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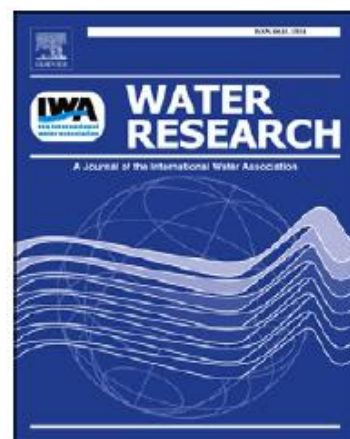
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Effect of wet oxidation on the fingerprints of polymeric substances from an activated sludge

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Abstract

Advanced oxidation processes (AOPs) are gaining importance as an alternative to the biological or physicochemical treatments for the management of leachates. In this work, it has been studied the effect of the characteristics of the leachate (content in humic acids, landfill age and degree of stabilization) on the wet oxidation process and final quality of the treated effluent. A high concentration of humic acids in the leachate had a positive effect on the COD removal because this fraction is more easily oxidizable. Additionally, it has been demonstrated that the simultaneous presence of humic acid and the intermediates generated during the oxidation process improved the degradation of this acid, since such intermediates are stronger initiators of free radicals than the humic acid itself. Similar values of COD removals (49% and 51%) and biodegradability indices (0.30 and 0.35) were observed, after 8 h of wet oxidation, for the stabilised leachate (biologically pretreated) and the raw one, respectively. Nevertheless, final colour removal was much higher for the stabilised leachate, achieving values up to 91%, whereas for the raw one only 56% removal was attained for the same reaction time. Besides, wet oxidation treatment was more efficient for the young leachate than for the old one with final COD conversions of 60% and 37%, respectively. Eventually, a triangular “three-lump” kinetic model, which considered direct oxidation to CO₂ and partial oxidation through intermediate compounds, was here proposed.

Keywords: advanced oxidation, biodegradability, humic and fulvic acids, landfill leachates, lumped kinetic model, toxicity

1. Introduction

Leachate is an aqueous liquid stream generated from waste landfill site due to the percolation of rainwater through the waste, inherent moisture, and biochemical reactions occurring within the disposed waste (Kurniawan et al., 2006a). Leachates are highly polluted aqueous wastes characterised by high concentrations of ammoniacal nitrogen, moderately high-strength of recalcitrant compounds and a medium-low ratio of BOD₅/COD, depending on their age. Untreated leachates can permeate groundwater or mix with surface waters and contribute to the pollution of soil, groundwater, and surface waters (Deng and Englehardt, 2006).

The potential dangers of these leaks have been confirmed and it is necessary to treat leachates properly so that they meet the standards for being discharged into sewer or natural waters. For this reason, the management of leachates has become a major environmental issue worldwide (Kurniawan et al., 2006b).

Leachate treatment technologies fall into two basic types, biological and physical/chemical methods. In larger systems and depending on the treatment goals, integrated systems which combine both techniques are often used (Raghab et al., 2013). Obviously, treatment technologies will depend on the strength and volume of leachate. Most of readily degradable organic matters that are present in the leachate, such as volatile fatty acids, could be removed efficiently by the traditional biological treatment processes. The drawbacks generally found in biological treatment are originated from operational problems such as foaming, metal toxicity, nutrient deficiency and sludge settling. Nevertheless, leachate also contains amounts of non-biodegradable matter, COD being in the range of 400 to 1500 mg/L, after the biological treatment, and should be removed further to attain the discharge limits (Kulikowska and Klimiuk, 2008).

Physicochemical treatment processes (coagulation–flocculation, chemical oxidation, air stripping, membrane process and adsorption on active carbon, among others) have also been employed, either individually or coupled with the biological treatment processes. Nevertheless these techniques are limited by high operation costs, difficult handling of the by-products, and low removal efficiency (Ziyang et al., 2014, Kurniawan et al., 2006a).

Advanced oxidation processes (AOPs) are receiving a growing interest due to its ability to enhance the biodegradability of the recalcitrant compounds in the leachate up to a suitable value for a subsequent and economical biological treatment (Renou et al., 2008). The oxidizing agent in advanced oxidation processes are free radicals, hydroxyl radicals ($\cdot\text{OH}$) being the most important. $\cdot\text{OH}$ is characterised by a high oxidation potential (2.8 V), being able to oxidize a variety of organic compounds to carbon dioxide and water. Therefore, it would be desirable to have a steady-state production of $\cdot\text{OH}$ in order to guarantee the effectiveness of AOPs (Ghazi et al., 2014; Deng and Englehardt, 2006; Kurniawan et al., 2006b).

Wet oxidation is a well-established technology with proven effectiveness in the treatment of industrial wastewaters with similar characteristics that those found in the landfill leachates (Oulego et al., 2014; Bhargava et al., 2006). Besides, this technique is very clean, since the oxidation process does not involve the use of any toxic chemical reagents and no harmful species, such as NO_x , SO_2 , HCl, dioxins, furans or fly ashes are produced. The main drawback of this technology, which is the high energy consumption, can be reduced using heat exchangers to recover the thermal energy of the treated effluent in order to preheat the feed, thus improving the operating costs and the feasibility of this technique.

Several works dealing with wet oxidation or other advanced oxidation techniques for the treatment of leachates can be found in the literature (Oulego et al. 2015, Garg and Mishra, 2010; Renou et al., 2008; Wiszniowski et al., 2006). All these studies are mainly focused on the effect of the operating conditions (temperature, dose of oxidant, addition of catalysts or promoters) on the depuration of a leachate from a specific landfill, or on the removal of a pure compound that was selected as model pollutant (usually, humic or fulvic acids). Nevertheless, the composition of landfill leachates varies greatly depending on various factors, such as landfill age, weather conditions, waste type, landfill design and operational practice, among others (Kurniawan et al., 2006b). As far as we know, there are not detailed studies about the effect of these factors. Consequently, a thorough study about the effect of the leachate composition on the performance of advanced oxidation processes would be very useful in order to establish conclusions for determining the best treatment process in each case.

In view of these considerations, the aim of this work was to obtain an in-depth knowledge about the effect of various characteristics of leachate (content in humic acids, landfill age and degree of stabilization) on the quality of the effluent treated by wet oxidation process (chemical oxygen demand (COD), biodegradability index (BOD_5/COD), average oxidation state of carbon atoms (AOSC), colour number (CN) and toxicity). This will allow us to evaluate the effectiveness of the oxidation as a function of the type of leachate employed. The use of a lumped kinetic model was also analysed with the aim of making simpler to understand the complex mechanism by which landfill leachates are oxidized.

2. Materials and methods

2.1. Landfill leachates

The leachates used in this study were taken from the sanitary landfill site La Zoreda (Asturias, Spain). Four different types of leachates were used: old, young, raw and stabilised after biological treatment. All of the samples were taken the same day in May 2014. Details of the sampling location and the landfill sections are shown in the section 1 of the Supplementary Material. Raw leachate consists of a mixture of old and young leachate (approximately 50% of each one). This raw leachate is treated at the landfill by means of a pressurised nitrification–denitrification process, being characterised by high volatile solids content (~14 g/L) and high oxygen solubility as a consequence of the pressure applied (2.5–3.0 bar). The biomass is subsequently separated using an ultrafiltration system. The stabilised leachate used in this work was the final permeate of the biological treatment. Depending on the age, the characteristics of the leachate in terms of COD, total organic carbon (TOC), biochemical oxygen demand (BOD₅), colour and pH are quite different. Thus, the old leachate is characterised by moderate COD values, low biodegradability and high pH, whereas the young one has high values of COD, high biodegradability and slightly acid pH. The composition of these leachates is very different and coincides with the characteristics reported by various authors for old and young leachates from municipal solid waste landfills (Kurniawan et al., 2006a). A detailed description of the characteristic of the landfill leachates used is shown in Table 1. It should be noted that each of the leachates employed is representative of those generated in the landfill site. Thus, the variability found in the physicochemical characteristics of such leachates and those found in the different leachates generated from 2007 to 2015 is low (see Table 1). Therefore, the results here obtained can be transferred to other time periods.

2.2. Preparation of humic and fulvic fraction from the biologically pretreated landfill leachate

The procedure used for the separation of humic and fulvic fractions from the biologically pretreated landfill leachate was based on methods described by Christensen et al. (1998). The details are explained in section 2 of the Supplementary Material.

2.3. Apparatus and procedure

Experiments were carried out in a 1 L capacity semi-batch reactor (Parr T316SS) equipped with two six-bladed magnetically driven turbine agitators. A more detailed description of the procedure can be found in Oulego et al. (2015). In a typical experiment, 0.7 L of landfill leachate or the humic or fulvic fractions were introduced into the reaction vessel, which was then heated and pressurized to the operating conditions under a continuous oxygen flow ($2.33 \times 10^{-5} \text{ Nm}^3/\text{s}$). In the experiments in which humic fraction or commercial humic acid (from Sigma-Aldrich, 53680 Aldrich) was injected, a concentrated solution was prepared. The concentration of this solution must be calculated so that, once it mixes with the reaction medium, gives the desired concentration. The oxygen was bubbled through the water reservoir in order to become saturated with water vapour before being sparged into the reaction vessel. A valve and a coil fitted to the top of the vessel allowed the withdrawal of samples during the reaction. Temperature and pressure were maintained constant during the course of each experiment. Two bubblers filled with concentrated sulfuric acid solution and another two bubblers filled with a concentrated sodium hydroxide solution were installed at the end of the gas line with the purpose of absorbing ammonia and carbonates, should they be formed.

2.4. Analytical methods

The concentration of COD was spectrophotometrically measured (at 600 nm) by dichromate method according to Standard Methods (APHA, 1999) using a DR2500 spectrophotometer (Hach Company). TOC analysis was performed using a TOC analyzer (Shimadzu TOC-V_{CSH}). BOD₅ was determined using a manometric respirometric measurement system, Lovibond® OxiDirect unit. pH was measured by means of a pH-meter Jenway 3510.

Bacterial toxicity assessment was performed with *Vibrio fischeri*. For these bioassays, the commercial assay Biofix®Lumi-10 was employed using a freeze-dried specially selected strain of the marine bacterium (NRRL number B-11177). Toxicity was analysed in samples diluted 1:5. The decrease in light emission of the bacteria after a contact period of 15 min was measured and compared to a toxicant-free control (2% NaCl solution). Temperature was kept at 15 °C by a thermo block and pH of the sample was adjusted between 6.5 and 7.5. Results are expressed as inhibition percentage (LI) according to ISO 11348-3. All analytical measurements were done at least in

triplicate, and the standard deviation was found to be below 5% in all cases.

2.5. Parameter calculations

In order to assess the oxidation state of the reaction mixture during the wet oxidation of landfill leachate, average oxidation state of carbon atoms (AOSC) was calculated. This parameter is defined as follows (Melero et al., 2009; Mantzavinos et al., 2000).

$$AOSC = 4 - 1.5 \left(\frac{COD}{TOC} \right) \quad (1)$$

where AOSC takes values from -4 (i.e. for methane) to +4 (i.e. for carbon dioxide) and COD and TOC values are expressed in mg O₂/L and mg C/L, respectively.

AOSC parameter only indicates the oxidation state at one given time and it cannot be used for assessing the progress of partial oxidations. Thus, COD removal by partial oxidation (COD_{partial}) was determined in order to know how much partial oxidation has taken place since the start of the reaction. This parameter was calculated by comparing COD/TOC ratios at different reaction times to the original ratio and it is shown as follows (Mantzavinos et al., 2000):

$$COD_{partial} = \left(\frac{COD_0}{TOC_0} - \frac{COD_t}{TOC_t} \right) \cdot TOC_t \quad (2)$$

where COD₀ and TOC₀ are the initial values and COD_t and TOC_t are the values for a reaction time, t.

The extent of COD removal by partial oxidation (α) is also an interesting parameter to follow the progress of partial oxidation and can be determined as follows:

$$\alpha = \frac{COD_{partial}}{COD_0} \quad (3)$$

It is also important to know the relation between COD partial and COD removed at any reaction time. Thus, it is defined the parameter, η , as follows:

$$\eta = \frac{COD_{partial}}{COD_0 - COD_t} \quad (4)$$

This parameter varies from 0, when only total oxidation occurs, to 1 when only partial oxidation takes places.

The colour number (CN), was used to monitor changes in the colour of the leachate during its oxidation (see equation 5). Spectral absorbance coefficients (SAC) are defined as the ratio of the values of the respective absorbance (Abs) over the cell thickness (x). This parameter was measured at 436, 525 and 620 nm using a UV/Vis spectrophotometer (Thermo Scientific, Helios γ).

$$CN = \frac{SAC_{436}^2 + SAC_{525}^2 + SAC_{620}^2}{SAC_{436} + SAC_{525} + SAC_{620}} \quad (5)$$

$$SAC_i = \frac{Abs_i}{x} \quad (6)$$

3. Results and discussion

3.1. Can a lumped kinetic model be applied to landfill leachate oxidation?

The mechanism by which wet oxidation occurs is very complex, even with a pure compound. Generally, the oxidation undergoes a very complicated pathway and leads to the formation of many different intermediates, such as refractory short-chain organic acids (Verenich et al., 2005). In the case of landfill leachates, this oxidation could be even more difficult since many compounds of various nature are present in their composition. In order to model their degradation, lumped reactions and lumped concentrations in the reaction mixture (TOC, COD or BOD₅) can be used to obtain the rate equation. Lumped kinetic models were also proposed by various authors to model industrial wastewaters such as debarking and phenolic effluents (Verenich et al., 2005; Martins et al., 2010). However, lumped kinetic models have not been applied before to landfill leachates. We proposed a “three-lump” triangular kinetic model for the description of the wet oxidation of landfill leachates. This model consists of a three-step mechanism (see Figure 1) with the compounds lumped into three groups: non-biodegradable parent compounds (A), biodegradable parent compounds or intermediates (B) and oxidation end-products, CO₂ (C).

FIGURE 1

The rate equations for this kinetic model can be expressed through a system of ordinary differential equations (ODEs), as follows. The mathematical arrangements that led to the obtention of these equations are shown in section 4 of the Supplementary Material.

$$-r_A = -\frac{dC_A}{dt} = (k_1 + k_2)(C_A - C_{A,u}) \quad (7)$$

$$-r_B = -\frac{dC_B}{dt} = -k_2(C_A - C_{A,u}) + k_3(C_B - C_{B,u}) \quad (8)$$

in which, k_1 , k_2 and k_3 are the kinetic constants due to total oxidation of A, partial oxidation of A and total oxidation of B, respectively. C_A and C_B are the concentrations of organic compounds in lump A and B. $C_{A,u}$ and $C_{B,u}$ are the final unoxidized fractions of organics in lump A and B. A more detailed explanation of these parameters and the calculation method for these concentrations are also explained in detail in section 5 of the Supplementary Material.

All the experimental data were successfully fitted to the proposed lumped kinetic model (equations 7 and 8) using Micromath Scientist 3.0, with a regression coefficient greater than 0.99 in all cases (see solid lines in Figures 2 to 6 and Figure S2 of the Supplementary Material). The kinetic constants obtained and the values of $C_{A,u}$ and $C_{B,u}$ are shown in Table 2. Consequently, the “three-lump” triangular kinetic model can be used to assess the behaviour of landfill leachates and their humic and fulvic fractions treated by wet oxidation. The data obtained during the heating-up period cannot be used for the fitting since the operating conditions are not constant. A detail explanation of the influence of the heating-up period has been included in the Supplementary Material (section 3).

3.2. Can the behaviour of the landfill leachate oxidation be simulated by using humic acid as model pollutant?

Numerous studies about the degradation of commercial humic acids by means of different advanced oxidation processes can be found in the literature (Rajca and Bodzek, 2013). For some of them, humic acid is considered as a model compound of the humic substances contained in the leachate. However, it must be taken into account that the humic acid fraction is not composed by a single acid. It is a complex mixture of many different acids containing carboxyl and phenolate groups. Additionally, leachates also contain other species such as volatile fatty acids and salts, among others. Thus, how reliable are these data? Can they be used as a simplification of the real behaviour of the humic fraction or, even, of the leachate? Or is it just a coarse approximation? In order to answer these questions, wet oxidations of commercial solution of humic acid (Sigma-Aldrich) and the humic fraction of the stabilized leachate (biologically pretreated), both with the same initial COD, were carried out.

As can be seen in Figure 2a, the humic fraction extracted from the leachate showed a higher mineralization rate than the commercial humic acid. Thus, the degree of mineralization achieved after 480 min were 72% and 54% for the extracted humic fraction and for the commercial humic acid, respectively. It should be noted that in this case with initial BOD practically negligible, the BOD for each time indicates the approximate concentration of intermediates. Although the induction periods were similar for both experiments, kinetic constant of humic fraction for the direct oxidation, k_1 , was 1.4 times higher than that of commercial humic acid $(9.66 \pm 0.03) \times 10^{-5} \text{s}^{-1}$. This means that the degradation rate directly to CO_2 is higher in the humic fraction than in the commercial one. In order to evaluate which pathway (direct oxidation to CO_2 or partial oxidation to intermediates) occur in a great extension, the reaction rate of the lump B was divided by the reaction rate of the lump A:

$$\frac{-r_{A \rightarrow B}}{-r_{A \rightarrow C}} = -\frac{dC_{A \rightarrow B}}{dC_{A \rightarrow C}} = \frac{k_2(C_A - C_{A,u})}{k_1(C_A - C_{A,u})} = \frac{k_2}{k_1} \quad (9)$$

The ratio k_2/k_1 ranges from 0 to ∞ , the value 0 indicating total oxidation to CO_2 directly and the value ∞ showing the oxidation through the formation of intermediate compounds. For this parameter, values of 0.10 and 0.48 were obtained for the commercial humic and the humic fraction, respectively. So, the pathway to form intermediate compounds occurs in a greater extension in the oxidation of the humic fraction.

Besides, it can be concluded that the intermediates formed in the wet oxidation of humic fraction are less refractory to oxidation in view of the higher value of k_3 for the humic fraction (see Table 2). This together with the fact that the pathway to yield intermediate compounds occurs in higher extension for the humic fraction, explain why α values were similar in both experiments (see Figure 2c).

These findings are also related to the experimental data observed in Figure 2d. As can be seen in this figure, the values of the parameter η for the humic fraction are lower than those obtained in the commercial humic acid, thus indicating a greater total oxidation in the case of humic acid. At first sight, this seemed to be illogical since the ratio k_2/k_1 was lower for commercial humic acid. However, these results make sense considering that a significant part of the total oxidation occurred by means of the oxidation of the intermediate compounds.

FIGURE 2

Humic fraction of the leachate and commercial humic acid presented similar initial values of AOSC; approximately -3.7 (see Figure 2b). During the oxidation process, AOSC values increased greatly and in a progressive way in both cases, although the improvement was more significant in the case of the humic fraction, obtaining a final value of around -0.8. These results suggested the formation of significant amounts of acetic acid during the wet oxidation since AOSC for this compound is 0. Several authors have reported the formation of short-chain carboxylic acids when industrial wastewaters are oxidized (Verenich et al., 2005; Martins et al., 2010). These compounds are easily biodegradable and probably they are also responsible for the higher biodegradability index obtained for the humic fraction at the end of the treatment (see Figure 2f). Additionally, this trend was also observed in Figure 2g, where luminescence inhibition was slightly lower for the humic fraction than for the commercial acid.

Regarding the colour, the higher colour number of the commercial humic acid indicates that the aromaticity was higher for the commercial humic acid. This fact would explain the significant differences observed in the results related to the wet oxidation of commercial humic acid and humic fraction (see Figure 2e).

It can be concluded that the use of a commercial humic acid with the aim of simulating the behaviour of the humic fraction of a leachate during an advanced oxidation should be done carefully, due to the existence of significant discrepancies. These differences can be probably attributed to the heterogeneity of the humic acids in terms of elemental composition, chemical functionality and molecular size distribution.

3.3. What leachate fraction is more refractory?

In order to know the effect of the composition on the degradation of the leachate, the wet oxidation of the stabilised leachate and its humic and fulvic fractions, previously separated, were carried out at 453 K and 6.0 MPa (initial pH 6.8). As can be seen in Figure 3a, the COD reduction during the wet oxidation of the leachate follows the usual pattern reported by several authors (Anglada et al., 2011).

So, a COD reduction of around 20% was quickly achieved, probably due to the loss of volatile organic compounds, as well as to the thermolytic decomposition of some unstable constituents of the effluent. After that, COD concentration remained approximately constant until 100 min and then decayed with gradually decreasing rate to reach a constant COD value (final degree of mineralization) of 51%. Several authors have reported the formation of refractory low molecular weight acids as final products after the oxidation of cyclic compounds, so explaining the asymptotic behaviour of COD for high reaction times (Bhargava et al., 2006).

FIGURE 3

Comparing these results with the wet oxidation of the isolated humic fraction, it is clear that their behaviours were different. So, the initial fast reduction in the COD was not observed during the wet oxidation of the humic fraction, which is in accordance with the absence of volatile or unstable compounds in the solution after the isolation. However, the induction period was detected in both cases, with similar duration (~90 minutes). Comparing the COD evolutions for both experiments after the induction periods, it should be noted that the degradation of the isolated humic acid was faster than that obtained for the leachate, achieving a final value of 72%. During this step, COD evolutions of both the stabilised leachate and the humic acid were successfully fitted to the three-lump kinetic model (see Table 2). The fitting parameters obtained also proved these experimental evidences. Thus, the kinetic constant of direct oxidation to CO₂ (k_1) for the humic fraction was approximately 1.3 times higher than that obtained for the leachate, $(1.09 \pm 0.03) \times 10^{-4} \text{ s}^{-1}$. In both cases, the kinetic constants for the formation of biodegradable compounds (k_2) and for their oxidation to CO₂ (k_3) exhibited lower values, these being slightly higher for the humic fraction (see Table 2). The low values of the kinetic constants would also explain the similarity of α and η values for the leachate and humic fraction.

Regarding the fulvic fraction, the results shown in Figure 3a indicate that its behaviour after wet oxidation treatment was similar to that obtained for the leachate. However, some differences can be readily seen. In this case the induction period of the fulvic acid was negligible and COD reduction occurred progressively in a single step.

Besides, the concentration of intermediates ($\text{COD}_{\text{partial}}$) was 7% of the initial COD, this percentage being half of the value for the stabilised leachate. The final degree of mineralization achieved was also slightly lower for the fulvic fraction (44%) than for the leachate (49%). Thus, the kinetic constants k_1 and k_2 were a bit lower for the fulvic fraction (see Table 2). However, the kinetic constant for the oxidation of the intermediates, $k_3 = (5.76 \pm 0.03) \times 10^{-5} \text{ s}^{-1}$, was higher than that obtained for the leachate. Therefore, the intermediates formed in the oxidation of fulvic fraction were faster degraded to CO_2 than those formed in the oxidation of the leachate. This is in agreement with the values of α observed, which decreased progressively after the heating period (~ 90 min). The presence of a significant decarboxylation was also observed in the parameter η , which showed a sharp drop (see Figure 3c and d).

On viewing these results, it seems reasonable to conclude that the humic acids constitutes the more easily oxidisable fraction of the leachates, this fraction being faster oxidized and yielding lower final refractory COD than the fulvic one. Several researchers have also observed a higher removal for the humic acids than for the fulvic acids using different advanced oxidation techniques, either for commercial pure compounds or for leachate (Rajca and Bodzek, 2013; Wu et al., 2011).

Regarding AOSC values, it increased in all cases from an initial value of approximately -3.7 to -3.1, -1.9 and -0.8, for the fulvic fraction, stabilised leachate and humic fraction, respectively (see Figure 3b). For the fulvic fraction, the value of AOSC increased during the heating period, keeping approximately constant during the oxidation process. This means that the intermediate compounds were formed in the heating period. The highest value was observed for the humic fraction. As it was previously commented, this result indicated the existence of short-chain carboxylic acids, such as acetic acid, which has an AOSC value of 0.

As reported by several authors (Aziz et al., 2007; Zouboulis et al., 2004; Tatsi and Zouboulis, 2002), the presence of humic substances is responsible for the dark colouration of municipal landfill leachates. Moreover, as can be observed in Figure 3e, the colour of the leachate is mainly due to the fulvic fraction. A fast decrease in the colour was observed in the three experiments.

There were no significant differences in the luminescence inhibition between the leachate and its separated fractions, which slightly increased during the oxidation process from 51% to around 65% after 8 hours (Figure 3g). Nevertheless, the variances in the biodegradability index for the leachate and its components were evident. As shown in Figure 3f, the leachate and the fulvic fraction showed similar final biodegradability indices (0.29 and 0.24, respectively), which increased rapidly at the beginning of the oxidation and then remained almost constant. Regarding the humic fraction, its biodegradability was a bit lower (0.20), but it progressively increased during the course of reaction (8 h). This fact suggests that the final products from the humic fraction could be more biodegradable (due to the presence of acetic acid as final reaction product) than those from the fulvic fraction or, even, than the leachate for longer reaction times.

3.4. Are there synergistic phenomena between fractions during the oxidation of leachate?

Synergism involves the simultaneous reaction of two species, one being easily oxidized and the other more refractory to oxidation, with the easily oxidized component effectively increasing the oxidation rate of the refractory compound. The existence of synergistic and inhibitory effects is common in aqueous-phase oxidations, such as wet oxidation, and may affect the overall oxidation rate of the process significantly (Collado et al., 2013). In order to find out the presence of a synergistic effect between the fractions of the leachate during the oxidation, the COD values measured during the wet oxidation of the separated humic and fulvic fractions has been added for each time and compared with the COD of the leachate for the same reaction time (Figure S3 of the Supplementary Material).

As can be seen, there is a slight decrease in the mineralization rate when both fractions are separately oxidized. This fact suggests the existence of a slight synergistic phenomenon between the leachate fractions, particularly during the first 30 min of reaction.

Another type of synergistic effect can occur due to presence of reaction intermediates. In this sense, the wet oxidation of commercial humic acid was carried out and, after 240 minutes of reaction, an injection of a concentrated solution of the same commercial humic acid was made without stopping the oxygen flow and without removing the products of the previous oxidation (Figure 4).

FIGURE 4

These two runs shown in Figure 4a were successfully fitted to the lumped kinetic model and the values for all the kinetic constants (k_1 , k_2 and k_3) were much higher after the injection (see Table 2). Consequently, both total and partial oxidation occurred faster, the quickest degradation being the direct oxidation to CO_2 (k_1 value was the highest). Considering the ratio k_2/k_1 , it should be noted that this parameter had a value of 0.10 before the injection and 0.18 after it, proving that the direct decarboxylation occurred in a greater extension before the injection than after it. The higher reaction rate observed can probably be explained as a result of the accumulation of intermediate compounds (Collado et al., 2010). This behaviour is usually attributed to the quinone-like compounds contained in the reaction medium, which are formed from the aromatic structures of the humic acid and can act as stronger initiators, thus increasing the generation of radicals and the degradation rate. As general conclusion and although kinetics of leachate removal in batch agitated reactors are useful data to clarify the characteristics of a given reaction, these results suggest the necessity of studying the effect of the backmixing. From a practical point of view, these findings involve that wet oxidation reactors with a high degree of backmixing will be more effective for the treatment of phenol-like compounds than reactors with flow patterns near to plug-flow or batch reactors, in which no backmixing occurs (Collado et al., 2013).

Nevertheless, the injection of humic acid had no effect on the final COD due to products. As can be seen in Figure 4a, the remaining COD after the injection (COD_∞) was approximately twofold higher than that measured before the injection, as it was expectable. However, differences were observed in the AOSC of the final products. Before the injection, the AOSC of the medium progressively increased achieving a value of around -2.8, whereas after the injection, the AOSC value was kept constant obtaining a final value of -2.7 (see Figure 4b). Thus, the second injection, or the selection of reactors with high degree of backmixing generates free radicals in the reaction medium. It seems reasonable that the intermediates present in the moment of the injection were attacked by this extra amount of free radicals formed after the injection, favouring the direct oxidation to CO_2 . This explains why AOSC values were constant. This is also in accordance with the values of α and η which decreased consecutively after the injection (see Figure 4c and d).

This drop was specially marked for η parameter which decreased from 0.4 to 0.09. On the other hand, the improvement in the biodegradability index can be explained by the presence of low molecular weight acids since k_2 value was higher after injection. This injection caused an increase in the biodegradability index from 0.08 to 0.12 (see Figure 4f). However, these differences were not observed in the toxicity measurements (Figure 4g). Regarding the colour, either with or without a second injection, the final effluent had no colour at the end of the process.

3.5. What is the effect of a biological pretreatment on the oxidation of the leachate?

Advanced oxidation processes were applied to raw leachate (Chemlal et al., 2014; Gotvajn et al., 2009) or to biologically stabilised leachate (Wang et al., 2014, Xiao et al., 2013). However, as far as we know, there are no studies comparing the oxidation obtained before and after the biological treatment. As a consequence, there is not data that allow evaluating if the findings collected in the bibliography using raw leachate can be extrapolated to the stabilised ones and viceversa. With this aim, Figure 5 shows the results obtained during the oxidation of the raw leachate (before this being treated biologically) and the stabilised leachate (after leaving the biological treatment).

FIGURE 5

Obviously, the initial COD and BOD₅ values were higher for the raw leachate than for the stabilised one (see Table 1). So, approximately a 77% COD reduction was reached during the biological treatment of this raw leachate, this value being similar to those reported by many authors under similar conditions (Renou et al., 2008; Sancha et al., 2014). Again, the experimental data were successfully fitted to the “three-lump” kinetic model. The value of k_1 (related to direct oxidation to CO₂) was a little bit higher for the raw leachate than for the stabilised one (see Table 2). However, k_2 value was negligible for the raw leachate in comparison to that obtained for the stabilised leachate. This low value obtained was due to the fact that the intermediate compounds were quickly formed during the heating period, these being oxidized to CO₂ after such period. This was also confirmed by the decreasing α values observed for the raw leachate (once the operating conditions were achieved), whereas for the stabilised one, α slowly increased from 0.08 to 0.14 (see Figure 5c). The value of k_3 was much higher for the raw leachate than for the stabilised one, which implied that intermediate compounds were faster degraded to CO₂ when the raw leachate was oxidized.

All this is in agreement with the η values obtained for the raw leachate, which drop importantly from 0.4 to 0.03 approximately, whereas the decrease of η values in the stabilised leachate was less noticeable (from 0.5 to 0.3, see Figure 5d).

The final COD reductions were quite similar, 49% for the stabilised leachate and 51% for the raw one, giving final COD values much higher for the raw leachate (see Figure 5a). In this sense, it should be noted that the biological treatment cannot be replaced by the oxidation process, at least in the conditions assayed.

AOSC evolutions for the raw and stabilised leachates confirmed our findings (see Figure 5b). Thus, AOSC of the raw leachate increased from -2.0 to -1.2 during the heating period, which proved the formation of intermediate compounds. After this period, the AOSC value decreased progressively achieving a final value close to the initial one (-1.7) coinciding with the oxidation of the intermediates to CO_2 . However, AOSC values for the stabilised leachate rose continuously achieving a final value of -1.9. Since AOSC values were similar, final biodegradability indexes were almost the same in both cases (see Figure 5f). It should be noted that wet oxidation improved significantly the biodegradability of the stabilised leachate. Hence, this treatment could be completed by a biological step, i.e. recirculating the effluent to the biological treatment.

Figure 5e shows the evolution of the colour number. As can be seen, the biological treatment allowed a significant colour reduction (73%), this value being higher than that obtained for the wet oxidation of the raw leachate (56%). The colour after the biological treatment was probably due to humic acids, which are coloured and non-biodegradable compounds (Rajca and Bodzek, 2013). When the wet oxidation was applied to the stabilised leachate, a complete colour removal was obtained, suggesting the whole elimination of these humic acids. However, the raw leachate after wet oxidation still presented an intense colour. It must be pointed out that colour removal for raw and stabilised leachates mainly occurred during the first 100 minutes, achieving a colour reduction for both cases of around 0.6 units. These facts showed that the humic acids of the raw or stabilised leachates were quickly attacked during the first minutes of the wet oxidation process. Nevertheless, most of the remaining colour in the raw leachate could not be removed by oxidation.

Sawyer et al.(2003) reported that colour of the raw leachate indicates the presence of large amounts of non-precipitable colloidal organic extracts. This colloidal matter is probably adsorbed and/or partially bioassimilated by the biomass during the biological treatment, explaining its absence in the stabilised leachate.

Eventually, the effect of the biological treatment of the raw leachate on the toxicity was negligible, as can be seen in Figure 5g at time zero. However, the evolution of the luminescence inhibition during the wet oxidation process differed. So, the toxicity of the stabilised leachate initially increased a bit and then remained approximately constant, as it was previously explained. On the other hand, the toxicity of the raw leachate slightly decreased, mainly due to the mineralization registered.

3.6. What is the effect of the landfill age on the oxidation of the leachate?

The characterization of a leachate is complicated since its composition varies greatly depending on the age of the landfill. Generally, leachate produced in younger landfills is characterised by the presence of substantial amounts of volatile acids, as a result of the acid phase of fermentation. In mature landfills, the great portion of organics in leachate is humic and fulvic-like species (Kulikowska and Klimiuk, 2008). However, this parameter is not usually discussed during the treatments of advanced oxidation of a specific leachate. How significant is the age of the landfill on the oxidation of leachates? In order to answer this question, wet oxidations of old and young leachates (collecting the same day in different parts of the landfill) were carried out and the results have been compared in Figure 6.

FIGURE 6

As it was expectable, young leachate showed the highest initial COD. During the first few years, the landfill is mainly in acidogenic phase and the concentration of organic carbon in the leachate is higher. Old landfills are generally in the methanogenic phase, generating leachates with lower COD values (Kulikowska and Klimiuk, 2008). Therefore, the easily biodegradable compounds have already been degraded anaerobically, remaining in the medium the less biodegradable species. Again, the data were successfully fitted to the lumped kinetic model. The age of the landfill had a negative effect on the oxidation rate, both total and partial, which can be derived from the lower values of the kinetic constants (k_1 , k_2 and k_3) obtained for the old leachates (see Table 2). Hence, the higher the

landfill age, the lower the percentage of COD removed. The value of k_2 was very low in both cases since the formation of intermediate compounds occurred during the heating period. After such period, these intermediates were oxidized to CO_2 , this oxidation being significant for the young leachate (high value of k_3). The values of α and η supports these facts. As we can see in Figure 6c and d, α and η values decreased importantly for young leachate (rapid oxidation to CO_2), whereas the reduction of such values was less marked for the old leachate since it is more refractory to oxidation.

Final COD reductions of 60% and 37% were observed for the young and old leachate, respectively (see Figure 6a). Reductions from 26% to 33% have been reported for advanced oxidation of old leachates when no catalyst was added (Kurniawan et al., 2009; Lin and Chang, 2000). However, it is remarkable that final values of COD at the end of the oxidations were very similar for the old leachate and the young one, with values of around 2000 mg O_2/L and 2800 mg O_2/L , respectively.

The effect of the landfill age on the AOSC was evident as well (Figure 6b). Initially, the young leachate showed higher AOSC than the older one. This is in accordance with the fact that the young leachate has a higher content in easily biodegradable compounds. In the case of the old leachate, the initial AOSC (-2.7) increased up to -1.6 during the heating period due to the formation of intermediates and afterwards kept approximately constant. Similar tendency was observed for the young leachate, although the improvement in the AOSC value in the heating period was more noticeable, decreasing again after this period due to the decarboxylation of this compounds. As expected, the value of the initial biodegradability was higher for the young leachate than for the older one and the same occurs after the oxidation process (see Figure 6f). Many authors have reported that the BOD_5/COD ratio of the leachate has a decreasing trend with age from a readily biodegradable ratio of 0.5 to a higher fraction of poor degradability with a value of 0.1 or less (Renou et al., 2008; Wiszniowski et al., 2006). Eventually, luminescence inhibition measurements also are in accordance with this finding, the old leachate being more toxic. As was expected, the colouration of the old leachate was more intense than that observed for the young one (Figure 6e). The higher content of humic acids and the iron oxidation from ferrous to ferric iron are the responsible of the colour changes from light yellow in young leachates to dark brown or black in older ones (Chian et al., 1976).

For the young leachate, the initial yellow colour was removed almost completely during the wet oxidation, whereas for the older one, the colour reduction was less evident due to its higher content in humic substances and non-precipitable colloidal organic extracts (Sawyer et al., 2003).

At this point, it must be taken into account that humic and fulvic acids are closely related to the age of the landfill. An older landfill usually has a greater content of humic and fulvic substances and as the leachate becomes older and more diluted, the ratio of humic/fulvic acids also increases (Christensen et al., 1998). Our findings suggest that the landfill age have two opposite effects on the refractory character of the leachate. On one hand, older landfills increase the total humic and fulvic acids content, reducing the degree of mineralization during the oxidation. On the other hand, the ratio humic/fulvic acids also increase and, according to our findings, the oxidability of the leachate was improved. So, for two different leachates from landfills with similar ages, it is expected that the advanced oxidation process is more efficient when is applied to those with higher percentage of humic acids.

4. Conclusions

The works found in the literature about advanced oxidation of leachates are mainly focused on the effect of the main operational conditions on the depuration of a leachate obtained from a specific landfill. In this work, it has been demonstrated the effect of several characteristics of leachate (content in humic acids, landfill age and degree of stabilization) on the oxidation process and, therefore, in the quality of the final effluent.

The use of commercial humic acid with the aim of simulating the behaviour of the humic fraction of a leachate during an advanced oxidation process should be done thoroughly. Significant discrepancies have been found in terms of COD removal, colour, final products and biodegradability. Besides, it would be desirable that the leachate contained a higher proportion of humic acids since this fraction is more easily oxidizable than the rest of the matrix. The synergistic effect of the matrix on fulvic and humic acids were not significant. However, an important effect was observed for the oxidation of humic acids due to the formation of intermediate compounds. This last statement implies that the degree of backmixing in the reactor should be considered as an additional variable during the experimentation and design.

The differences between the wet oxidation of raw and stabilised leachates were not important either in terms of final AOSC value or biodegradability index. However, most of the remaining colour in the raw leachate can only be removed by biological treatment and important differences were also found in the COD of the effluent after the oxidation process.

Regarding the effect of the age of the selected leachate on the wet oxidation treatment, the findings revealed that the older the raw leachate, the lower the mineralization rate and the lower the biodegradability after oxidation treatment. This is because in older landfills increases the total humic substances content (humic and fulvic acids), reducing the degree of mineralization during the oxidation.

Consequently, experimental results obtained during the advanced oxidation of a specific leachate cannot be extrapolated to other cases without a thorough analysis of the specific characteristic of the leachate to be treated.

All experimental data were successfully fitted to a triangular “three-lump” kinetic model, which consider that the leachate can be directly oxidized to CO₂ or to intermediate compounds which can also be degraded to CO₂.

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Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proofs

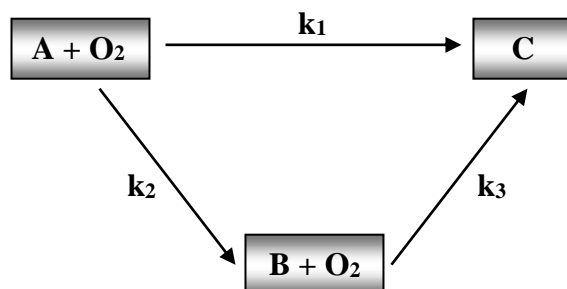
Figure 1**Figure 1.** Triangular lumped kinetic model proposed.

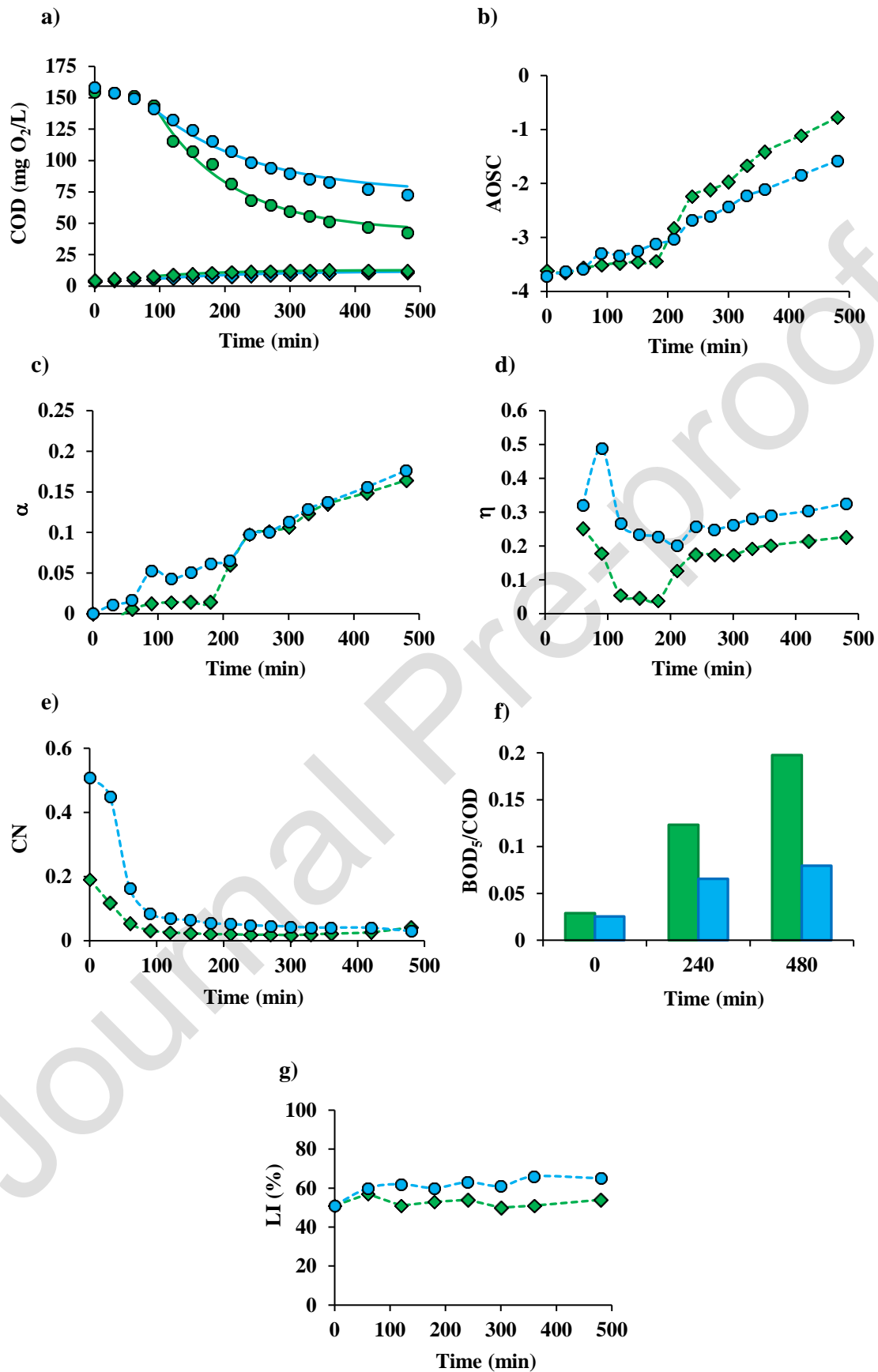
Figure 2

Figure 2. Evolution of the a) COD (●) and COD of the intermediate compounds (◆), b) AOSC, c) extent of partial COD removal (α), d) efficiency of partial COD removal (η), e) colour number (CN), f) biodegradability index (BOD_5/COD) and g) luminescence inhibition (LI) during the wet oxidation of the commercial humic acid (blue symbols) or the humic fraction of the stabilised leachate (green symbols). In both cases: $T = 453\text{ K}$, $P = 6.0\text{ MPa}$, without initial pH adjustment (6.8). Solid lines in a) denote model curve according to Table 2.

Figure 3

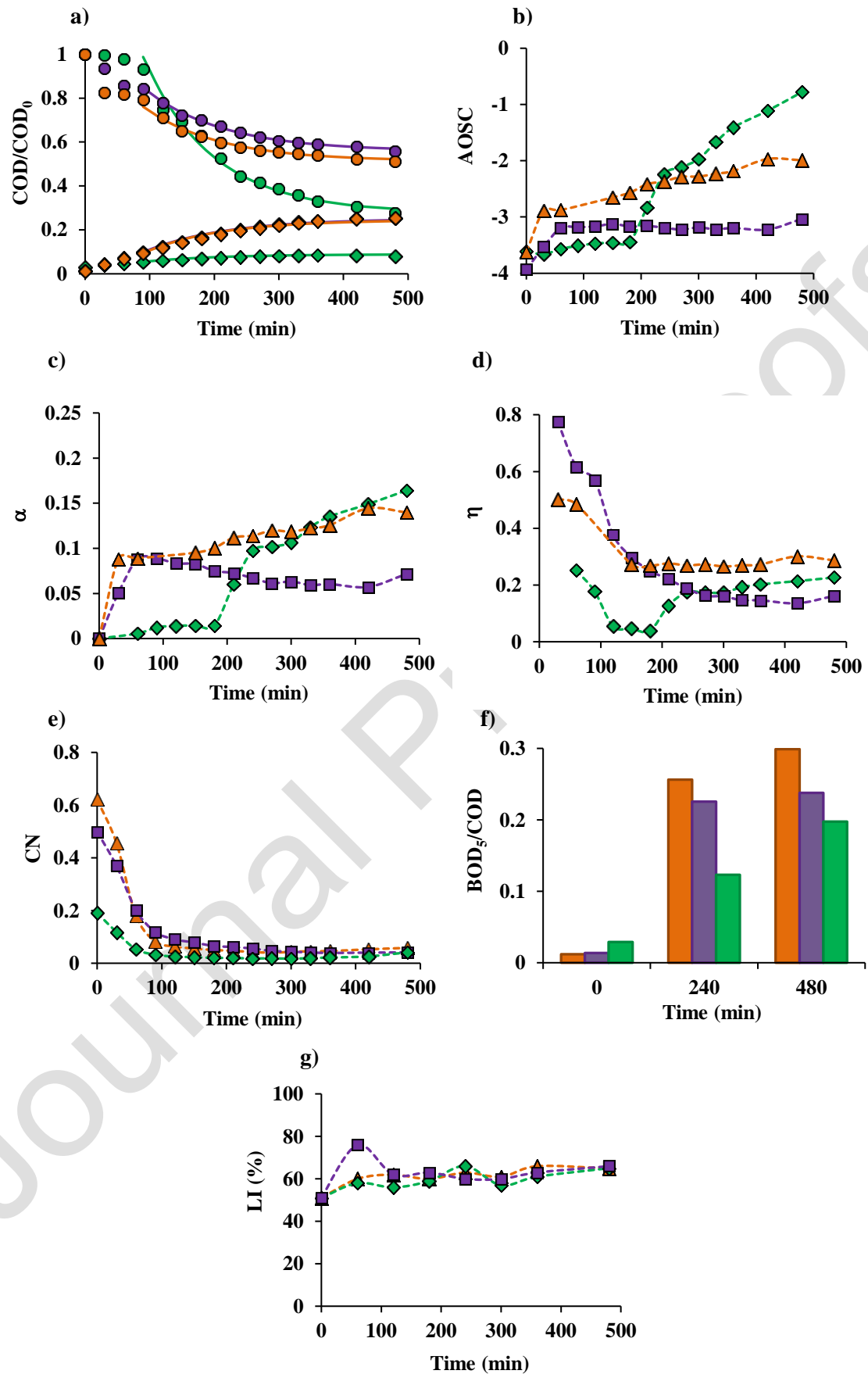


Figure 3. Evolution of the a) COD (●) and COD of the intermediate compounds (◆), b) AOSC, c) extent of partial COD removal (α), d) efficiency of partial COD removal (η), e) colour number (CN), f) biodegradability index (BOD_5/COD) and g) luminescence inhibition (LI) during the wet oxidation of the stabilised leachate (orange symbols) and the humic (green symbols) and fulvic fractions (purple symbols) previously separated by means of acidic precipitation. In all cases: $T = 453\text{ K}$, $P = 6.0\text{ MPa}$, without initial pH adjustment (6.8). Initial concentration of the stabilised leachate, fulvic and humic fraction: $1143\text{ mg O}_2/\text{L}$, $989\text{ mg O}_2/\text{L}$ and $155\text{ mg O}_2/\text{L}$, respectively. Solid lines in a) denote model curve according to Table 2.

Figure 4

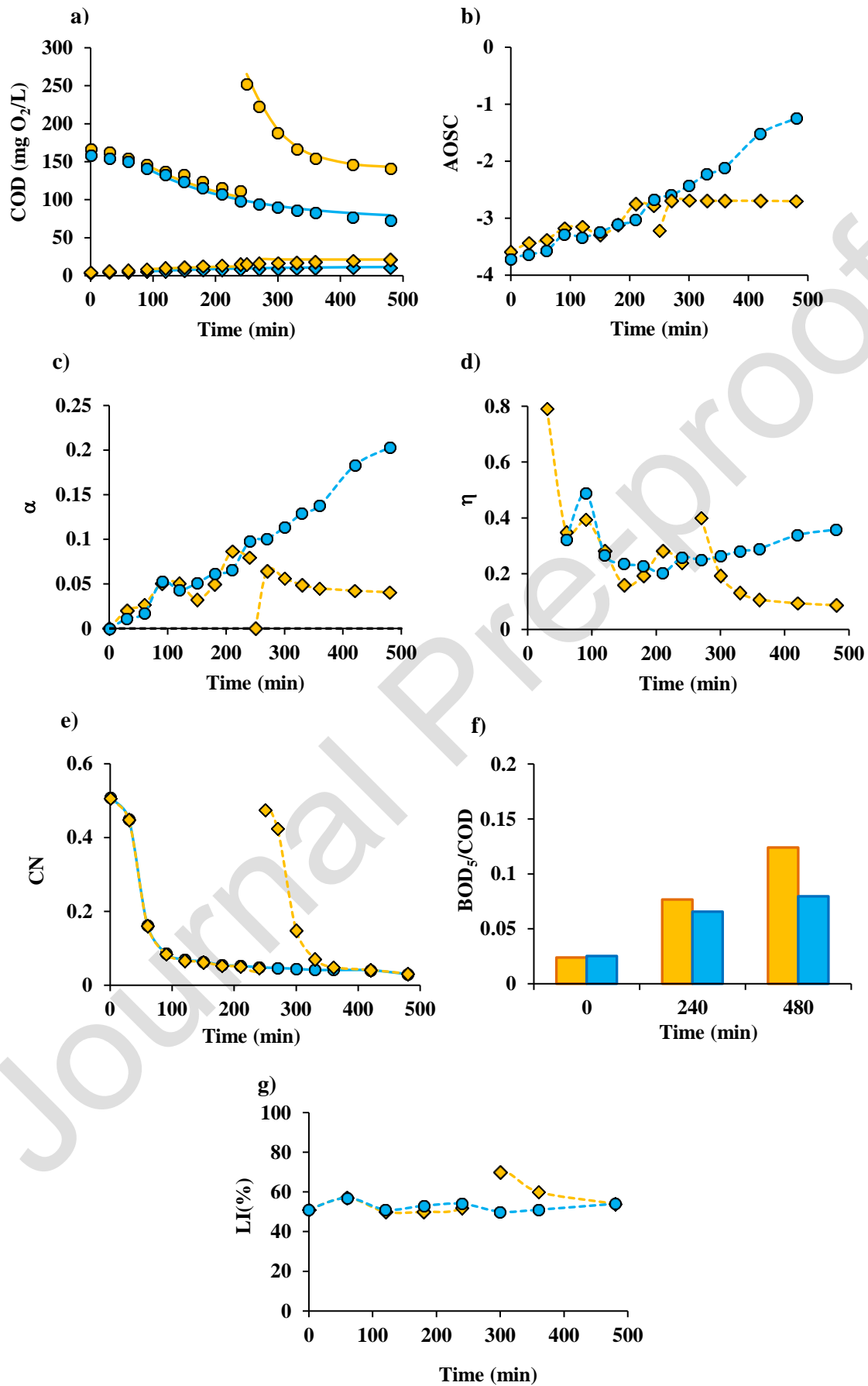


Figure 4. Evolution of the a) COD (●) and COD of the intermediates (◆), b) AOSC, c) extent of partial COD removal (α), d) efficiency of partial COD removal (η), e) colour number (CN), f) biodegradability index (BOD_5/COD) and g) luminescence inhibition (LI) during the wet oxidation of commercial humic without (blue symbols) or with a second injection of humic acid after 240 minutes of reaction (yellow symbols). In both cases: $T = 453\text{ K}$, $P = 6.0\text{ MPa}$, without initial pH adjustment (6.8). Solid lines in a) indicate model curve according to Table 2.

Figure 5

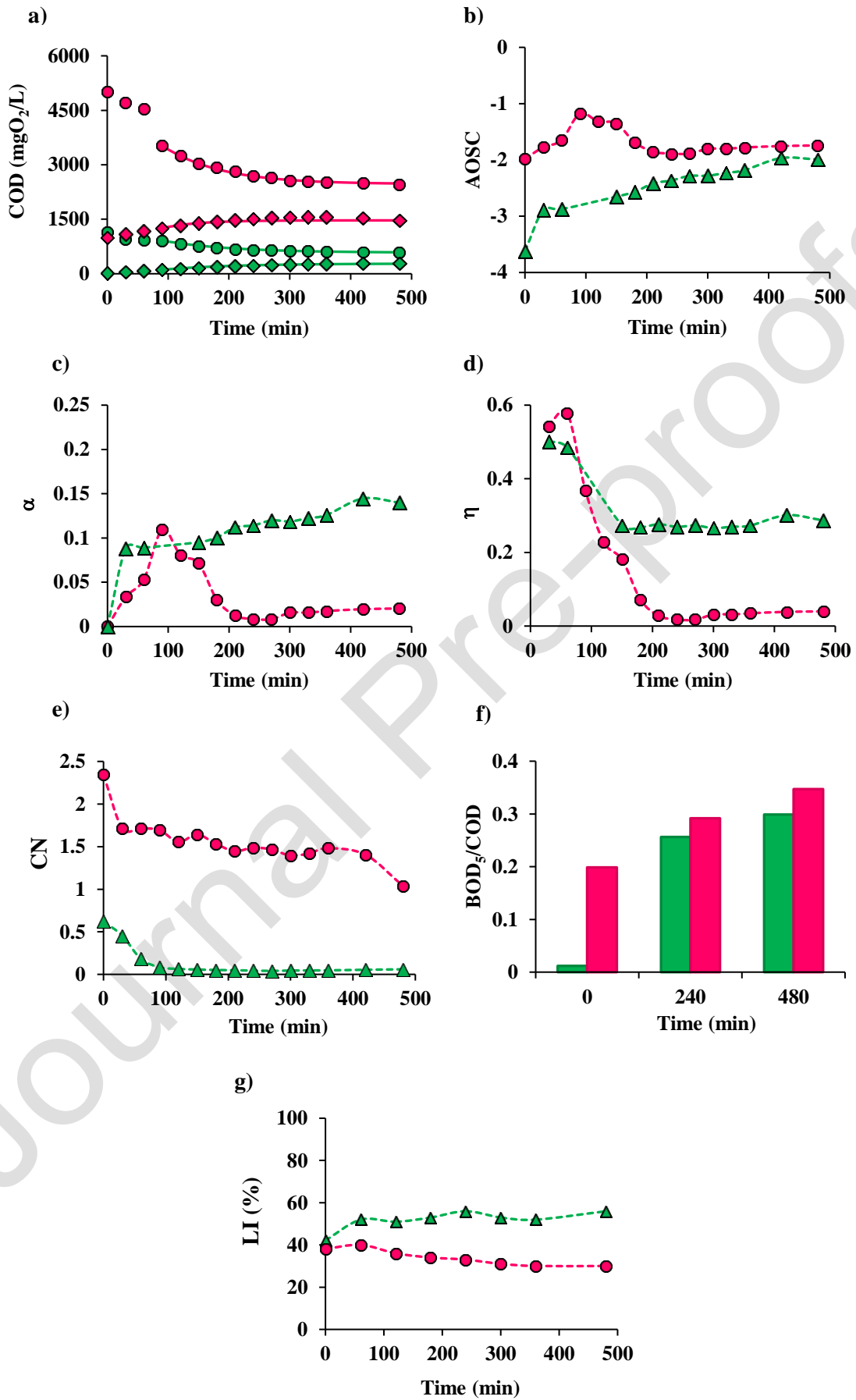


Figure 5. Evolution of the a) COD (●) and COD of the biodegradable compounds (◆), b) AOSC, c) extent of partial COD removal (α), d) efficiency of partial COD removal (η), e) colour number (CN), f) biodegradability index (BOD_5/COD) and g) luminescence inhibition (LI) during the wet oxidation of a raw leachate (fuchsia symbols) or a biologically pretreated leachate (green symbols). In both cases: $T = 453$ K, $P = 6.0$ MPa, without initial pH adjustment. Solid lines in a) indicate model curve according to Table 2.

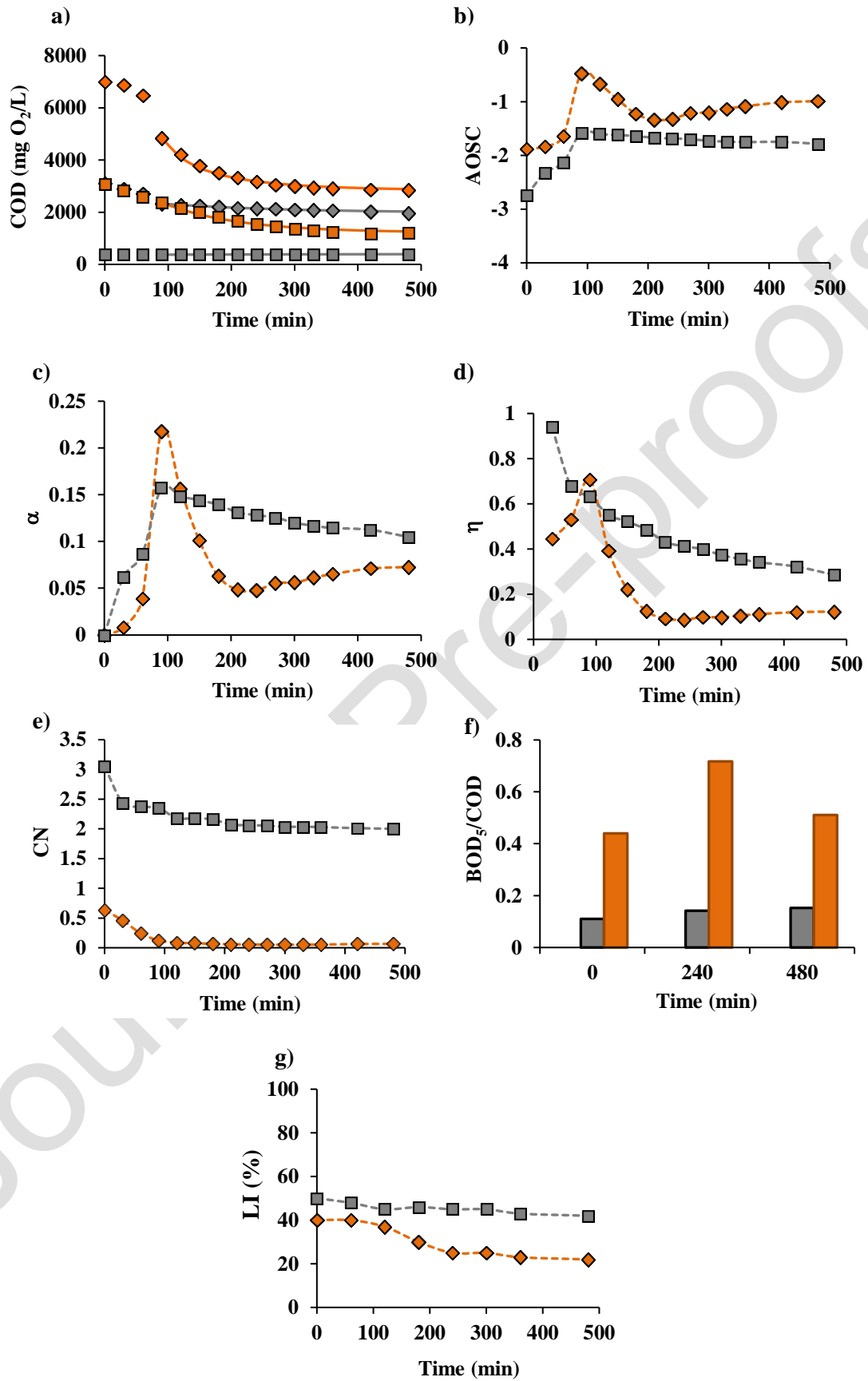
Figure 6

Figure 6. Evolution of the a) COD (\blacklozenge) and COD of the biodegradable compounds (\blacksquare), b) AOSC, c) extent of partial COD removal (α), d) efficiency of partial COD removal (η), e) colour number (CN), f) biodegradability index (BOD_5/COD) and g) luminescence inhibition (LI) during the wet oxidation of raw leachate with different ages: young (orange symbols) or old (grey symbols). In both cases: $T = 453\text{ K}$, $P = 6.0\text{ MPa}$, without initial pH adjustment. Solid lines in a) indicate model curve according to Table 2.

Tables**Table 1.** Average values of physicochemical characteristics of the landfill leachates generated in “La Zoreda” landfill site (from 2007 to 2015) and values measured in the leachates used in this work (in brackets).

Parameter	Type of landfill leachate			
	Old leachate	Young leachate	Raw leachate	Stabilized leachate ⁽¹⁾
pH	8.4 ± 0.1 [8.9]	n/a [6.7]	8.5 ± 0.2 [7.8]	6.6 ± 0.3 [6.8]
COD (mg O₂/L)	3006 ± 552 [3105]	6709 ± 1863 [6991]	4636 ± 1132 [5011]	1296 ± 297 [1143]
TOC (mg/L)	n/a ⁽²⁾ [641]	n/a [1784]	n/a [1257]	n/a [225]
BOD₅ (mg O₂/L)	451 ± 105 [372]	2076 ± 619 [3077]	1526 ± 575 [1093]	23 ± 15 [14]
Colour Number	n/a [3.052]	n/a [0.631]	n/a [2.345]	n/a [0.622]
Suspended solids (mg/L)	22 ± 12 [30]	87 ± 82 [100]	50 ± 34 [55]	10 ± 5 [0]
NH₄⁺ (mg/L)	2189 ± 317 [2323]	2813 ± 441 [3247]	2253 ± 333 [2739]	7±6 [5]
NO₃⁻ (mg/L)	n/a	n/a	n/a [20]	526 ± 196 [698]
NO₂⁻ (mg/L)	n/a	n/a	n/a [< 1]	1.5±0.6 [1]
PO₄³⁻ (mg/L)	n/a	n/a	n/a	9 ± 3 [10.8]
Alkalinity (mg CaCO₃/L)	212 ± 28 [221]	258 ± 36 [281]	212 ±30 [242]	16 ± 7 [18.7]
Conductivity (µS/cm)	23517 ± 2912 [23800]	25941 ± 2992 [19990]	23504 ± 2990 [25900]	12036 ± 1607 [13460]

⁽¹⁾after biological treatment; ⁽²⁾n/a= not available

Table 2. Relevant kinetic data for the non-catalytic wet oxidation of different types of landfill leachates.

	Biologically pretreated			
	Components			Real
	Commercial Humic acid	Humic Fraction	Fulvic Fraction	Stabilized Leachate
T (K)	453	453	453	453
P (MPa)	6.0	6.0	6.0	6.0
C_{O₂} (M)	4.8×10^{-2}	4.8×10^{-2}	4.8×10^{-2}	4.8×10^{-2}
k₁ (s⁻¹)	$(9.66 \pm 0.03) \times 10^{-5}$	$(1.36 \pm 0.08) \times 10^{-4}$	$(1.03 \pm 0.07) \times 10^{-4}$	$(1.09 \pm 0.03) \times 10^{-4}$
k₂ (s⁻¹)	$(8.97 \pm 0.02) \times 10^{-6}$	$(6.50 \pm 0.05) \times 10^{-5}$	$(2.65 \pm 0.09) \times 10^{-5}$	$(4.44 \pm 0.04) \times 10^{-5}$
k₃ (s⁻¹)	$< 10^{-8}$	$(4.17 \pm 0.02) \times 10^{-6}$	$(5.76 \pm 0.03) \times 10^{-5}$	$(1.18 \pm 0.06) \times 10^{-6}$
C_{A,u} (ppm)	62 ± 4	31 ± 5	307 ± 6	300 ± 8
C_{B,u} (ppm)	11 ± 3	12 ± 4	245 ± 7	287 ± 4
r²_{a;b;c}	0.998;0.991;0.997	0.997;0.9996;0.998	0.9996;0.998;0.996	0.9997;0.997;0.997

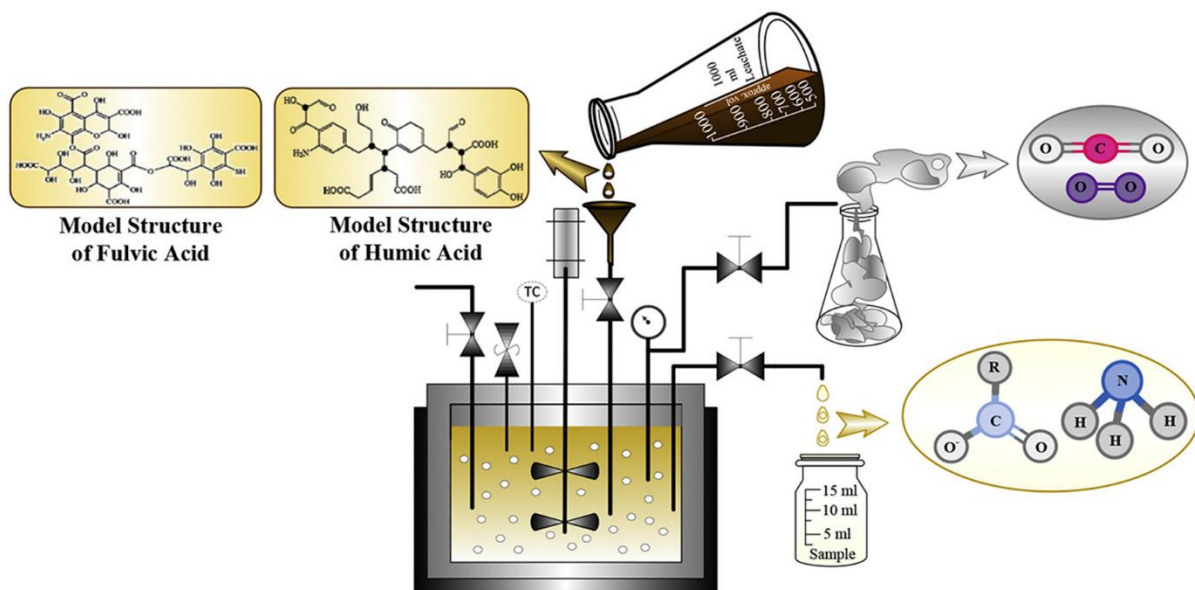
a;b;c referred to the goodness of k₁, k₂, k₃ fitting

	Non-Biologically pretreated		
	Real		
	Old leachate	Young leachate	Raw leachate
T (K)	453	453	453
P (MPa)	6.0	6.0	6.0
C_{O₂} (M)	4.8×10^{-2}	4.8×10^{-2}	4.8×10^{-2}
k₁ (s⁻¹)	$(8.20 \pm 0.06) \times 10^{-5}$	$(4.70 \pm 0.06) \times 10^{-4}$	$(1.88 \pm 0.03) \times 10^{-4}$
k₂ (s⁻¹)	$< 10^{-8}$	$< 10^{-8}$	$< 10^{-8}$
k₃ (s⁻¹)	$(6.53 \pm 0.02) \times 10^{-5}$	$(1.40 \pm 0.08) \times 10^{-4}$	$(2.82 \pm 0.03) \times 10^{-4}$
C_{A,u} (ppm)	1577 ± 4	1632 ± 7	999 ± 6
C_{B,u} (ppm)	393 ± 5	1216 ± 5	1468 ± 2
r²_{a;b;c}	0.9998;0.9997;0.997	0.9997;0.998;0.9998	0.998;0.998;0.9996

a;b;c referred to the goodness of k₁, k₂, k₃ fitting

	Commercial Humic acid	
	Before injection	After injection
T (K)	453	453
P (MPa)	6.0	6.0
C_{O₂} (M)	4.8×10^{-2}	4.8×10^{-2}
k₁ (s⁻¹)	$(9.66 \pm 0.03) \times 10^{-5}$	$(2.67 \pm 0.06) \times 10^{-4}$
k₂ (s⁻¹)	$(8.97 \pm 0.02) \times 10^{-6}$	$(4.81 \pm 0.06) \times 10^{-5}$
k₃ (s⁻¹)	$< 10^{-8}$	$(1.68 \pm 0.02) \times 10^{-2}$
C_{A,u} (ppm)	62 ± 4	120 ± 2
C_{B,u} (ppm)	11 ± 3	21 ± 1
r²_{a;b;c}	0.998;0.991;0.997	0.991;0.998;0.997

a;b;c referred to the goodness of k₁, k₂, k₃ fitting

Graphical abstract

Highlights

- High effect of leachate composition on the efficiency of wet oxidation treatment.
- The humic fraction is more easily oxidizable than the rest of the leachate matrix.
- The degree of backmixing is an important design variable in leachate wet oxidation.
- Lower mineralization rates and removals were obtained in older leachates.
- A lumped kinetic model considering total and partial COD oxidation was proposed.

Supplementary Information for

‘Effect of wet oxidation onto fingerprints of polymeric substances from an activated sludge’

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(11 Pages, 5 Figures, 1 Table)

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- 3. Effect of the EPS hydrophilicity/hydrophobicity on the MOC (Table S1).**

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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 $\sim 3300\text{ cm}^{-1}$ (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 $\sim 2900\text{ cm}^{-1}$ in the LB-EPS fraction, confirming that carbohydrates are present in a low concentration. This band was not 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 $\sim 3100\text{ cm}^{-1}$ were overlapped with those from carbohydrates at ~ 3350 and $\sim 2900\text{ cm}^{-1}$, 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 $\sim 80\%$ C-O stretching, $\sim 10\%$ 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 ($\sim 60\%$ N-H bending vibration and $\sim 40\%$ 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).

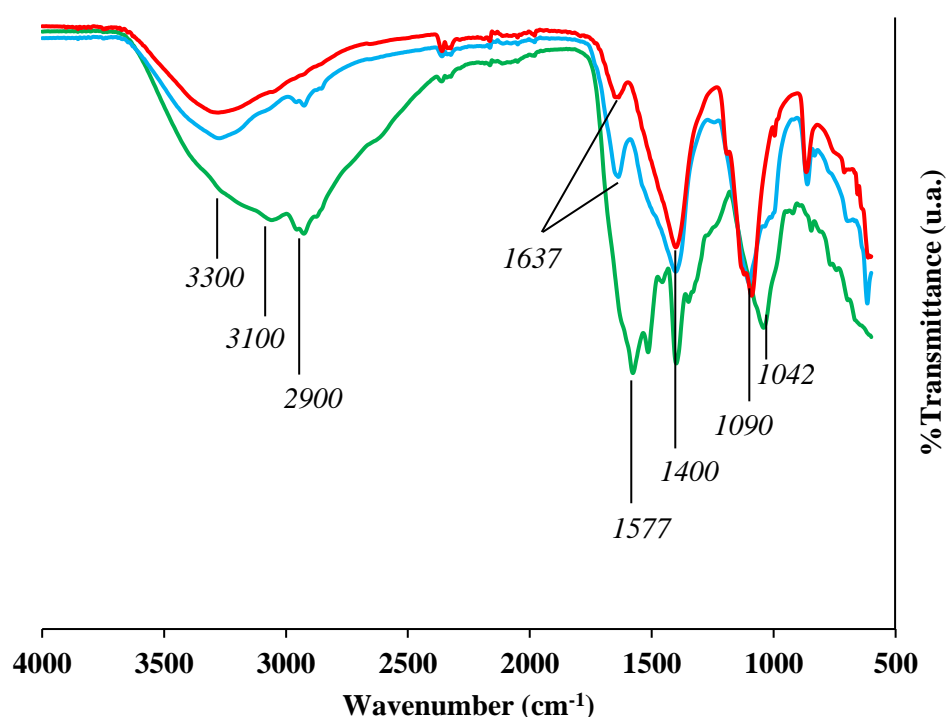


Figure S1. FTIR spectra of the initial fractions of SMP (■), LB-EPS (■) and TB-EPS (■).

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.

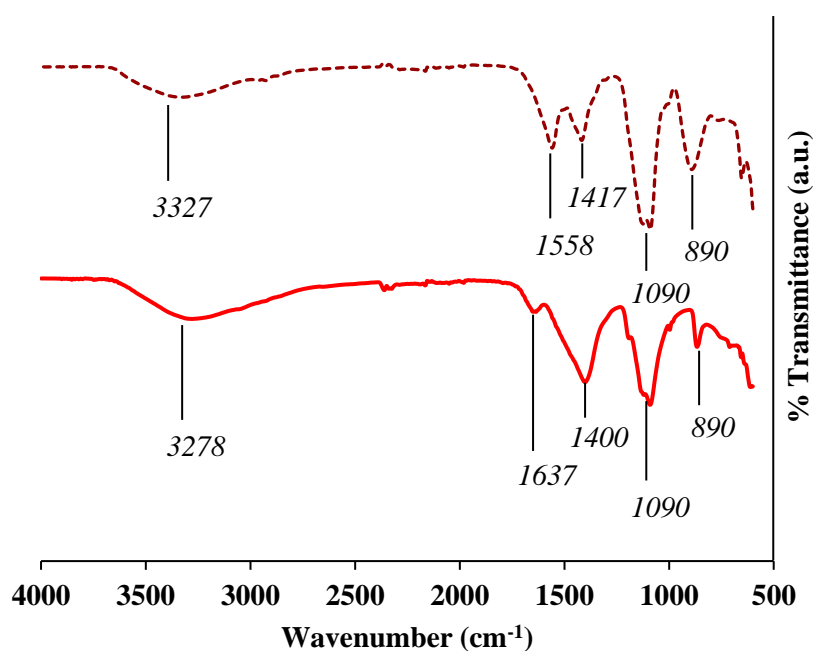


Figure S2. FTIR spectra of the initial (- -) and final (—) fractions of SMP.

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 $\sim 890\text{ cm}^{-1}$, 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.

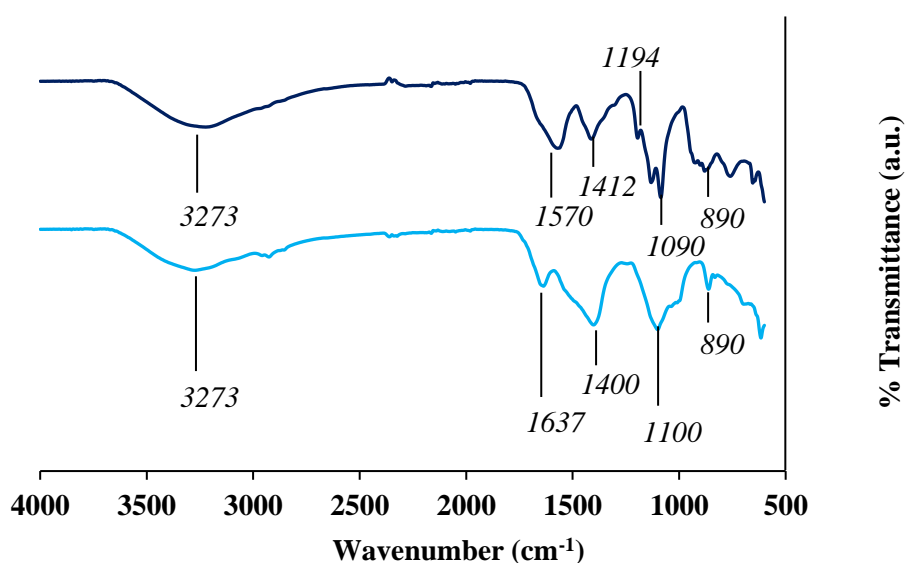


Figure S3. FTIR spectra of the initial (■) and final (■) fractions of LB-EPS.

Considering TB-EPS, the initial fraction showed a broad band at $\sim 3300\text{ cm}^{-1}$ (amide A), whereas, in the final fraction, both bands amide A and B was identified at ~ 3300 and $\sim 3100\text{ cm}^{-1}$, 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 $\sim 1600\text{ cm}^{-1}$. 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.

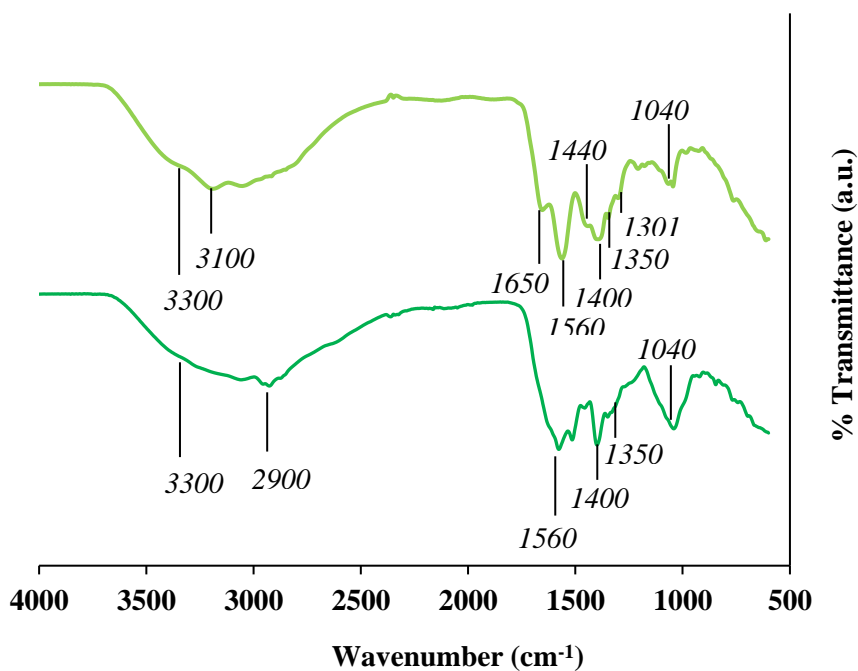


Figure S4. FTIR spectra of the initial (■) and final (■) fractions of TB-EPS.

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 S1) 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.

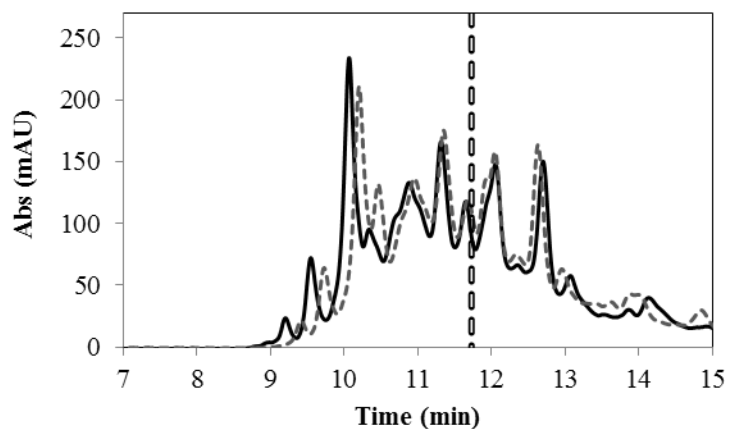


Figure S5. Polarity effect of mobile phase on fingerprints of activated sludge fractions. Result for naked cell after 210 min of WO at 190 °C and 65 bar. — Mobile phase buffer, - - - mobile phase buffer with 5% methanol, . . . total column volume. UV absorbance at 260 nm.

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.

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

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