and HMF were achieved after 30 min, with values of 1.1 g HMF/kg TSSo and 0.2 g furfural/kg TSSo for wet oxidation. Nevertheless, if the end of the treatments (120 min) is taken as the reference time, the following values were obtained: 0.2 g HMF/kg TSSo and 0.1 g furfural/kg TSSo. So, after wet oxidation, the percentage of sb/nbDQO due to HMF or furfural was half for the former or a third for the latter of the percentages obtained at the end of thermal hydrolysis. The concentration at which these compounds start to affect the growth of microorganisms is around 1 g/L [40]. In this case, the concentrations did not exceed 43 mg HMF/L and 19 mg furfural/L, so the inhibitory effects could be discarded. Moreover, given the low concentrations registered, the possible compounds formed by their degradation or oxidation would also be at negligible concentrations.

When it comes to phenolic compounds, their formation was also favoured under an inert atmosphere and short times of reaction, as they are products of carbohydrate hydrolysis [40]. As an example, the maximum concentration of phenolics was achieved after 30 min of treatment with 7.0 g/kg TSSo, before falling to 0.8 g/kg TSSo after 120 min in presence of oxygen. On the contrary, phenolic compounds showed a continuous upward trend during the 120 min of the thermal hydrolysis, reaching a final concentration of 20.3 g/kg TSSo. In terms of percentage of sb/nbCOD, in phenol equivalent, phenolics would represent 5.5% after wet oxidation and 69.7% after thermal

hydrolysis. Due to their heterogeneity, they are usually divided into groups according to their structure and, in general, the higher the molecular weight, the lower the toxicity to microorganisms [40].

Finally, it is interesting to point out that the physicochemical properties of the liquid fraction, especially viscosity, are related to the biopolymers and compounds released from the flocs [29]. Regarding density (see Figure S.1. in Supplementary Material), it hardly changed throughout the hydrothermal treatments, regardless of the atmosphere tested. This parameter showed a slight increasing trend, although the increment was less than 1% after 120 min of reaction. Moreover, the values obtained were almost identical to the density of water at the temperature of measurement (25 °C), since the initial relative density was 1.001 and at the end of the treatments, 1.007 after wet oxidation and 1.009 after thermal hydrolysis. Regarding viscosity (see Figure S.1. in Supplementary Material), the raw liquid fraction of sewage sludge had a pseudoplastic behaviour, with $\tau_0 = 0$ and $n = 0.82 (< 1)$. During the first hour of reaction, this pseudoplastic character became more pronounced due to the solubilisation of biopolymers and the cellular lysis [29]. This was more noticeable in absence of oxygen owing to the lack of oxidation reactions decomposing the soluble intermediates. In fact, the flow behaviour index reached a minimum between 30 and 60 min of treatments, achieving values of 0.66 and 0.54 in presence and absence of oxygen, respectively, while the flow consistency index increased, from 0.002 Pa·sⁿ to 0.006 Pa·sⁿ and 0.015 Pa·sⁿ, after one hour of reaction. Afterwards, the behaviour tended towards that seen initially, especially during wet oxidation. The final values achieved for n were 0.79 and 0.67 after 120 min of wet oxidation and thermal hydrolysis, while for K they were 0.002 Pa·sⁿ and 0.006 Pa·sⁿ. In parallel with these results, in Figure S.4. (Supplementary Material) it can be seen that apparent viscosity followed the same trend

as the flow behaviour index. So, the highest values were obtained after 30 min and in absence of oxygen. In addition, the results demonstrated that sludge hydrolysate did not exhibit thixotropic behaviour at any time throughout the experiments.

4. Conclusions

A classification based on chemical characteristics and biodegradability of the sludge products in hydrothermal treatments has been made. Furthermore, this work has deepened understanding of the role of oxygen in the reaction mechanisms and the characterisation of the composition of hydrolysates obtained. In general, after the initial stage of solubilisation (30 – 60 min), the presence of oxygen caused the mineralisation of some intermediates and the precipitation of insoluble complexes; while its absence favoured other processes, such as Maillard or condensation reactions. This explains the distribution of compounds and COD values in the fractions of thermally treated sludge. After 120 min of hydrothermal treatments at 180 °C and 80 bar, inorganic compounds represented 67% and 38% of TSS in the oxidised and hydrolysed solid phase, of which Ca, P and Fe were predominant. Moreover, a higher reduction of TSS was achieved under an oxidising atmosphere (75% compared to 50% under an inert one). Concerning the final liquid phase, readily assimilable molecules, but also poorly biodegradable compounds were in higher concentration after thermal hydrolysis. However, it is noteworthy that wet oxidation favoured the formation of VFA due to the conversion of some intermediate compounds into acetic acid, while thermal hydrolysis promoted the formation of inhibitors.

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Supplementary Material

A PROPOSAL FOR THE CLASSIFICATION OF SLUDGE PRODUCTS THROUGHOUT HYDROTHERMAL TREATMENT

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Table S.1. Physicochemical properties of liquid fraction from sludge.

Parameter	Liquid fraction
Soluble chemical oxygen demand (sCOD) (g O ₂ /L sludge)	0.8 ± 0.2
Readily biodegradable chemical oxygen demand due to carbon oxidation (rbcCOD) (mg O ₂ /L sludge)	360 ± 50
Theoretical readily biodegradable chemical oxygen demand due to nitrogen oxidation (rbcCOD) (mg O ₂ /L sludge)	510 ± 10
Non-volatile hydroxy acids (mg/L sludge)	32 ± 3
Volatile fatty acids (mg/L sludge)	81 ± 7
Reducing sugars (mg/L sludge)	56 ± 8
Organic nitrogen (mg/L sludge)	98 ± 1
Non-protein organic nitrogen (mg/L sludge)	85 ± 1
Ammonia (mg N-NH ₃ /L sludge)	26.6 ± 0.4
Nitrites (mg N-NO ₂ ⁻ /L sludge)	0.143 ± 0.001
Nitrates (mg N-NO ₃ ⁻ /L sludge)	5.58 ± 0.03
Proteins (mg/L sludge)	83.3 ± 0.7
Humic acids (mg/L sludge)	31.8 ± 0.8
Complex carbohydrates (mg/L sludge)	26 ± 6
Hydroxymethylfurfural (mg/L sludge)	1.8 ± 0.5
Furfural (mg/L sludge)	2.4 ± 0.8
Phenolic compounds (mg/L sludge)	1.1 ± 0.1
Uronic acids (mg/L sludge)	4.1 ± 0.2
Density (ρ) (g/mL)	0.998 ± 0.001
Flow behaviour index (n)	0.82 ± 0.04
Flow consistency index (K) (Pa·sn)	0.002 ± 0.001

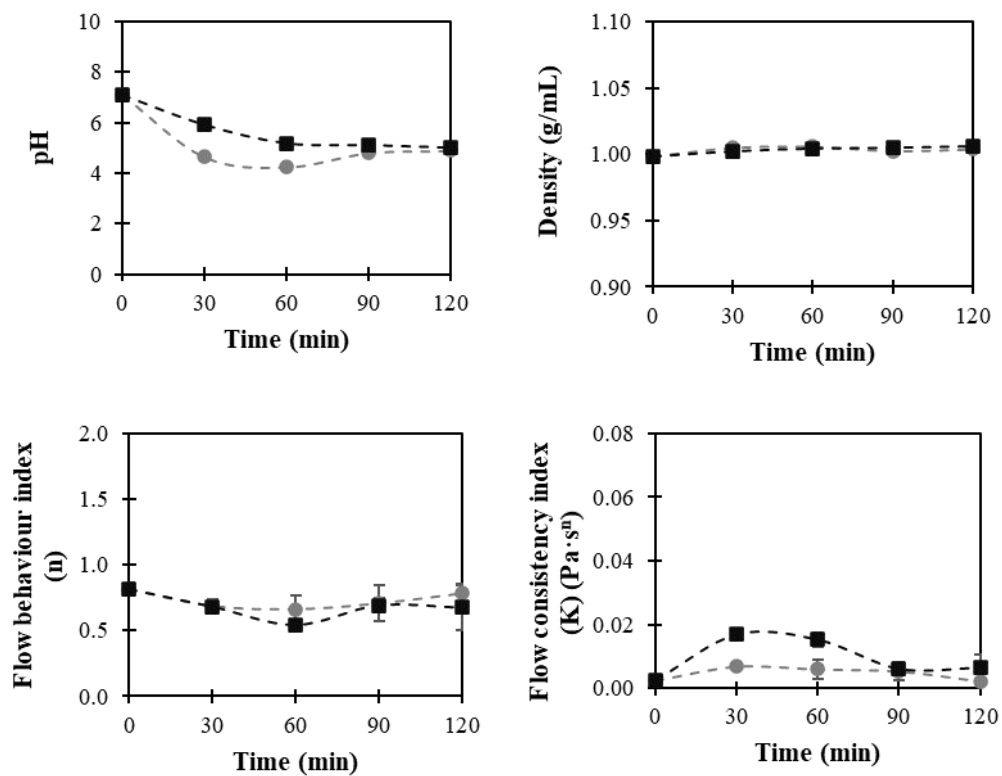


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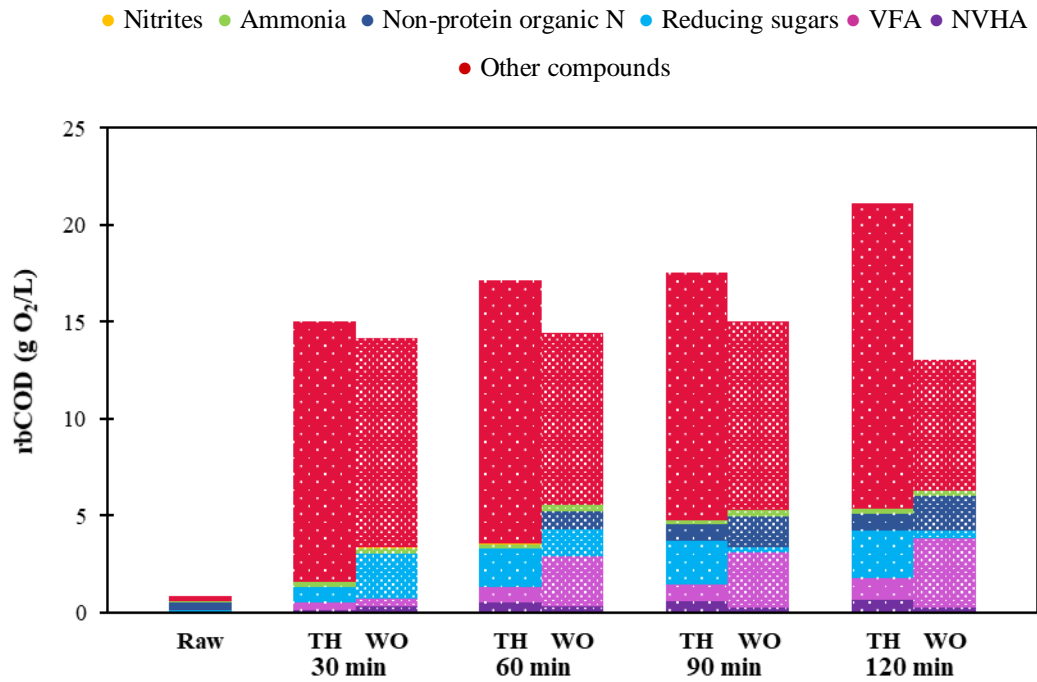


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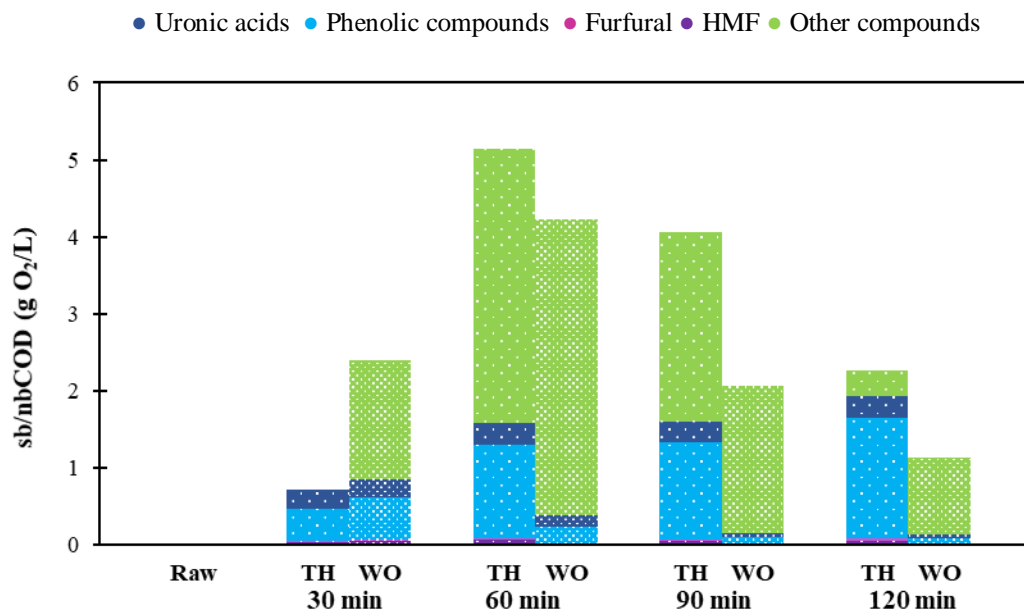
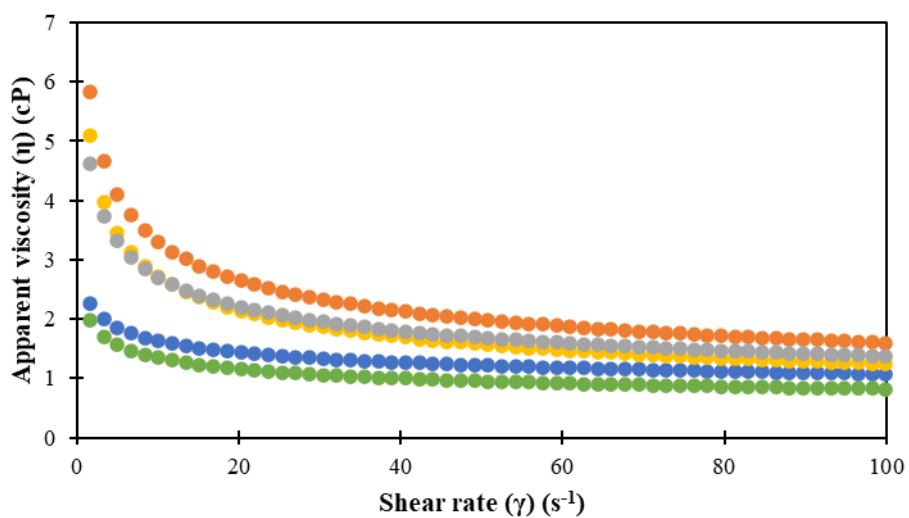


Figure S.3. Changes in sb/nbCOD compounds during wet oxidation (WO) and thermal hydrolysis (TH) of sludge. In all cases: temperature, 180 °C; pressure, 80 bar; stirrer speed, 150 rpm; gas flow rate, 1,800 mL/min.

a) Wet oxidation



b) Thermal hydrolysis

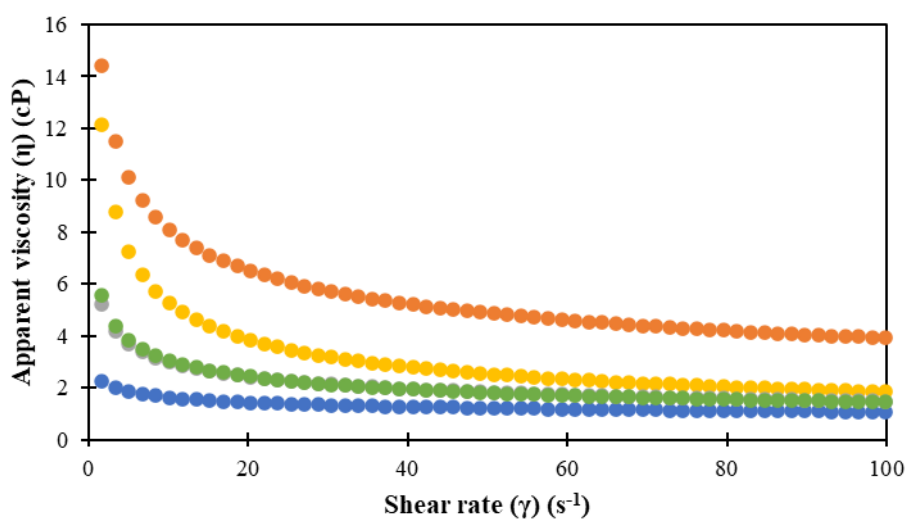


Figure S.4. Changes in apparent viscosity of liquid fraction at 25 °C with shear rate.

Samples: raw supernatant (●) and 30 (●), 60 (●), 90 (●) and 120 min (●) of wet oxidation (a) and thermal hydrolysis (b). In all cases: temperature, 180 °C; pressure, 80 bar; stirrer speed, 150 rpm; gas flow rate, 1,800 mL/min.