Evaluation of *Phanerochaete chrysosporium* for swine wastewater treatment

Ana Isabel Díaz [a], Adriana Laca [a,*], Mercedes Sánchez [b], Mario Díaz [a]

[a] Department of Chemical and Environmental Engineering. University of Oviedo, C/Julián Clavería, s/n, Oviedo 33006, Asturias, Spain
[b] Department of Agroforestry Sciences, ETSIIAA, University of Valladolid, Avenida de Madrid 56, Palencia, Castilla y León, 34004 Spain

ABSTRACT

The swine slurry is a mixture of manure, urine, and cleaning water from pig livestock farms, characterized by its high chemical oxygen demand (COD) and ammonia concentration. This effluent needs to be treated before being spilled to avoid negative environmental impacts as eutrophication of surface water reservoirs. In this study, an untreated slurry and two pig slurries pre-treated by anaerobic digestion with and without ammonia trapping were treated biologically by *Phanerochaete chrysosporium*. The effect on the biological treatment of pre-treating the raw slurry by sieving and centrifugation was evaluated. Samples were inoculated with 3 g/L (dry matter) of fungus and incubated at 26 °C during 10 days in a batch reactor. The activity of ligninolytic enzymes (LiP, MnP and Lac), colour, COD, total nitrogen and BOD_5 evolution during the treatment were analysed. For undigested slurries, the best results were obtained for sieved slurry, reaching removal efficiencies for soluble COD and colour around 68% and 78%, respectively. For digested effluents, the digestate coming from the reactor with ammonia trapping membrane showed the best results with efficiencies of 38% for soluble COD and 35% for colour. These results provide new insights into the application of this fungus as complementary method to treat swine wastewaters.

1. Introduction

The swine industry has expanded rapidly in Spain, currently reaching around 40% of final livestock production [1]. Consequently, the volume of swine wastes generated by this sector has been increased. The swine wastewater is a farming effluent resulting from the mixture of pig manure, urine and the water used to clean the pigsty, which is characterized for its high nitrogen, phosphorus, colour and organic matter concentration. [2,3]. Swine wastewater usually has a high content of organic materials in the form of volatile solids (VS). Within this organic matter, the largest fraction is represented by carbohydrates, followed by proteins, lipids, lignin and volatile fatty acids (VFA) [4]. Lignocellulosic matter is mainly present in manure fraction and corresponds to complex compounds that have not been fully digested by the animals. In addition, this piggery effluent also contains feed and bedding residues (sand, straw, sawdust) [5] and some phenolic compounds such as p-cresol, p-ethylphenol and phenol, which are known to contribute to unpleasant chemical odors from swine wastewater [6].

Normally, this wastewater is used as biofertilizer, precisely due to its high content of nutrients. However, because of the high slurry production in intensive farms, a large amount of this effluents needs to be managed and stored until be treated. Important environmental problems, such as acidification, soil contamination, water eutrophication, as well as the emission of gases that increase the greenhouse effect, results from a wrong management and discharge into the environment of this effluent [7,8]. This situation has encouraged the development of different technologies to treat this effluent at low costs and without environmental risks.

Different physico-chemical techniques such as ozonation, photocatalysis, electrochemical oxidation, electrocoagulation and ultrasounds, among others, have been evaluated to treat this effluent in order to reduce its chemical oxygen demand (COD) or/and improve its biodegradability and nutrients concentration [9,10]. Nevertheless, most of these methods require high energy demand and the addition of chemicals to the process, increasing operational costs [11]. Thus, the use of anaerobic and aerobic biological methods has drawn extensive

attention. In particular, the anaerobic digestion (AD) of slurry to produce biogas has been applied worldwide [12]. However, it is difficult to make the process economically sustainable. The high concentration of pollutants, as well as the complexity of the lignocellulosic materials present in the slurry, makes necessary a pre-treatment to improve the final performance of the process. Moreover, the digestate resulting from the process contains still high concentration of phosphorus, nitrogen, recalcitrant matter and residual antibiotics which needs to be treated before being discharged [5]. Recent research has focused on the use of microorganisms capable of growing under both aerobic and anoxic conditions, such as purple bacteria or phototrophic algae [3,13,14]. However, the problem of the wine slurry and wastewaters are still far to be solved. For this reason, some countries have promoted the creation of thermoelectric power plants using natural gas to dry the slurries. Nevertheless, this solution needs of the economic support of Government.

The use fungi as complement for biological treatments, has several advantages over the use of just bacteria since they can survive in more adverse conditions of pH and nutrient requirements. Fungi are able to synthesize extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) or laccase (Lac), which allow them to degrade complex or recalcitrant compounds that could not be used for bacteria [15,16]. The application of fungi for the processing of swine wastewaters and pig manures have been mainly focused on its use as pre-treatment to accelerate composting processes [17–19] or enhance biogas production [20–22]. Moreover, considering the ability of fungi to produce extracellular enzymes, some studies have focused on the possibility of using the swine wastewaters as raw material for the production of lignolytic, cellulolytic and proteolytic enzymes with important biotechnological applications [23,24]. In addition, fungal treatments have been reported as promising alternative to remove toxic metals, such as copper or zinc [25]. In addition, these microorganisms can degrade antibiotics present in swine farms such as tiamulin [26]. The presence of antibiotics in swine wastewaters hinders others biological treatment processes, such as anaerobic digestion, due to its compounds inhibited the volatile fatty acids degrading bacteria and acetoclastic methanogens [27]. Other studies open the possibility of using swine wastewaters as growth medium for algae cultivation. In this regard, Liu et al. [28] reported a reduction of the endogenous microbial diversity in a digested piggery wastewater inoculated with *Phanerochaete chrysosporium*. This reduction increased the growth of algae around 70% in comparison to the growth obtained in untreated wastewater. Several authors have been described that swine wastewaters are rich in pathogenic enteric bacteria like *Listeria monocytogenes, Cryptosporidium, Escherichia coli, Salmonella, Citrobacter freundii, Yersinia,* and *Campylobacter,* among others [5].

The fungus *Phanerochaete chrysosporium* has been a model of study for many authors due to its capacity to degrade lignin and other xenobiotic compounds thanks to the extracellular enzymes it synthesizes, such asMnP and LiP. This fungus has been shown to be very promising for treating effluents from paper and pulp [29], textile [30] and olive oil industries [31], and digestates from piggery wastewater [28] and anaerobic sewage sludge treatment [15]. For all these reasons, this fungus is a good candidate for the treatment of the samples evaluated in this work.

The aim of this work was to evaluate the use of *Phanerochaete chrysosporium* fungus to treat three different effluents, a raw slurry and two anaerobically digested swine wastewaters. The effect of the fungal treatment on the high COD, colour, biological oxygen demand (BOD$_5$), and total nitrogen concentration has been studied. Also, the Lac, MnP and LiP enzymatic activities were determined. In addition, the effect of a physical pre-treatment of the slurry on the fungus activity was also evaluated.

2. Material and methods

2.1. Samples description

Three different swine wastewaters were used for the experiments. Slurry resulting from the mixture of pig manure and pigsty washing water was collected from a pig farm sited in Castilla y Leon (Spain), and used for S1, S2 and S3. A digestate from a conventional anaerobic digestion process of pig slurry and a digestate from an AD process of pig slurry coupled with a gas-permeable membrane made of polytetrafluoroethylene (ePTFE), which allowed to recover ammonia [32], was used for test D4 and D5, respectively. The initial characterization of these samples was showed in Table 1.

2.2. Microorganisms

The basidiomycete white-rot fungus, *Phanerochaete chrysosporium* Burdsall 1974 (CECT 2798) was used for the biological treatment. The recovery of freeze-dried strain as well as the obtention and conservation of fungus pellets were carried out according to the methodology described by Díaz et al. [15].

2.3. Fungal treatment

Five batch tests were performed to evaluate the potential of *Phanerochaete chrysosporium* on the swine wastewaters bioremediation. Three experiments were carried out with fungus and a slurry directly coming from the pig farm. Firstly, this effluent was subjected to fungal treatment without any physical pre-treatment (**Test S1**). Then, with the aim of assess the influence of sieving or centrifugation processes as physical pre-treatments on subsequent biodegradation, two additional experiments were assayed using as raw materials the slurry sieved by 0.5 cm (**Test S2**) or the supernatant obtained from the slurry centrifugation at 10000 rpm during 15 min (**Test S3**).

Finally, two tests were carried out using *P. chrysosporium* to treat an anaerobically digestated slurry from a conventional AD process (**Test D4**) or a digested slurry from a digester with an ammonium trapping membrane (**Test D5**). Likewise, due to all the experiments were performed with unsterilized samples, for all the conditions tested, control experiments without fungus addition were carried out to assess the impact of fungus addition in relation with the activity of the endogenous microbiota. These control experiments correspond to **C1**, **C2**, **C3**, **C4,** and **C5**.

The experiments S1, S2 and S3 were carried out in 1 L Erlenmeyer flasks containing 200 mL of unsterilized sample, while S4 and S5 were performed in 250 mL Erlenmeyer flasks with 50 mL of unsterilized sample. Erlenmeyer flasks were incubated at 26ºC for 10 days in an orbital shaking at 135 rpm. In all cases, according to Hu et al. [33], the sample was initially adjusted to pH = 6.0 using 1 M NaOH or 1 M HCl. The amount of fungus inoculated was 3 g/L (dry matter) in all the conditions tested. The degradation tests were carried out in duplicate. The data shown are the average values of both experiments. In all cases, standard deviations were lower than 13% with respect to average value.

The evolution of the following parameters in the supernatant was

Table 1
Characteristics of the initial samples subjected to fungal treatment.

	Swine wastewater			Digested slurry	
	S1	S2	S3	D4	D5
Total COD (mg O₂/L)	21632	19442	3638	5362	4125
Soluble COD (mg O₂/L)	3916	3850	3638	3246	2852
Soluble BOD₅ (mg O₂/L)	724	695	672	213	234
Biodegradability Index (BI)	0.18	0.18	0.18	0.07	0.08
pH	8.6	38.59	8.55	7.58	8.03
Colour (CN)	2.418	2.232	2.240	2.122	2.106

studied: COD, BOD$_5$, pH, colour, total nitrogen, and fungal enzymatic activities (LiP, MnP and Lac).

2.4. Analytical methods

Samples were taken periodically each 24 h and centrifuged at 10000 rpm during 30 min. The pellet obtained after centrifugation process was dismissed, and the supernatant was used for the analytical measurements, which were done at least in triplicate.

The concentration of soluble chemical oxygen demand (sCOD) was measured by dichromate method according to Standard Methods [34]. The soluble biochemical oxygen demand (sBOD$_5$) was measured using the respirometric method by measuring oxygen pressure decrease by Oxitop bottles. The biodegradability index (BI) was calculated as the ratio of sBOD$_5$ over sCOD.

For the measurement of Lac, LiP and MnP enzymatic activities the methodology described by Lisboa et al. [35] was followed.

Concentrations of nitrogen as nitrites, nitrates, ammonium and organic nitrogen in the supernatant were measured using a Segmented Flow Self-Analyzer, SKALAR SAN PLUS, based on colourimetric reactions.

Finally, the change in the colour of slurry, defined as colour number (CN), was assessed according to Díaz et al. [36] and the value of pH was measured by means of a pH-meter.
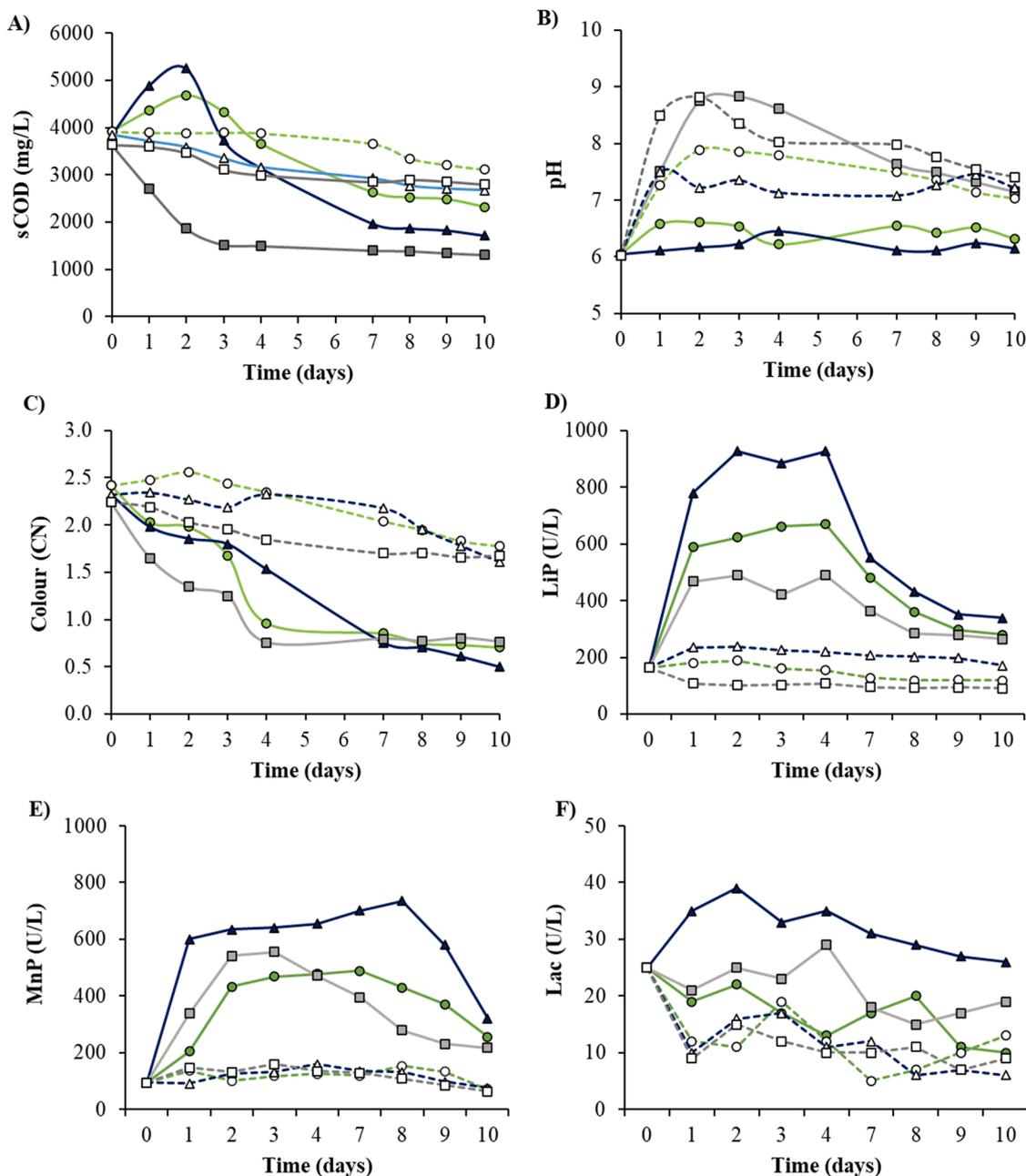
Fig. 1. Evolution of different parameters during the swine wastewater treatment by *P. chrysosporium*. **A)** soluble COD **B)** pH **C)** Colour number (CN) **D)** LiP enzymatic activity **E)** MnP enzymatic activity **F)** Lac enzymatic activity. The standard deviation (SD) of the experimental data were in all cases less than 10% of mean value. In A, B, and C, empty markers and dashed lines show the control test, C1 (∘ •), C2 (△) and C3 (■) and filled markers and solid lines show the inoculated tests, S1 (●), S2 (▲) and S3 (■).

3. Results and discussion

3.1. Fungal treatment of undigested slurry

The evolution of sCOD concentration during the fungal treatments is shown in Fig. 1A and significant differences can be observed depending on the pretreatment. The highest sCOD removals were obtained for S3 test, where centrifuged slurry was used. In this case, a 59% of sCOD degradation was achieved just after three days of incubation. Then, the sCOD value remained almost constant, reaching final percentages of removal around 65%, whereas only 20% of the initial sCOD was removed in the control samples (without fungus inoculation).

With respect to S1 and S2 tests, final sCOD removals of 41% and 55% were achieved, respectively, in relation with the initial value. As can be seen in Fig. 1, during the first two days of treatment, an increase of sCOD was observed in both test S1 and test S2, achieving values of 4685 ± 132 mg/L for and 5251 ± 202 mg/L, respectively. This effect was not observed in the control experiments.

Slightly higher concentrations of soluble organic matter were measured in the slurry pre-treated by sieving (test S2). It seems that the removal of the biggest particles allowed a better contact of the remaining particles with the fungus, favoring the solubilization of the solids and increasing the sCOD of the sample [37,38]. However, the fungus, probably with certain contribution of endogenous microbiota, consumed these compounds as they were produced.

It should be noted that, in S3, in just 5 h, the fungus was able to drop the sCOD from 5251 mg/L to 1715 mg/L, which corresponds to a sCOD removal of 67%, similar to that obtained in S3. For non-inoculated samples, C2 and C3, sCOD degradation was a 23% and 30%, respectively, which proves that the observed degradation was mainly caused by the inoculated fungus.

Regarding the $sBOD_5$ concentration, a decrease throughout the fungal treatment was observed in all cases. From a value around 700 mg/L (Table 1), final values of 244 ± 11 mg/L, 283 ± 17 mg/L and 143 ± 11 mg/L were achieved for S1, S2, and S3, respectively. These concentrations were higher than those reached for the control samples, with final values lower than 80 mg/L in all cases. Consequently, because of biodegradable matter consumption, the BI decreased with the treatment, giving final effluents with a BI values between 0.11 and 0.17 for inoculated tests and below to 0.03 for controls.

Significant differences were also observed in the pH evolution (Fig. 1B). For tests S1 and S2, where the fungal treatment was carried out in the presence of suspended solids, the pH value showed a slight increase after 24 h of treatment and then remained almost constant, reaching maximum pH values of 6.6. However, for the S3 experiment, which was carried out with the centrifuged swine wastewater, the increase in pH was significantly greater reaching pH values around 9.0 after 48 h of treatment, which progressively dropped to a pH value of 7.2. Regarding the control samples C1, C2 and C3, an increase in pH values was also observed, greater for the C3 sample, which reached values close to 9.0 after 48 h of treatment. It seems that the activity of microorganisms tends to increase the pH values, but this basification is balance by the solubilization of solid that occurred in S1 and S2.

With respect to the changes in CN (see Fig. 1C), decolourizations over 65% were achieved in all the inoculated samples reaching the highest removals in S2 test (78%). The CN is a parameter that is closely related to the concentration of phenolic compounds. Generally, when the concentration of phenolic compounds increases, the CN is higher [39,40]. Thus, it could be considered that, in this case, the use of the fungal treatment reduced the concentration of phenolic compounds because the CN of the sample was decreased. Regarding control tests, the decolourization efficacies were just 30%. These results revealed that there was a synergistic effect between the fungus *P.chrysosporium* and the endogenous microorganisms present in the slurry. The extracellular enzymes released by the fungi allowed to break the complex organic compounds that then, could be degrade by fungus and endogenous

microflora, reducing the colour. Lac, Lip and MnP activities have been measured and can be seen in Fig. 1. The inoculation of *P. chrysosporium* substantially increased the enzymatic activity (see Fig. 1D, 1E and 1F). Lac and MnP enzymatic activities have mainly been related with colour removal and LiP with COD degradation processes [41].

In this work, the highest enzymatic production corresponded to LiP. In the case of S1 and S2, where the sample contained solids, an increase in sCOD was observed during the first 2 days of treatment, which corresponded to the high LiP values measured. Surely, the breakdown and solubilization of the complex compounds present in the solids increased the sCOD. In addition, it was observed that from the 7th day on, LiP synthesis was reduced while sCOD degradation remained almost stable. In the case of S3, the highest sCOD removal also corresponded to the highest LiP values.

The fungal enzymes production in the experiments carried out with the sieved slurry (S2) gave the highest values of LiP, MnP and Lac enzymatic activities, with a maximum activity achieved of 927 ± 11 U/L, 736 ± 17 U/L, and 39 ± 8 U/L, respectively. These results indicated that the sieving process was key for optimal enzyme synthesis and agree with the values reported by Meehnian et al. [29]. These authors studied the influence of the particle size of cotton stalks on the synthesis of ligninolytic enzymes by the fungus *Daedalea flavida*. They reported higher enzyme activity when the particle size was 0.5 mm, while for particle sizes of 1 mm or 10 mm the activity was lower, which would explain the differences observed in this work for S1 and S2.

Several authors have reported the pH range of 4.5–6 as optimal for the enzymatic synthesis by *P. chrysosporium* [15,33,42]. In this work, S1 and S2 test, remained within this pH values throughout the treatment, whereas in S3 pH was above 7 after the first day of treatment. This fact, together with the absence of solids, could explain the lower enzymatic activity measured in S3.

Regarding the total nitrogen (TN) concentrations in the treated samples (Table 2), the lowest concentrations were obtained for S3 with a final value of 487 mg/L. Comparing this value with C3 (1456 mg/L), it can be observed that the inoculation of the fungus allowed a reduction in the final TN concentration of around 67%. When the concentrations of the different nitrogen species are analyzed, it can be observed that the differences between TN concentrations in S3 and C3 were mainly due to the removal of inorganic nitrogen by *P. chrysosporium*, with ammonium and nitrates concentration 4 times and 10 times lower, respectively, in S3 than in C3. In S1 and S2, final TN concentration were also lower than in the controls, but the differences were less marked. In these cases, the lower TN concentration were due to the degradation of dissolved organic nitrogen. Hu et al. [33], who evaluated the effect of pH on the efficiency of ammonium removal by *P. chrysosporium* for landfill leachates, described greater removals when the fungal treatment was performed at pH values higher than 7.0. This fact could explain the higher NH_3 removals obtained in S3 compared to S1 and S2.

With respect to the phosphorous, a decrease in its concentration was also detected, as can be observed in Table 3. The higher removals took place for the raw (S1) and the sieve (S2) swine wastewater, with efficacies around 66%. The lower TP concentrations in the samples treated

Table 2
Concentrations of nitrites (NO_2), nitrates (NO_3), ammonium (NH_3), total inorganic nitrogen (TIN), dissolved organic nitrogen (DON) and total nitrogen (TN) for the inoculated (S1-S3) and control (C1-C3) samples after 10 days of swine wastewater treatment.

Sample	NO_3 (mg/L)	NO_2 (mg/L)	NH_3 (mg/L)	TIN (mg/L)	DON (mg/L)	TN (mg/L)
S1	174	72	817	1063	97	1160
S2	117	51	789	957	53	1010
S3	13	0,3	207	221	266	487
C1	87	1,1	910	998	728	1726
C2	32	0,7	859	892	506	1398
C3	163	1,3	874	1039	417	1456

Table 3

Concentrations of inorganic phosphorus (IP), dissolved organic phosphorus (DOP) and total phosphorus (TP) for the inoculated (S1-S3) and control (C1-C3) samples after 10 days of swine wastewater treatment.

Sample	IP (mg/L)	DOP (mg/L)	TP (mg/L)
S1	174	39	213
S2	164	47	210
S3	336	81	417
C1	545	83	628
C2	538	98	636
C3	533	94	628

with fungi was mainly due to the removal of the inorganic phosphorus, which was also in higher percentage in the initial sample.

A pretreatment of centrifugation to remove the solids, followed by fungus treatment allowed decreases higher than 60% in COD, TN and colour, respect to the initial one. These results open the possibility of using *P. chrysosporium* to treat pig slurries in an economical and eco-friendly way. The effluent obtained could be employed for irrigation or being incorporated to aerobic conventional biological treatments Other possible utility of the fungal treatment would be to use it as a pretreatment with the aim of increasing the dissolved organic matter in the effluent so that the biogas production is improved in a subsequent anaerobic digestion process [18,22,43]. As can be observed in Fig. 1A, a fungal treatment of sieved slurry (S2) for just 48 h increased the sCOD concentration in around 30%.

3.2. Fungal treatment of digested slurry

The treatment of swine wastewater by anaerobic digestion (AD) as well as its co-digestion with other agri-food wastes to produce biogas has been widely studied [4,22,44]. However, the resulting digestate still contains a high recalcitrant organic load that could not be degraded by anaerobic bacteria during the anaerobic digestion process. Considering the ability of *P. chrysosporium* to synthesize fungal enzymes capable of breaking down these recalcitrant compounds, the fungal treatment could be an interesting alternative [15].

Regarding the results obtained for sCOD concentration (Fig. 2A), both digestates showed very similar trends, reaching greater degradations in the inoculated samples (D4 and D5) than in the control samples (C4 and C5). With respect to the sCOD removal for D5 test, carried out with the digestate from the membrane reactor, a degradation of 32% was achieved after four days of treatment. Afterwards, the sCOD concentration decreased slowly until achieving a final percentage of removal of 38%, substantially higher than the 11% reached in the control sample (C5). Regarding to the digestate from the conventional AD process, removals of 23% and 9% were achieved for D4 and C4, respectively. These results seem to indicate that the presence of an ammonium trapping membrane in the reactor favors the subsequent fungal treatment process, with final COD concentrations lower than those obtained with the conventional digestate (1780 mg/L for D5 and 2500 mg/L for D4). In this sense, high concentrations of ammonia have been associated with important reductions in fungal enzyme activities [45].

Regarding the treatment of anaerobically digested swine wastewater in previous studies, Deng et al., [46] reported COD removal of about 10% using a sequencing batch reactor. Daverey et al., [47] reported higher removals of COD (64.0%–83.0%), by the simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) process, using again a sequencing batch reactor (SBR). However, 250 days were necessary for the treatment process. Wen et al., [48] investigated the biological treatment of an anaerobically digested swine wastewater by two strains of photosynthetic bacteria, *Rhodobacter*

blasticus and *Rhodobacter capsulatus*. They reported COD removal percentages of around 83%. The removal obtained in this work is much lower. It should be noted that, in this case, the anaerobically digested swine wastewater used was not centrifuged and/or sterilized, as in other published works.

With respect to the concentration of BOD_5, the values were reduced in all cases, reaching final values around 80 mg/L in the inoculated samples (D4 and D5) and around 170 mg/L in the controls (C4 and C5). In this case, the final inoculated samples showed lower BOD_5 values than the control samples, and in all cases the final effluents are low biodegradable with a BI values lower than 0.07.

Accordingly to the removal of colour (Fig. 2C), again higher degradations were obtained when the effluent was treated by *P. chrysosporium*. However, the colour removals achieved with the anaerobic digestates were lower than those obtained for the undigested slurry, with percentages around 30% for D4 and D5.

The LiP and MnP enzymatic activities are shown in Fig. 2D and 2E, respectively. For D5, the digestate from the membrane reactor, slightly higher values of LiP and MnP were obtained than for the conventional digestate, whereas Lac enzymatic activity was undetectable for all cases. The values obtained with the digestates were quite lower than those obtained for the undigested slurry. In the treatment of recalcitrant effluent by fungi, the importance of counting with easily assimilable carbon sources to favor enzymatic synthesis has been described by several authors [15,49]. It should be noted that the digested slurries were less biodegradable than the undigested ones, as indicated by the $sBOD_5$ shown in Table 1. In addition, pH was not controlled during the treatment, reaching values higher than 6.5 in all cases, which could also decrease the synthesis of fungal enzymes (see Fig. 2B), as pH values were above the range reported as optimal.

4. Conclusions

Results obtained in this work showed the potential of *P. chrysosporium* to treat swine slurries and wastewaters, either effluents directly from the pig farm or effluents previously subjected to anaerobic digestion processes. A short fungal treatment of the raw slurry (2 days) caused the solubilization of solids, increasing the sCOD concentration for a subsequent anaerobic treatment. If the fungal treatment continues for 7 days, percentages of sCOD removal (calculated with respect to the initial concentration) of 41% were achieved. Additionally, the use of centrifugation as previous step allowed decreases higher than 60% in COD, TN and colour, so that the effluent can be more easily used for irrigation or treated in conventional processes. Moreover, high enzymatic activities of LiP and MnP were measured after 2 days of fungal treatment, in particular the highest values were obtained when the sieved slurry was treated, which opens the possibility of using this residue as a raw material to synthetize fungal ligninolytic enzymes with large industrial applications. Regarding the treated digestates, even though they were low-biodegradable effluents (BI < 0.1), the fungal treatment was able to eliminate 23–32% of the initial sCOD concentration and around 30% of the colour. The possibilities of coupling fungal treatment with other biological processes are wide, however further specific research should be carried out.

CRediT authorship contribution statement

Ana Isabel Díaz: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Adriana Laca:** Conceptualization, Methodology, Validation, Formal analysis, Supervision, Data curation. **Mercedes Sánchez:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Supervision. **Mario Díaz:** Conceptualization,
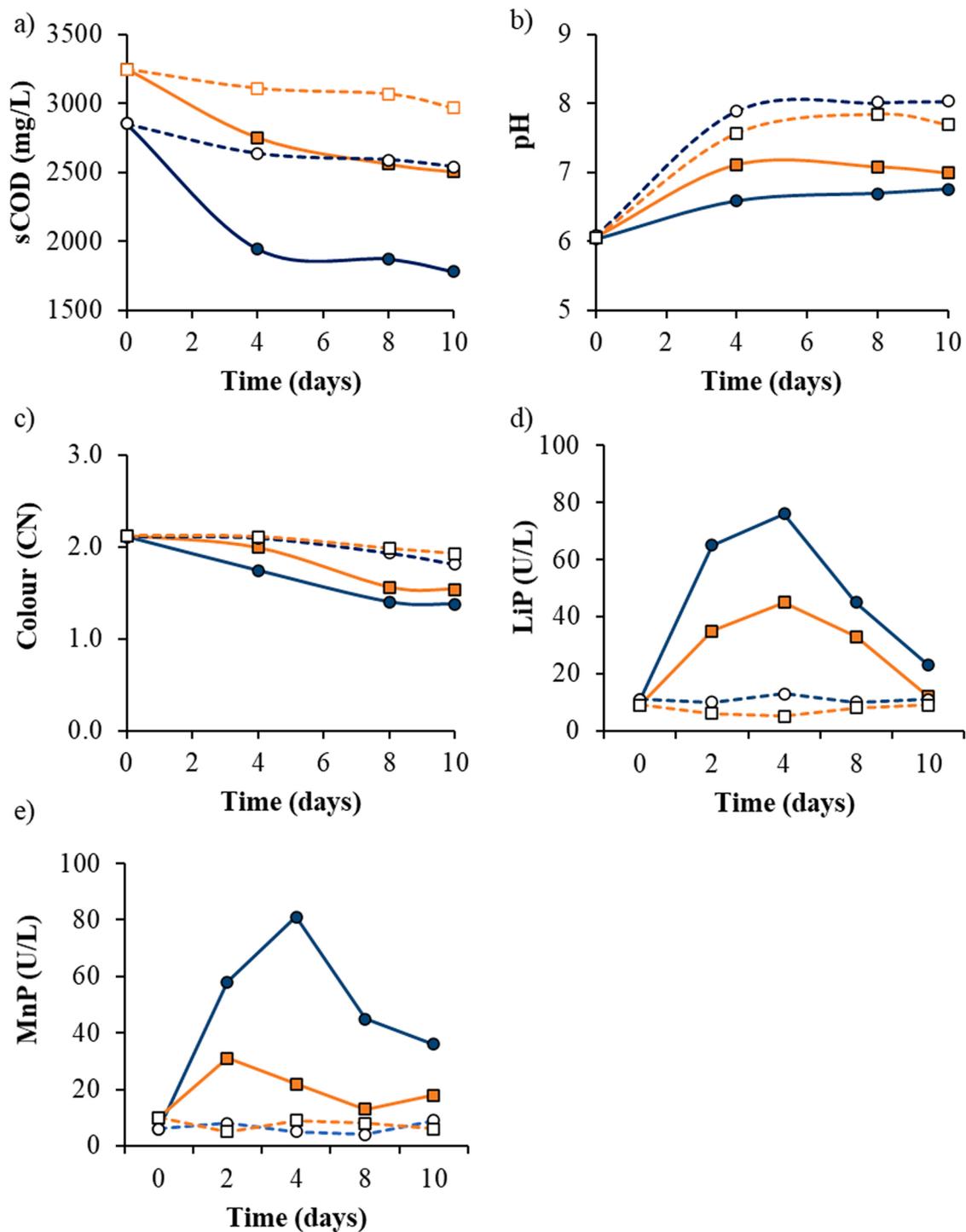
Fig. 2. Evolution of different parameters during the digestated slurry treatment by *P. chrysosporium*. **A)** soluble COD **B)** pH **C)** Colour number (CN) **D)** LiP enzymatic activity **E)** MnP enzymatic activity. The standard deviation (SD) of the experimental data was in all cases less than 13% of mean value. Empty markers and dashed lines show the control test C4 (□) and C5 (○) and, filled markers and solid lines show the inoculated tests, D4 (■) and D5 (●).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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