

The wet oxidation of aqueous humic acids

Manuel García^a, Sergio Collado^a, Paula Oulego^a, Mario Díaz^{a*}

^a Department of Chemical and Environmental Engineering. University of Oviedo.

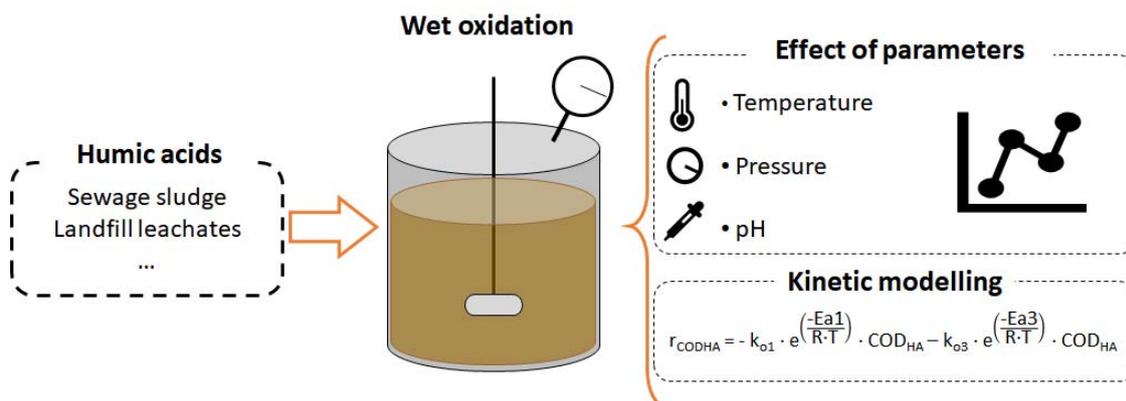
C/ Julián Clavería s/n. E-33071 Oviedo, Spain. Tel.:34985103439; Fax: 34985103434

* Mario Díaz. Department of Chemical and Environmental Engineering. University of Oviedo.

C/ Julián Clavería s/n. E-33071 Oviedo, Spain. Tel.: +34 985 10 34 39; Fax: +34 985 10

34 34 mariodiaz@uniovi.es

GRAPHICAL ABSTRACT



HIGHLIGHTS

Higher temperatures enhanced formation of acetic and oxalic acids

No effect of oxygen pressure (65-95 bar) on the removal

Alkaline medium: higher HA removal but more refractory intermediates

Alkaline medium favours the formation of formic, lactic, maleic and pyruvic acids

A lumped kinetic model was successfully fitted to the experimental data

ABSTRACT

Humic acids are highly distributed in aqueous environments. This article examines in depth the advanced oxidation of humic acid aqueous solutions, in order to understand more complex oxidation processes such as those of the sewage sludge or landfill leachate, or the matrix effects triggered by the humic acids of natural organic matter (NOM) in the oxidation of other aqueous compounds as herbicides.

Humic acids were efficiently oxidized; higher temperatures (180-220°C) involved higher mineralization, the formation of intermediates with lower colour and also led to a higher concentration of organic acids at the end of the treatment, particularly acetic and oxalic ones. Nevertheless, humic acid wet oxidation was not sensitive to changes in the pressure, at least in the range tested (65-95 bar), but the initial pH (4-13) was found to be a key factor. Thus, alkaline media accelerated the humic acid removal, but more refractory intermediates were generated, and the organic acids, excepting malic acid, were more stable than in neutral or acidic media. Eventually, a lumped kinetic model was proposed and successfully fitted to the experimental data, including the effect of all the operating variables studied.

KEYWORDS: humic acid, lumped kinetic model, NOM, organic acids, wet oxidation

1. Introduction

Humic acids (HAs) are extremely complex molecules. Its structure, which usually comprises quinones, phenolic, carboxylic, enolic and ether functional groups, but also peptides and carbohydrates [1], is characteristic of its biological origin, age and environment (among other factors). One of the main characteristics of these compounds is that they are partially soluble in water, totally insoluble in acidic media, but fully soluble in alkaline media.

HAs naturally occur in soils [2], but can also be found in both sea and land waters [3], constituting the main components of the Natural Organic Matter (NOM) [4]. Their high distribution throughout the environment is not surprising, taking into account that they derive from the highly transformed part of the residues of dead plants, animals, microorganisms and their degradation products, although the specific mechanisms of the formation of humic acids as well as their structure are still a subject of discussion and controversy [5].

Regarding their occurrence due to anthropogenic activities, humic acids are mainly found in some wastewaters in higher concentrations to those reported in terrestrial soil,

natural water, and sediments. So, humic acids are present in sewage sludge due to the hydrolysis of organic residues and as a result of the cellular lysis during post-production processing. The humic acids from NOM also get adsorbed to extracellular polymeric (EPS) matrix of sludge by different functional groups like carboxylic and phenolic ones [6–8]. Because of the different determination methods and influent sources used, the percentage of humic substances in the sludge composition varies in the range from 8% to 29% (expressed as % volatile solids) [9]. Humic acids are not anaerobically biodegradable, therefore, as well as increasing the disposal cost of sewage sludge, their presence decreases the biomethane production potential and generates a more polluted concentrate during the sludge anaerobic digestion [10,11].

Humic acids are also the main pollutants in landfill leachates. Specifically, the organic load, and toxicity, of mature leachates is largely caused by them, due to their very poor biodegradability [12]. This fact, together with the high volumes generated, explains the potential dangers of landfill leachates and the necessity to treat it so as to meet the standards for discharge in receiving waters [13].

Even when humic acids are naturally found in waters as a component of the NOM, their presence is also linked to some environmental concerns, mainly related to drinking water quality and its treatment processes, such as a deterioration in its organoleptic properties, higher costs of desalination and disinfection or the production of harmful disinfection by-products [14,15].

Ultimately, it is evident that the removal of humic acids is necessary, whether they are present in sewage sludge, leachates or as NOM. Different methods have been studied to this end such as fungal biodegradation [12,16], adsorption [17,18] or biosorption [6], coagulation [19,20]... Among them, advanced oxidation processes appear as ones of the most promising technologies for the removal of humic acids [21]. These processes are based on utilizing in-situ generated hydroxyl and/or sulphate radicals for the degradation of pollutants [22]. This technology is found to be particularly effective for the degradation of recalcitrant compounds, such as humic acids, by increasing biodegradability and reducing toxicity. Although available literature about advanced oxidation of humic acid-containing waters (sludges, leachates, NOM...) is abundant, to the best of our knowledge, there are no studies specifically dealing with the advanced oxidation of pure humic acids, even when this information should be very useful by several reasons. For example, this knowledge enables one to study the mechanisms involved during the oxidation of humic acid, avoiding the matrix effects caused by the other compounds present in the real

wastewater. Similarly, taking into account that humic acids (as part of NOM) are ubiquitous [23] in natural aquatic and terrestrial environments, they are also the main responsible for the matrix effects during the oxidation of other compounds, such as emerging contaminants or micro-pollutants in drinking water treatment [24]. Therefore, in order to understand either the oxidation of humic acids in wastewaters or their matrix effects on the oxidation of other pollutants in natural waters, examining in depth the advanced oxidation of purified humic acid is highly recommended as a first step towards achieving these objectives.

In consequence, the aim of this work is to investigate, for the first time ever, the oxidation of humic acid by hydroxyl radicals, using wet oxidation as AOP and paying special attention to the effect of the main process on either the reaction pathway or the kinetic model.

2. Material and methods

2.1. Reagents

The commercial humic acid (CAS 1415-93-6) used in the experiments was provided by Sigma-Aldrich. A 1000 ppm humic acid stock solution was prepared with distilled water. This concentration was chosen because it is an average value among those found in old landfill leachates, a waste that is mainly composed of humic acids [25].

2.2. Experimental setup

Wet oxidation experiments were performed with a PARR 4520 series semi-batch reactor (Parr Instrument Company, Illinois, USA), of one litre capacity. The reactor was equipped with a six-bladed stirrer. The gas line included a gas humidifier of two litre capacity upstream. For security, both reactor and humidifier were filled only to 70% of their maximum capacity. A PID controller allowed to select and maintain a constant temperature in both reactor and humidifier. Pressure was handled by a back-pressure valve located at the end of the gas line. A schematic view of the reactor setup can be found in Urrea *et al* [26].

Temperatures assayed during wet oxidation experiments ranged from 180 to 220 °C and pressures, from 65 to 95 bar, these being typical in conventional wet oxidation processes [27]. Different initial pH values between 4 and 13 were tested as well, which were adjusted using HCl 1 M or NaOH 1 M.

2.3. Analytical methods

Chemical Oxygen Demand (COD) and pH measurements were carried out according to Standard Methods [28]. Colour was evaluated in terms of colour number (CN), which was calculated using equation 1 [29]:

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

where SAC_i corresponds to the spectral absorption coefficient at a wavelength of i nanometres. The absorbance at each wavelength was determined with an AnalytikJena Spectrophotometer. HA concentration was spectrophotometrically measured according to Lowry modified method [30] with an AnalytikJena Spectrophotometer. Total organic carbon (TOC) was obtained using a TOC analyser (Shimadzu TOC-VCSH, Japan). The Average Oxidation State of Carbon (AOSC) was obtained by applying equation 2 [31]:

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

Attending to AOSC, is important to point out that in the text will be used the term MOC as well, which stands for Mean Oxidation number of Carbon. The difference between AOSC and MOC is that AOSC is referred to a mixture whereas MOC is used for pure compounds.

Concentrations of organic acids were determined by HPLC (Agilent 1200, Agilent Technologies Inc., California, USA) equipped with a Refractive Index Detector (RID). The column employed was a Coregel ION300 (Concise Separations, San Jose, USA), using 0.450 mM sulphuric acid (pH 3.1) as mobile phase at a flow rate of 0.3 mL min⁻¹. The following organic acids were detected and measured: formic, acetic, lactic, malic, maleic, oxalic and pyruvic.

3. Results and discussion

3.1. Effect of temperature

At first, the effect of temperature on the humic acid wet oxidation was analysed. In this regard, figure 1 shows the most relevant results obtained at three different temperatures.

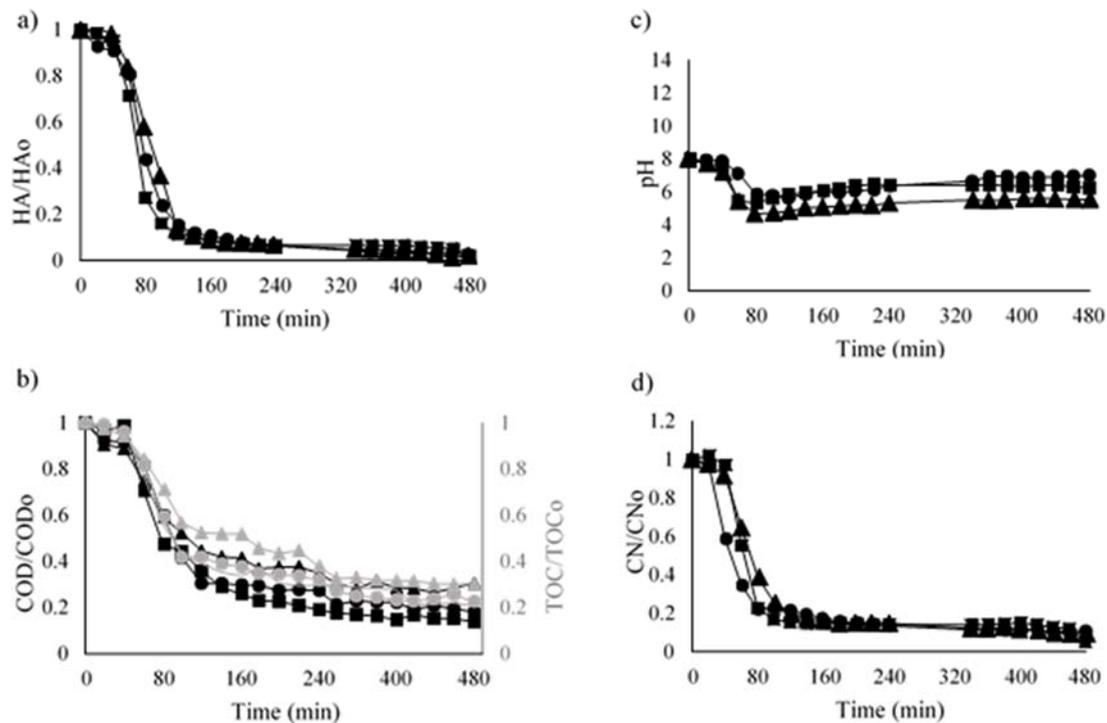


Figure 1. Evolution of: a) HA concentration, b) TOC and COD, c) pH, d) colour number during the wet oxidation of humic acids at three different temperatures: 180 °C (▲), 200 °C (●) and 220 °C (■). In all cases: 80 bar, initial pH 8 and initial concentration of 1 g/l.

From figures 1a, 1b, 1c and 1d, it is easily deduced that the evolution of pH, COD, TOC, CN and HA concentration showed three distinguishable stages: an initial phase, where there was no variation; a second stage, where the corresponding parameter sharply decreased; and a final stage, where its value remained almost constant.

It should be noted that the first minutes, where no variations were observed, corresponded to the heating phase until the operating conditions were reached. The main differences in the evolution of these parameters at different temperatures were found in the second stage when oxidation reactions prevailed. The third stage was probably related to the formation and accumulation of compounds highly refractory to oxidation [32].

Focusing on the organic load, expressed as either COD or TOC (figure 1b), this did not significantly depend on temperature during the preheating period, that is to say, the first 40 minutes of treatment. However, its decrease during the second stage was remarkable, being the removal faster at high temperatures. Regarding the third stage, the values at the end of the experiment corroborated the effect of temperature on the mineralization: the higher the temperature, the higher the organic load removed (70%, 82%, 86% of COD reduction at 180 °C, 200 °C and 220 °C, respectively; and 70%, 78%,

80% of TOC eliminated at 180 °C, 200 °C and 220 °C, respectively). These values are in the range of those reported in other works carried out with molecules with similar structures to humic acids, such as lignin, with COD removals around 80% [33]. However, the TOC removals reported for lignin were lower than those here obtained for HA, probably owing to the fact that lignin is chemically more complex than humic acids.

Attending directly to HA concentration, an increase in temperature also led to a faster abatement of this; at minute 100 (the end of drop stage), 84% HA was degraded at 220 °C, 76% at 200 °C and only 60% at 180 °C. In fact, humic acid was almost completely degraded at all temperatures if a sufficiently long reaction time is employed. This behaviour also proved that the remaining organic load is mainly due to refractory products and not to unreacted HA. The HA solutions are brownish, so its degradation is highly related to the evolution of colour number, as can be seen in figures 1a and 1d. As expected, higher temperatures led to faster CN reductions. However, there is not a direct proportionality between both parameters during the experimentation (figure S1a). It was found in the first stages that CN decreased faster than the HA concentration for all the temperatures assayed. This phenomenon could be surprising; however, it has been demonstrated that the colour of a humic acid solution is related to their size: the higher the size, the higher the colour [34]. In the final stages of the treatments (near point 0,0 in figure S1a), it can be observed that the curves went into the CN zone, indicating the presence of coloured intermediates. In this sense, a slight increase in colour was also detected during the first minutes of wet oxidation, especially at the highest temperature, whereas the humic acid concentration slightly decreased at this time, thus suggesting the formation of highly coloured intermediates in the first instants of reaction. Other authors have reported similar behaviours when carrying out WO of debarking wastewater, which contains important amounts of lignin [35]; or phenol itself [36], for example. These molecules, as well as humic acids, are initially oxidized into benzoquinones which are highly coloured and highly toxic [37]. The fact that COD lowered faster than TOC also suggested the presence of intermediates since oxygen was being consumed faster than carbon was being released.

Regarding pH (figure 1c), it suffered an initial decrease, reaching a minimum at 100 minutes. This drop on pH values is associated with the formation of organic acids and intermediates with low pKa, that decreased the global pH value, as will be subsequently evidenced by HPLC measurements. From minutes 100 to 480, pH increased, especially for experiments at 200 °C and 220 °C. Similar results were found in other works [38,39].

This phenomenon is explained by the reaction routes of WO, which include the formation of the mentioned organic molecules and CO₂ [40]. It is crucial to point out that the reactions mentioned are not sequential, but a balance between the generation of acidic molecules and their release from the liquid phase as CO₂ after being totally oxidized. This fact is supported by the findings of other authors, who reported CO₂ generation even in the first moments of the wet oxidation of phenol solutions [40].

These results were contrasted through the measurement of acids by HPLC (table S1). It was observed the predominance of acetic acid at the end of the reaction. This molecule was also observed as a final product in almost all the studies involving wet oxidation [33,41,42]. Increasing the temperature had a positive effect on the formation of acetic, lactic, maleic and oxalic acid and a negative one on formic, malic and pyruvic. The AOSC is a parameter that is strongly related to organic acids. As expected, AOSC was increased in all temperatures tested, being this increase higher at higher temperatures, corroborating the results mentioned for organic acids production, which have positive MOC values (excepting acetic acid which have a MOC = 0).

In conclusion, higher temperatures involved an increase in COD and TOC reductions and led to a faster formation of organic acids, except for formic, pyruvic and malic acids, which were less stable at high temperature.

3.2. Effect of pressure

Figure 2 show the main results obtained during the WO of humic acid at different pressures.

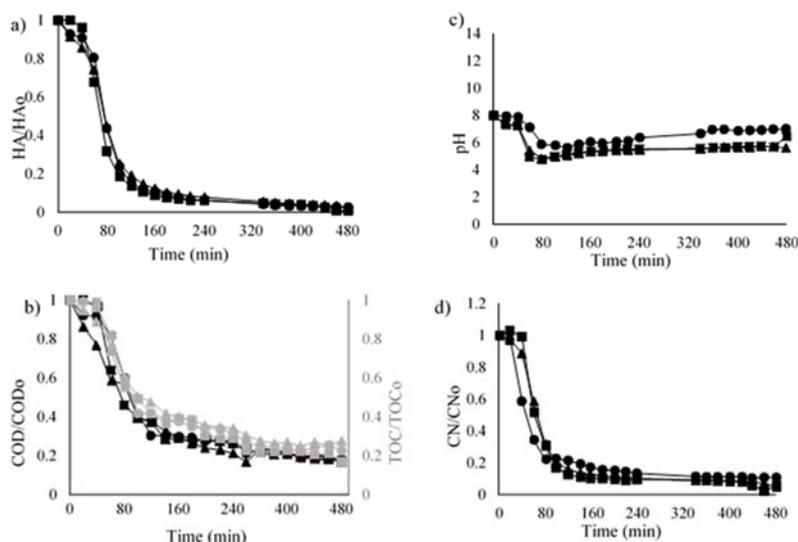


Figure 2. Evolution of: a) HA concentration, b) TOC and COD, c) pH, d) colour number during the wet oxidation of humic acids at three different pressures: 65 bar (▲), 80 bar (●) and 95 bar (■). In all cases: 200 °C, initial pH 8 and initial concentration of 1 g/l.

As in the case of temperature, the results for pH, COD, TOC, CN and HA concentration during the wet oxidation of commercial humic acid at different pressures showed again the previously explained three phases. Nevertheless, the effects of different pressures on the evolution of these three stages were negligible (figure 2b). So, an 84% of COD reduction was achieved at the end of the experiments, regardless of pressure. These results were similar to those reported by other authors, where pressure was not found to be significant in the COD removal of other organic materials, like sewage sludge, which is rich in humic acids [43,44].

The effect of pressure on the non-identified intermediates was also negligible, since a 49%, 51% or 54% of final COD values (at 65 bar, 80 bar and 95 bar, respectively) were attributed to the organic acids identified.

The evolution of pH (figure 2c) showed again similar values for 65 bar and 95 bar and a totally different evolution for 80 bar, probably due to the different distribution of acids obtained, predominating at this pressure those with higher pKa, such as malic acid.

To sum up, the use of higher pressures during the wet oxidation of humic acid did not show improvements in the evolution of either COD, TOC or CN.

3.3. Effect of initial pH

Finally, the effect of the initial pH on the wet oxidation of humic acid was analysed as well, testing initial pH values ranging between 4 and 13 (figure 3).

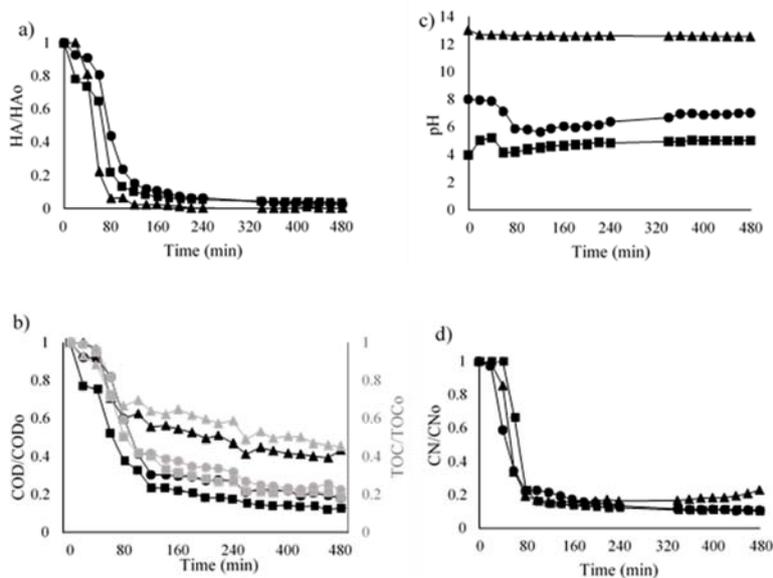


Figure 3. Evolution of: a) HA concentration, b) TOC and COD, c) pH, d) colour number during the wet oxidation of humic acids at three different initial pH values: 13 (▲), 8 (●) and 4 (■). In all cases: 200 °C, 80 bar and initial concentration of 1 g/l.

The results revealed that initial pH was the variable with the highest impact on the wet oxidation of commercial humic acid. As in the case of temperature and pressure sections, the evolutions of COD, TOC and HA followed the three phases already explained. In terms of COD, an acidic initial pH of 4 showed the highest mineralization (88% of initial COD was abated after 480 minutes), whereas the higher the initial pH, the lower the COD reduction, with COD reductions of 72% and 60% at initial pH values of 8 and 13, respectively. This behaviour was also corroborated by the TOC evolution; the reductions in this parameter were 55%, 78% and 82% for initial pH values of 13, 8 and 4, respectively. Studies dealing with the WO of phenolic compounds also obtained lower COD and TOC reductions in alkaline media [45].

Surprisingly, just attending to humic acid concentration, adjusting initial pH to 13 led to its fastest removal, achieving a complete abatement of the compound in 120 minutes, whereas for the other pH values tested, although degradation was high, the removal rates were slower (95% of HA degraded for initial pH 4 and 8 in 120 minutes) than at an initial pH of 13. In all cases, colour was quickly reduced (80% of the initial CN after 100 minutes). However, it is interesting to point out that some colour remained at the end of the treatments, especially in those experiments carried out in alkaline medium. In fact, at pH 13, CN even increased in the last minutes. This is associated with the formation of coloured intermediates (phenolic derivatives), as has been already mentioned during the discussion about the effect of temperature. Comparing humic acids and colour number evolutions (figure S1c), it was found that the removal of humic acid was faster than the colour reduction when an initial pH of 13 was selected, whereas this tendency reversed when the initial pH was 8. At acidic pH, the reduction of HA and colour was proportional during the experiment, indicating the no formation of a significant amount of coloured intermediates at acidic pH, thus also suggesting a less toxic degradation pathway of humic acid at lower pH [46].

These compounds (benzoquinones, hydroquinones...) are easily oxidized toward colourless molecules in neutral or acidic media, but their stability is increased at alkaline pH. In this light, it has been reported that phenolic molecules were much more slowly degraded at strong alkaline conditions [47], thus maintaining the colour we detected even at long reaction times. This increase can be linked with the increase in the CN at the same time.

The evolution of the oxidation state, measured as AOSC, was similar for the three pHs assayed. As the experiment advanced, the generation of oxidized molecules, such as

organic acids, raised AOSC values. This increase was particularly high for the experiment at pH = 4 since it started at negative a value (-0.7) and at the end of the experiment it turned into 0.75. The surprisingly low initial value could be due to the low pH, that led to the precipitation of a part of humic acids, lowering the COD value and therefore the AOSC value.

Regarding organic acids production, an alkaline medium strongly favoured the generation of some organic acids such as formic, lactic or pyruvic; whereas an acidic one led to worse generation, even some acids were not detected, for instance, pyruvic acid. These behaviour has already been reported in other works and is due to the effect of pH in the dissociation of the acids [45]. Moreover, our results coincide with works where phenol was subjected to WO. Santos et. *al* reported an enhancement in the production of formic, oxalic and pyruvic acid employing a catalytic WO treatment [48]. For example, at acidic pHs, formic acid and not formate was present, thus being easily degradable via non-oxidative thermal treatment and also via oxidation but, at the dissociated form, it is hard to degrade, because the only possible way is an oxidation reaction [49].

As seen in the previous paragraphs, there is a visible difference in the behaviour of all the parameters when an alkaline medium was employed. Excepting for HA, all parameters showed worse results, in terms of mineralisation and structure breakage. Moreover, the production of some organic acids was enhanced. This is mainly related to two phenomena. On the one hand, it has been reported that alkaline pH strongly affected the reaction pathways in wet oxidation experiments, since WO reactions are known to occur via free radical reactions [50] and the generation rate of these radicals is slower at alkaline pH [51]. On the other hand, humic acids tend to form pseudomicelles as pH decreases [52], so they are more difficult to attack.

In conclusion, the effect of pH on the WO of humic acid is clear. An alkaline pH led to worse mineralization and humic acids were completely degraded into coloured intermediates. In contrast, it allowed to obtain a higher concentration of organic acids at the end of the treatment. An acidic pH caused high COD and TOC reductions, but it also led to a lower final concentration of organic acids.

3.4. Kinetic modelling

Based on the findings, a lumped kinetic model was proposed, assuming the sequences of reactions shown in Figure 4. According to the reaction pathway proposed, the commercial HA is initially oxidized into non-identified intermediates (reaction 1) or directly oxidized into organic acids and CO₂, (reaction 3). In turn, the non-identified

intermediates can be further degraded to produce organic acids (reaction 2). Finally, organic acids can be completely mineralized into CO₂ (reaction 4).

The concentrations of humic acids, non-identified intermediates and organic acids were expressed as COD in order to simplify the calculations. The COD of HA and organic acids were calculated by using their theoretical COD, which can be easily obtained from the stoichiometry for the complete oxidation of the corresponding molecule to CO₂ and H₂O. The COD values for CO₂ (COD_{CO₂}) and non-identified intermediates (COD_{Int}), these were calculated according to the following equations:

$$\text{COD}_{Int} = \text{COD}_T - \text{COD}_{HA} - \text{COD}_{OA} \quad (3)$$

$$\text{COD}_{CO_2} = \text{COD}_{TI} - \text{COD}_{Tt} \quad (4)$$

where COD_{Int} is the COD of intermediates, COD_T is the total COD at any time, COD_{HA} and COD_{OA} are the COD values due to humic acid and organic acids, respectively, COD_{TI} is the initial total COD value and COD_{Tt} is the total COD value at each time.

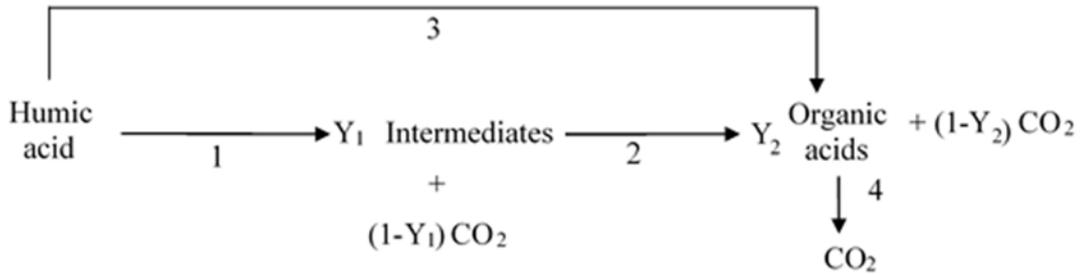


Figure 4. Scheme of the reactions proposed for the WO of humic acids. Numbers in the reactions indicate: 1, humic acids oxidation into non-identified intermediates and CO₂; 2, oxidation of the intermediates into organic acids and CO₂; 3, oxidation of humic acids directly into organic acids and CO₂; 4, oxidation of organic acids into CO₂. Y₁ and Y₂ are coefficients that represent the part of humic acid and intermediates that are converted into intermediates and organic acids respectively.

The software employed to obtain the kinetic constants was Micromath Scientist, by adjusting the model to the data obtained from the experiments at different temperatures or initial pH values.

3.1.1. Effect of temperature

According to the reaction pathway proposed, the next kinetic equations were deduced:

$$r_{\text{COD}_{HA}} = -k_{01} \cdot e^{\left(\frac{-Ea1}{R \cdot T}\right)} \cdot \text{COD}_{HA} - k_{03} \cdot e^{\left(\frac{-Ea3}{R \cdot T}\right)} \cdot \text{COD}_{HA} \quad (5)$$

$$r_{\text{COD}_{Int}} = Y_1 \cdot k_{o1} \cdot e^{\left(\frac{-Ea1}{R \cdot T}\right)} \cdot \text{COD}_{HA} - k_{o2} \cdot e^{\left(\frac{-Ea2}{R \cdot T}\right)} \cdot (\text{COD}_{Int} - \text{COD}_{Int,R}) \quad (6)$$

$$r_{\text{COD}_{OA}} = Y_2 \cdot k_{o2} \cdot e^{\left(\frac{-Ea2}{R \cdot T}\right)} \cdot (\text{COD}_{Int} - \text{COD}_{Int,R}) + k_{o3} \cdot e^{\left(\frac{-Ea3}{R \cdot T}\right)} \cdot \text{COD}_{HA} - k_{o4} \cdot e^{\left(\frac{-Ea4}{R \cdot T}\right)} \cdot \text{COD}_{OA} \quad (7)$$

It is important to point out that, as can be checked in figure 1b, some COD remained after the wet oxidation treatment. This residual value was taken into account during the modelization, as $\text{COD}_{Int,R}$. The proposed model was successfully fitted to the experimental data, as can be seen in figures S2a, S2b and S2c.

Table 1. Parameters of the Equations (5, 6 and 7), estimated from the kinetic constants.

In all temperatures $\text{COD}_{Int,R} / \text{COD}_{TI}$ was 0.1.

<i>Reactions (i)</i>	$\overline{K_{0i}}$ (s ⁻¹)	$\overline{Ea_i}$ (J/mol)	$\overline{Y_i}$
<i>1</i>	$(3.4 \pm 0.1) \times 10^5$	$(6.17 \pm 0.01) \times 10^4$	0.32 ± 0.05
<i>2</i>	$(3.4 \pm 0.2) \times 10^4$	$(5.07 \pm 0.07) \times 10^4$	0.40 ± 0.10
<i>3</i>	<i>Not applicable.</i>		
<i>4</i>			

From the fitting parameters obtained (table 1), several findings can be deduced. Thus, the main reactions of the mechanism were the direct oxidation of humic acid to non-identified intermediates (1) and the subsequent oxidation of these to organic acids (2). The low value of the kinetic constants 3 and 4 revealed that the direct transformations of humic acid into organic acids (3), as well as the degradation of organic acids into CO₂ (4), were not significant. The values obtained for Y_1 and Y_2 in combination with the high k for reactions 1 and 2 and the low k value obtained for reaction 4, suggest that the oxidation of the humic acid into intermediates is the main responsible for the CO₂ generation.

3.4.2. Effect of pressure

As explained in previous paragraphs, pressure did not produce noticeable effects on the COD values. For this reason, the effect of pressure was not modelled, since in all cases the results would have been similar to the results obtained for the modelling of the wet oxidation at 200 °C, 80 bar and initial pH equal to 8.

3.4.3. Effect of pH

Regarding the modelling of the experimental data obtained at different initial pH values (section 3.3), and due to the strong effect of an alkaline or acidic media in the WO of humic acid, it is necessary to take into account the effect of the different initial pH values tested on the kinetic model. The high effect of the pH on the wet oxidation suggests that the reaction mechanisms may well be different depending on the pH, so activation energies previously calculated for pH 8 cannot be extrapolated to pH 4 or, particularly, pH 13. Therefore, the data at different pH were employed to fit the kinetic model, assuming that the temperature remained constant during the experimentation, thus obtaining the pseudo-first kinetic constants instead of the corresponding pre-exponential factors and activation energies. Therefore, the next equations were proposed:

$$r_{\text{COD}_{\text{HA}}} = -k_1 \cdot \text{COD}_{\text{HA}} - k_3 \cdot \text{COD}_{\text{HA}} \quad (8)$$

$$r_{\text{COD}_{\text{Int}}} = Y_1 \cdot k_1 \cdot \text{COD}_{\text{HA}} - k_2 \cdot (\text{COD}_{\text{Int}} - \text{COD}_{\text{Int,R}}) \quad (9)$$

$$r_{\text{COD}_{\text{OA}}} = Y_2 \cdot k_2 \cdot (\text{COD}_{\text{Int}} - \text{COD}_{\text{Int,R}}) + k_3 \cdot \text{COD}_{\text{HA}} - k_4 \cdot \text{COD}_{\text{OA}} \quad (10)$$

This model was successfully fitted to the experimental data, as can be observed in figures S3a, S3b and S3c. Table 2 shows the values of the kinetic constants calculated at the different pH values tested.

Table 2. Parameters of the Equations (8, 9 and 10), estimated from the kinetic constants. COD_{IntR} values from figure 3b are showed as well.

<i>Parameter</i>	<i>pH 4</i> \bar{x}	<i>pH 8</i> \bar{x}	<i>pH 13</i> \bar{x}
<i>k₁</i>	5.9 x 10 ⁻²	5.2 x 10 ⁻²	2.0 x 10 ⁻¹
<i>k₂</i>	2.3 x 10 ⁻²	8.4 x 10 ⁻²	2.5 x 10 ⁻³
<i>k₃</i>	~0	~0	~0
<i>k₄</i>	~0	~0	1.4 x 10 ⁻²
<i>Y₁</i>	0.29	0.32	0.48
<i>Y₂</i>	0.35	0.40	0.05
<i>COD_{IntR}</i>	0.02	0.11	0.23

Observing the results in table 2, it can be corroborated that the alkaline medium enhanced the oxidation of the humic acid, being *k₁* at pH 13 two-fold higher than at pH 8 or pH 4. However, reaction 2 is less favoured at higher pH values. As in the case of the effect of temperature, the conversion of humic acid to CO₂ was found negligible at any pH. Unexpectedly, at alkaline pH values, the final oxidation of organic acids to carbon

dioxide (reaction 4) was significant, owing to the decomposition of organic acids to formic acid, a molecule that cannot be easily oxidized at alkaline pHs [45].

In alkaline medium, the production of carbon dioxide was mainly associated with the oxidation of the non-identified intermediates, as Y_2 coefficient shows. Nevertheless, the production of CO_2 from the direct oxidation of humic acids was also significant in acidic medium. The effect of the different pH in the structure of humic acids and the formation of free radicals has been profusely discussed in the effect of pH section, so those explanations can also be applied here.

4. Conclusions

Results revealed that humic acid can efficiently be removed by means of wet oxidation for all the conditions tested. It was found that the higher the temperature, the faster the humic acid removal and the mineralization, although around a 10% of the initial COD always turned out to be refractory to oxidation. High temperatures also involved a higher decolourization of the medium, enhancing the formation of acetic and oxalic acids as final products and reducing the stability of formic and pyruvic ones

On the other hand, pressure effect was negligible during the humic acid wet oxidation in the range tested (65 bar – 95 bar), not observing better mineralization or a faster humic acid degradation.

Finally, the initial pH turned out to be the key parameter during the wet oxidation of humic acids. When oxidation was carried out in an alkaline medium, humic acid was faster oxidized, but also gave place to more refractory intermediates than those obtained at either neutral or acidic media, as well as favoured the formation of organic acids as final products.

Finally, a reaction pathway based on the sequential oxidation of humic acids \rightarrow quinone-like compounds \rightarrow organic acids \rightarrow carbon dioxide was proposed on the basis of the experimental observations and employed to deduce the corresponding kinetic model, which was successfully fitted to the experimental data.

5. Acknowledgements

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SUPPLEMENTARY MATERIAL

THE WET OXIDATION OF AQUEOUS HUMIC ACIDS

Manuel García^a, Sergio Collado^a, Paula Oulego^a, Mario Díaz^{a}*

^a Department of Chemical and Environmental Engineering. University of Oviedo.

C/ Julián Clavería s/n. E-33071 Oviedo, Spain. Tel.:34985103439; Fax: 34985103434

* Mario Díaz. Department of Chemical and Environmental Engineering. University of Oviedo.

C/ Julián Clavería s/n. E-33071 Oviedo, Spain. Tel.: +34 985 10 34 39; Fax: +34 985 10

34 34 mariodiaz@uniovi.es

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Figure S1. Effect of a) temperature: 200 °C (—), 220 °C (- -) and 180 °C (· ·), in all cases pressure was 80 bar and initial pH was 8; b) pressure: 80 bar (—), 95 bar (- -) and 65 bar (· ·), in all cases temperature was 200 °C and initial pH was 8; and c) pH: 8 (—), 4 bar (- -) and 13 (· ·), in all cases temperature was 200 °C and pressure was 80 bar; on the relationship between HAs and CN.

Table S1. Effect of the different conditions on the evolution of the concentration of the measured organic acids. In effect of temperature: ● is 200 °C, ■ is 220 °C, ▲ is 180 °C; in all cases pressure was 80 bar and initial pH was 8. In effect of pressure: ● is 80 bar, ■ is 95 bar, ▲ is 65 bar; in all cases temperature was 200 °C and initial pH was 8. In effect of pH: ● is initial pH = 8, ■ is initial pH = 4, ▲ is initial pH = 13; in all cases temperature was 200 °C and pressure was 80 bar. Y axes have been deliberately adjusted to improve the view of the figures.

Figure S2. Fitting of the model to the experimental data for the different temperatures: a) 200 °C, b) 220 °C and c) 180 °C. In all cases ● represents experimental points of total COD, ■ is the experimental values for COD of intermediates, ▲ is the COD of organic acids, ◆ shows the COD loss as CO₂, (—) is the calculated total COD, (- -) is the calculated

intermediates COD, (- ·) is the calculated COD of organic acids and (· ·) is the calculated COD loss as CO₂.

Figure S3. Fitting of the model to the experimental data for the different initial pH values: a) initial pH = 8, b) initial pH = 13 and c) initial pH = 4. In all cases ● represents experimental points of total COD, ■ is the experimental values for COD of intermediates, ▲ is the COD of organic acids, ◆ shows the COD loss as CO₂, (—) is the calculated total COD, (- -) is the calculated intermediates COD, (- ·) is the calculated COD of organic acids and (· ·) is the calculated COD loss as CO₂.

S.1. Comparison between humic acids and CN decrease

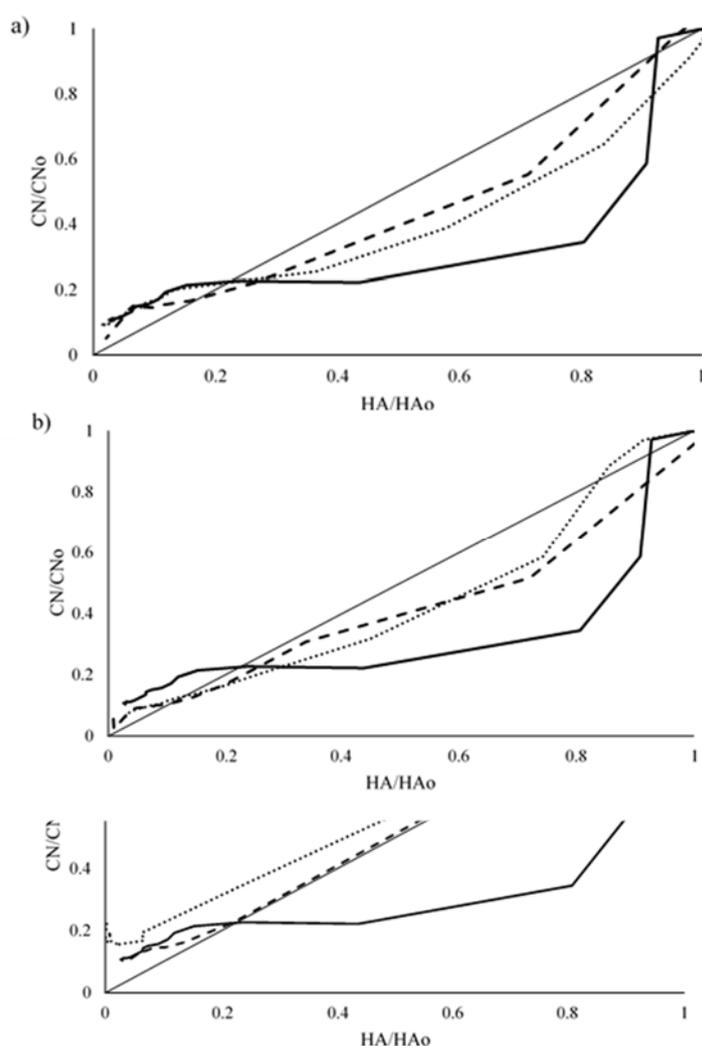
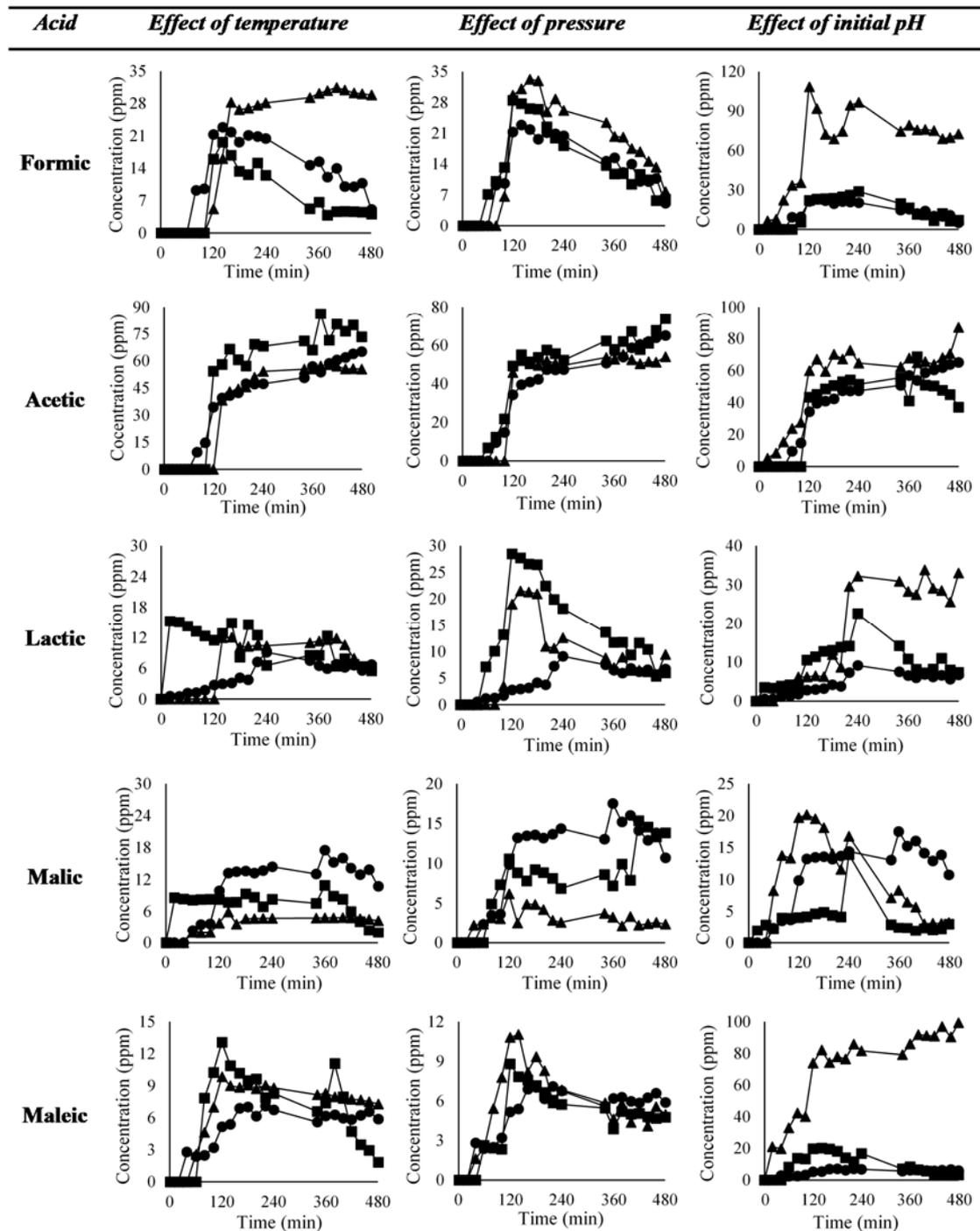


Figure S1. Effect of a) temperature: 200 °C (—), 220 °C (- -) and 180 °C (· ·), in all cases pressure was 80 bar and initial pH was 8; b) pressure: 80 bar (—), 95 bar (- -) and 65 bar (· ·), in all cases temperature was 200 °C and initial pH was 8; and c) pH: 8 (—), 4 bar (- -) and 13 (· ·), in all cases temperature was 200 °C and pressure was 80 bar; on the relationship between HAs and CN.

S.2. Organic acids production

Table S1. Effect of the different conditions on the evolution of the concentration of the measured organic acids. In effect of temperature: ● is 200 °C, ■ is 220 °C, ▲ is 180 °C; in all cases pressure was 80 bar and initial pH was 8. In effect of pressure: ● is 80 bar, ■ is 95 bar, ▲ is 65 bar; in all cases temperature was 200 °C and initial pH was 8. In effect of pH: ● is initial pH = 8, ■ is initial pH = 4, ▲ is initial pH = 13; in all cases temperature was 200 °C and pressure was 80 bar. Y axes have been deliberately adjusted to improve the view of the figures.



S.3. Modelling

S.3.1. Effect of temperature

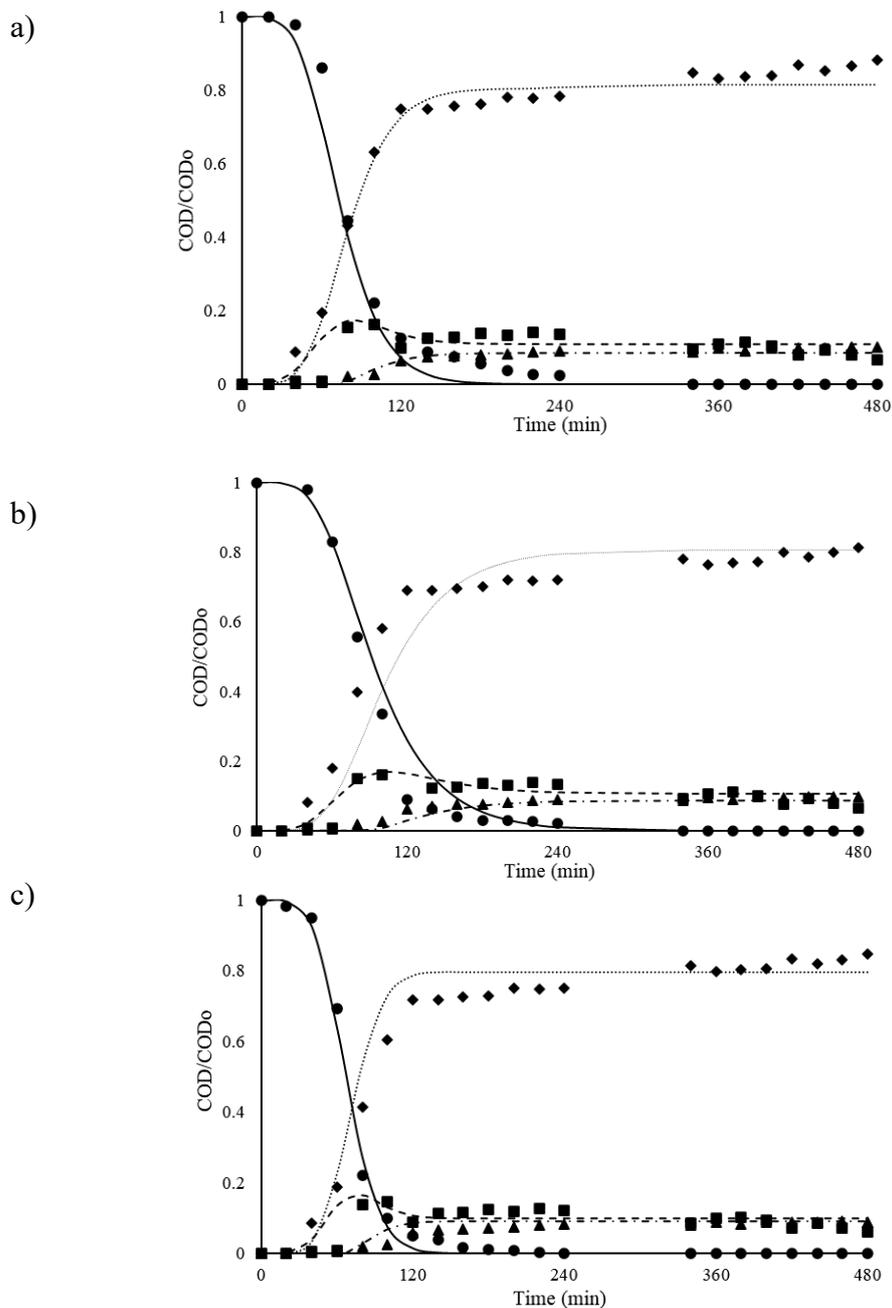
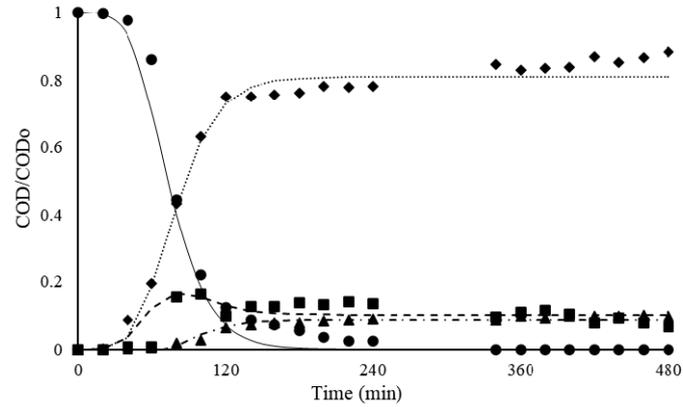


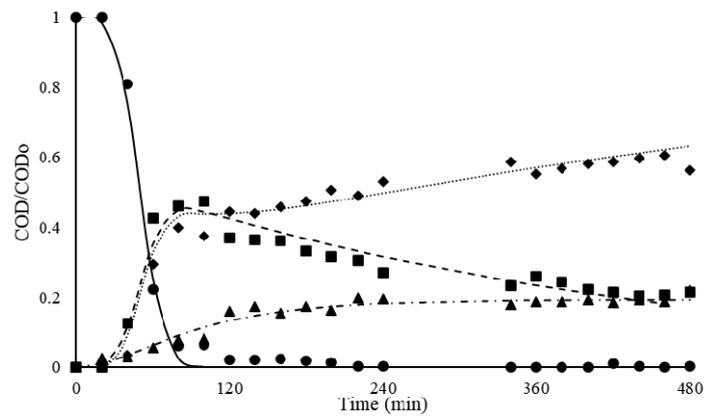
Figure S2. Fitting of the model to the experimental data for the different temperatures: a) 200 °C, b) 220 °C and c) 180 °C. In all cases ● represents experimental points of total COD, ■ is the experimental values for COD of intermediates, ▲ is the COD of organic acids, ◆ shows the COD loss as CO₂, (—) is the calculated total COD, (---) is the calculated intermediates COD, (- · -) is the calculated COD of organic acids and (· · ·) is the calculated COD loss as CO₂.

S.3.2. *Effect of pH*

a)



b)



c)

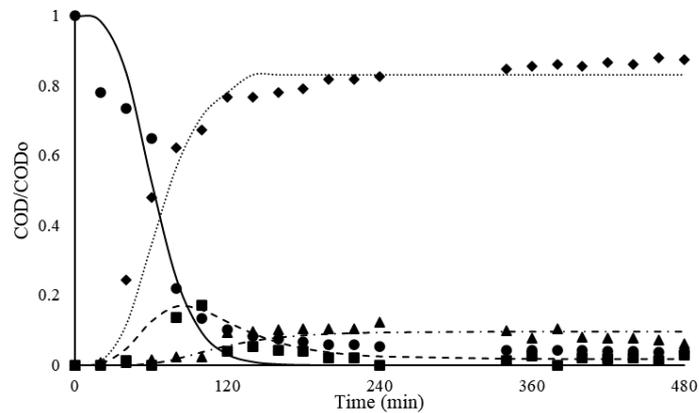


Figure S3. Fitting of the model to the experimental data for the different initial pH values: a) initial pH = 8, b) initial pH = 13 and c) initial pH = 4. In all cases ● represents experimental points of total COD, ■ is the experimental values for COD of intermediates, ▲ is the COD of organic acids, ◆ shows the COD loss as CO₂, (—) is the calculated total COD, (- -) is the calculated intermediates COD, (- ·) is the calculated COD of organic acids and (· ·) is the calculated COD loss as CO₂.

4.5. USO DE HIDROLIZADOS DE LODOS. PRODUCCIÓN DE ENZIMAS

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Estado: En revisión por la revista.

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Con la información obtenida en la caracterización de los tratamientos hidrotérmicos de los lodos de depuradora, se pudo comprobar que los hidrolizados son ricos en proteínas y carbohidratos, así como en ácidos húmicos. Estas moléculas son potenciales fuentes de carbono, por lo que es factible utilizar el hidrolizado como medio para fermentaciones que permitan obtener productos de interés. Es necesario indicar que los hidrolizados utilizados se obtuvieron al tiempo adecuado de tratamiento obtenido en el trabajo del apartado 4.2. Por ello, se plantea la inoculación del hidrolizado con un microorganismo capaz de sobrevivir en los hidrolizados, para lo que debe ser capaz de asimilar proteínas y ácidos húmicos, es decir, secretar proteasas y lacasas que le permitan utilizar las proteínas y los ácidos húmicos. Además, estas enzimas tienen alto valor comercial, pues las proteasas son ampliamente usadas, por ejemplo, en la industria de detergentes, en industria textil, industria química; y la lacasa se puede usar en descontaminación de aguas textiles o en industria alimentaria (Razzaq et al., 2019; Rodríguez Couto and Toca Herrera, 2006).

Uno de los microorganismos que cumple estas características es *Bacillus licheniformis* CECT 20. Además, es de nivel de bioseguridad 1, su genoma está secuenciado y su cultivo es sencillo.

Por lo tanto, el objetivo de este capítulo es evaluar la posibilidad de utilizar el hidrolizado obtenido tras la OH y la HT de lodos de depuradora como medio de fermentación para obtener enzimas de interés, abriendo así otra vía para la revalorización del lodo.

La publicación inicial de este trabajo fue como comunicación oral en el congreso “5th EUROPEAN CONFERENCE ON SLUDGE MANAGEMENT (ECSM’2019), OCTOBER 2019, Liège”, a partir de la cual fue seleccionada para un número especial en *Journal of Environmental Management*.