Estimation of Biological Kinetic Parameters from a Continuous Integrated Ozonation–Activated Sludge System Treating Domestic Wastewater

Fernando J. Beltrán, Juan F. García-Araya, and Pedro M. Álvarez

Departamento de Ingeniería Química y Energética, Universidad de Extremadura, 06071 Badajoz, Spain

The feasibility of treating municipal wastewater by a combined ozone-activated sludge continuous flow system was studied. Lab-scale experiments of both single activated sludge and combined ozone-activated sludge processes were carried out to determine the kinetic coefficients of the biological stage. The results obtained indicated a clear improvement in the kinetic parameters of the aerobic oxidation when a pre-ozonation stage was applied. Particularly, COD removal and nitrification rates were highly increased. The biokinetic parameters were also used to simulate and optimize the continuous reaction system. From the model prediction it was concluded that the integrated process (i.e., ozone-ASP) may significantly increase the waste reduction capacity. The results presented here provide a useful basis for further scaling up and efficient operation of ozone-ASP units in wastewater treatment processes.

Introduction

The activated sludge process (ASP) has traditionally been the usual approach for the treatment of municipal sewage and wastewater from many industrial activities. A number of variations of the basic system have been developed, which allow a wide range of operational circumstances to be applied (1). However, ASPs as conventional biological processes are not feasible to treat toxic and recalcitrant substances, which reduce the biodegradation performance and eventually could inhibit the process. Moreover, conventional ASPs are not able to remove nonreadily biodegradable substances that would require long hydraulic retention time to be mineralized, and therefore effluents sometimes contain organic material above the specified limits. Also, primarily as a result of environmental regulatory pressures the degree of domestic sewage purification to be achieved in wastewater treatment plants will likely increase in the future. In this sense, the development of integrated chemical-biological processes for the treatment of wastewater has created considerable interest. Thus, depending on the organic load and nature of the wastewater, the chemical treatment can effectively assist the cheaper biological process (2, 3).

Ozone, a known powerful oxidizing agent, presents some applications when used in conjunction with a conventional aerobic biological treatment of wastewater (i.e., ASP). In fact, nowadays ozone is used as oxidant and disinfectant agent in wastewater treatment processes for water reuse purposes (4). Recent works have also demonstrated the feasibility of using ozone as pretreatment just before an ASP or even integrated at the biological step. It has been found that ozone can be used to (a) improve particle flocculation through different mechanisms (5), (b) remove color and compounds toxic to bacteria (6, 7), (c) enhance wastewater biodegradability (8), (d) prevent foamability in the aeration basin of the ASP (9), (e) avoid excess of sludge that would require further treatment and improve sludge settling characteristics (10), and (f) lead to a satisfactory nitrification at the biological stage, avoiding inhibiting effects (11). In light of all these advantages the optimization of the operation strategy for a cost-effective combined ozone-ASP deserves special attention. However, little information on the kinetics of ozone-ASP applications is available to date.

The present paper reports on the study of a continuous ozone-ASP for domestic wastewater treatment with special focus on the kinetics of the biological stage, a necessary step for the design, scale-up, and optimal operation of the combined treatment on an industrial scale. ASP and combined ozone-ASP reactor performance have been then compared by simulating the continuous biological reaction system. Therefore, the goal of the present study was to investigate the role of the pre-ozonation step on the biological kinetic coefficients and reactor performance of the subsequent ASP.

Materials and Methods

Materials. Domestic wastewater used in this work was taken from the municipal wastewater treatment plant at Badajoz (Spain). The feed wastewater was collected from the discharge effluent of the primary clarification unit. Average BOD, COD, and NH$_4^+$-N concentrations at this point were 158 ± 13, 274 ± 25, and 35 ± 6 mg/L, respectively. Other features of the wastewater can be found in a previous work (12). Sludge for the biological treatment was obtained from the activated sludge returning line of the municipal plant and then acclimated in a laboratory reactor running in fill and drawn mode to the particularly reaction conditions (sludge concentration, temperature, dissolved oxygen concentration, aerobic sludge age, etc.). A second acclimation stage was carried out to adapt part of the sludge to ozonated wastewater. After each acclimation stage, the
sludge presented good metabolic and settling characteristics. Thus a yield coefficient, Y_{S/X}, of about 0.5 g BOD removed per g of volatile suspended solids (VSS) generated and sludge volume index, SVI, between 60 and 120 mL/g were obtained after acclimation. Ozone was produced from air in a 500 Fisher laboratory ozone generator.

**Experimental Setup and Procedure.** All experiments were performed in a continuous ozone-ASP reaction system described elsewhere (12). The ozonation reactor consisted of a 2.5-L bubble column (i.d. 9 cm; length 45 cm) provided with a porous plate (16–40 µm pore diameter) situated at its bottom to facilitate the diffusion of the gas supplied. A peristaltic pump was used to continuously recirculate the wastewaters at 20 L/h flow rate, thus providing good mixing conditions within the ozonation bubble column. The biodegradation was carried out in a 20-L continuously stirred reactor charged with a mixed culture (i.e., activated sludge) able to perform carbon oxidation and nitrification. Mechanical paddle stirrers provided mixing so that the aeration tank contents were well mixed and uniform throughout. Compressed air was supplied through bubble diffusers at 200 L/h to allow dissolved oxygen levels (DO) above 2.5 mg/L, avoiding anoxic zones in the reactor. Temperature in both chemical and biological reactors was kept constant in 20 ± 0.5 °C by means of thermostatic baths.

In a typical ozone-ASP run a constant flow rate of 2–18 L/h of feed wastewater was continuously delivered with the aid of a peristaltic pump from the feed tank to the top of the ozonation column. At the same time, an oxidant gas flow rate of 30 L/h was fed to the bottom of the column from the ozone generator so that liquid and gas phases worked in counter-current. Before entering the aeration basin, the ozonated wastewater went through a 2-L glass vessel where 100 L/h airflow rate was supplied in order to strip the dissolved ozone. This ozone would be dangerous for the microorganisms in the activated sludge. It may be noted that for single ASP experiments the feed wastewater was directly delivered from the storage tank to the digester. The effluent from the digester went to a clarifier, where the sludge was settled down and separated from the clarified water. The clarified water was discharged as effluent, while the settled sludge was returned to the aeration basin at 100% of the input flow rate. Mixed liquor volatile suspended solids (MLVSS) in the digester were kept constant through each experiment by removing the excess of sludge. Effluent samples were collected and centrifuged, and the clear supernatant analyzed for COD every 2 h during the course of each experiment. Continuous operation was kept on until the system reached steady state, which was realized when three consecutive COD and NH_{4}^{+}-N measurements on the effluent were approximately the same. Once steady state was attained the following parameters were determined in the last effluent sample: pH, oxygen demand (COD and BOD), and NH_{4}^{+}-N. Also, suspended solids and specific oxygen uptake rate (SOUR_{app}) were quantified in the mixed liquor of the activated sludge system.

**Analytical Methods.** The analysis of the wastewater was performed according to standard methods (13). Changes of pH in the mixed liquor of the aeration basin were monitored in a Radiometer Copenhagen PHM-82 pH meter and dissolved oxygen was measured with an oxygen probe connected to a YSI 58 dissolved oxygen meter. MLVSS was determined using a HT100 probe connected to a Dr. Lange turbidimeter, calibrated according to the standard method (13). SOUR_{app} was measured by removing 400 mL of mixed liquor from the aerobic digester, aerating the sample for 5 min to increase the dissolved oxygen level to 5–7 mg/L, and then following the DO decrease with time. Finally, the ozone concentration both in the gas entering and leaving the ozonation column and dissolved in the water entering the aerobic digester were determined with a UV photometer (Anseros Ozomat GM-109) and by the Indigo method, respectively (14).

**Biokinetic Parameter Analysis.** Many models for the suspended growth processes have appeared in the wastewater treatment literature (15). In this work, a simple but plausible steady-state model has been adapted to the specific case and used to predict the biodegradation rate in the activated sludge system. Global parameters, such as BOD, COD, and NH_{4}^{+}-N were used as substrate for evaluation under the assumption that the removal was exclusively due to aerobic biodegradation.

Mass balances to describe the substrate utilization and microbial growth rates in the continuous activated sludge reactor are as follows:

\[ Q(S_0 - S) - UXV = 0 \]  
\[ Q\Delta X - \mu XV = 0 \]

where Q is the wastewater flow rate; S_0 and S are the input and output substrate concentrations, respectively; U and \( \mu \) are the specific substrate utilization rate and specific growth rate of heterogeneous microorganisms, respectively; X is the MLVSS in the digester, \( \Delta X \) is the net growth of microorganism, expressed as mass of VSS formed per liter of wastewater treated; and V is the actual reaction volume.

It is known that the biodegradable fraction of domestic wastewater is made of multiple organic compounds, the biodegradation of each individual compound following the Monod’s kinetics. As the concentration of each compound is usually higher than the half-saturation parameter, the degradation of individual components approaches zero-order kinetics. Hence, as a result of the summation of multiple zero-order reactions, a pseudo first-order law can express the rate of a multicomponent substrate degradation (16):

\[ U = k \frac{S}{S_0} \]

Combining eqs 1 and 3 and taking into account the definition of hydraulic retention time, \( t_h = V/Q \), eq 4 is obtained:

\[ U = \frac{(S_0 - S)}{X_{t_h}} = k \frac{S}{S_0} \]

If COD is chosen as substrate for kinetic modeling, a nonbiodegradable fraction of substrate, \( S^* \), must be considered, and therefore the specific removal rate is better predicted by eq 5:

\[ U = \frac{(S_0 - S)}{X_{t_h}} = k \frac{S - S^*}{S_0} \]

As far as nitrification is concerned (i.e., conversion of ammonium to nitrite and, in a second step, conversion of nitrite to nitrate), under steady-state conditions the rate-limiting step is the oxidation of ammonium to nitrite by the genus Nitrosomas (i.e., nitrifiers). In this situation,
the growth of nitrifying bacteria can be well represented by Monod’s kinetics (15). However, from the practical point of view, when a wastewater containing both carbonaceous and nitrogen compounds is treated in an aerobic reactor, there is a limiting value of \( X_b \), below which, due to their slow growth rate, nitrifiers are washed out from the system. Moreover, nitrification is also affected by other environmental factors that can inhibit the process, including low temperature, extreme pH, or compounds toxic to nitrifying bacteria. In the absence of inhibiting factors, the effluent nitrogen-ammonium concentration for a combined carbon oxidation and nitrification in a continuous running ASP follows eq 6 (17):

\[
\ln \frac{[\text{NH}_4^+\text{-N}]_a}{[\text{NH}_4^+\text{-N}]_b} = k_N (X_{t_h} - X_{l})
\]  

where \([\text{NH}_4^+\text{-N}]_b\) is the available nitrogen to nitrifiers, \( k_N \) is the nitrification constant and \( X_l \) is the critical \( X_b \) below which nitrification does not develop. To estimate \([\text{NH}_4^+\text{-N}]_b\), it should be kept in mind that not only is aqueous ammonium removed from wastewater by nitrification but also microorganisms use this source to synthesize new cellular material. Given the fact that nitrogen represents about 12 wt % of the average mass of microorganisms, according to the cell formula C\(_{6}\)H\(_{7}\)NO\(_2\), for a steady-state process, \([\text{NH}_4^+\text{-N}]_b\) can be estimated as follows:

\[
[\text{NH}_4^+\text{-N}]_b = [\text{NH}_4^+\text{-N}]_a - 0.12\Delta X
\]  

where the subscript 0 refers to the input concentration.

On the other hand, microorganisms use the substrate for biomass synthesis, maintenance, and product formation. As a result, the net specific growth rate of heterogeneous microorganisms, \( \mu \), is represented by the contributions of microbial growth and endogenous decay:

\[
\mu = Y_{\text{XS}} U - b
\]  

\( b \) and \( Y_{\text{XS}} \) being the decay and substrate yield coefficients, respectively, the latter defined as the amount of VSS synthesized per unit of substrate. When combining eqs 2 and 8, the following is obtained:

\[
\mu = \frac{\Delta X}{X_{t_h}} = Y_{\text{XS}} U - b
\]

Also, during the metabolism of the aerobic heterotrophic organisms in activated sludge, oxygen is required as an electron acceptor. To estimate the oxygen requirement of an ASP, two contributions are considered: the oxygen necessary to synthesize the new cellular material and that corresponding to oxidize a fraction of this cellular material to satisfy the energy of maintenance requirement. If nitrification takes place there is additional oxygen consumption due to the oxidation of ammonium to nitrite and nitrite to nitrate. Therefore, the net specific oxygen uptake rate, SOUR, is given by eq 10:

\[
\text{SOUR} = \text{SOUR}_{\text{app}} - \text{NOD} = a_1 U + b_1
\]  

where SOUR\(_{\text{app}} \) is the measured oxygen uptake rate, NOD represents the specific nitrification oxygen demand, and \( a_1 \) and \( b_1 \) are the oxygen-use coefficients for synthesis and energy maintenance, respectively. The oxygen demand due to nitrification can then be estimated by considering the global reaction of nitrification:

\[
\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}
\]  

The nitrogenous oxygen demand can be estimated from the stoichiometry of reaction 11 to be approximately 4.57 g O\(_2\)/g \( \text{NH}_4^+\text{-N} \) removed by nitrification. Therefore, a conservative figure for the specific nitrification oxygen demand (NOD) can be estimated as follows:

\[
\text{NOD} = 4.57 \frac{[\text{NH}_4^+\text{-N}]_b - [\text{NH}_4^+\text{-N}]}{X_{t_h}}
\]

Results and Discussion

A series of experiments were made at various hydraulic retention times, between 1 and 10 h, and MLVSS, between 1 and 4 g/L, to estimate the biokinetic parameters of single ASP and combined ozone-ASP treating domestic wastewater. It should be stressed that for combined experiments an ozone dose of 40 g/L was always applied at the wastewater pretreatment stage. This dose was found to be the optimum in a previous work because it led to the most biodegradable effluent (12). As far as ozonation is concerned, about 17% COD removal was achieved while the BOD slightly increased. This fact is in accordance with the results previously reported on the effect of small ozone doses on the wastewater biodegradability (8, 18). Since the ratio BOD/COD represents the fraction of readily biodegradable organic material, the application of ozone to wastewater results in an increase of this ratio so that the compounds of the wastewater become more amenable to microorganisms. Small reductions of \( \text{NH}_4^+\text{-N} \) were also observed after ozonation, likely due to the low reaction rate between molecular ozone and ammonium (19).

Estimation of Parameters. Figure 1 depicts the variations of specific BOD and COD utilization rates with the normalized residual effluent BOD and COD at the steady state in experiments performed by single ASP and combined ozone-ASP. A linear relationship can be observed in all cases, indicating the agreement between experimental results and the kinetic model used (see eqs 4 and 5). From Figure 1, it is also observed that, regardless of the process, lines corresponding to BOD and COD are almost parallel. This means that the ASP, as a biological treatment, is only able to oxidize the amount of biodegradable material, which in turn depends on whether the pretreatment is applied or not. After least-squares regression analysis values of \( k_{\text{BOD}} \) and \( k_{\text{COD}} \) were obtained from the slopes of the straight lines plotted. Table 1 shows the values of the bioreaction rate constant referred to BOD and COD (i.e., \( k_{\text{BOD}} \) and \( k_{\text{COD}} \)). It should be noted that the value of \( k_{\text{COD}} \) for single ASP is lower than others found in the literature (20). This fact is likely due to inhibition effects because of the nature of the wastewater. The inhibitors are thought to be some compounds present in agro-industrial wastewater that also entered the wastewater treatment plant where samples of this study were taken. From \( k_{\text{COD}} \) data in Table 1, it is also observed that pre-ozonation significantly improved the substrate utilization rate constant. Thus, for the combined process, \( k_{\text{COD}} \) was found to be about 1.5 times higher than that for the single ASP. On the other hand, the intercepts of BOD lines with the X-axis in Figure 1 are close to zero because of the biodegradable nature of BOD. On the contrary, COD lines are far to intercept on the origin of the plot, due to the
for low values of origin, suggests that nitrification was not achieved at all was applied or not. The first line, drawn from the plot two straight lines regardless of whether pre-ozonation was calculated for nonozonated wastewater entering the reactor volume lower than that of a single ASP to reach the same effluent quality.

Concerning nitrogen removal, eq 6 was used to determine the rate constants of nitrification. Figure 2 shows that a plot of the left-hand side of eq 6 versus $X_t$, yields two straight lines regardless of whether pre-ozonation was applied or not. The first line, drawn from the plot origin, suggests that nitrification was not achieved at all for low values of $X_t$. From the linear regression analysis of the second straight line, $k_N$ and $X_t$ could be determined from the slope and intercept according to eq 6 (see Table 1 for values). In the same way, as commented before, for

### Table 1. Values of Estimated Biokinetic Parameters for Single ASP and Combined Ozone-ASP at 20°C and pH 7–8

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Single ASP</th>
<th>Combined Ozone-ASP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$ [h$^{-1}$]</td>
<td>0.03 ± 0.12</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>$a_1$ [g COD/g COD]</td>
<td>0.58 ± 0.02</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>$b_1$ [g COD/g gVSS h]</td>
<td>1.80 ± 0.06</td>
<td>3.61 ± 0.18</td>
</tr>
</tbody>
</table>

Regarding nitrogen removal, eq 6 was used to determine the rate constants of nitrification. Figure 2 shows that a plot of the left-hand side of eq 6 versus $X_t$, yields two straight lines regardless of whether pre-ozonation was applied or not. The first line, drawn from the plot origin, suggests that nitrification was not achieved at all for low values of $X_t$. From the linear regression analysis of the second straight line, $k_N$ and $X_t$ could be determined from the slope and intercept according to eq 6 (see Table 1 for values). In the same way, as commented before, for...
ASP because of the increase of the biological reaction rate after wastewater ozonation. However, SOUR was nearly the same for both processes (see Figure 4) while the oxygen-use coefficient to synthesis, $a$, was found lower for the combined process. It should be noted that ozonation mechanisms lead to more oxygenated products which therefore require less oxygen for their further oxidation to CO$_2$ and H$_2$O.

**Process Modeling.** The aforementioned kinetic model and the values of the parameters of Table 1 were used to predict the process performance in terms of effluent concentrations, excess of biomass produced, and oxygen requirements. It has to be noted, however, that such a prediction should be used with caution, since rate coefficients are variable functions because their values depend on process conditions, such as temperature, and the characteristics of the substrate being treated.

The quality of ASP effluents in terms of carbonaceous substrate or nitrogen removal is expected to depend on the MLVSS and the hydraulic retention time in the aeration basin. The higher the MLVSS or $t_h$, the better the quality of the effluent. However, the performance of ASP is also affected by the settlement of the sludge that must be separated in a clarifier after the biological oxidation (21). Hence, in operational terms, the sludge age or the associated parameter food-to-microorganism ratio, $f/m$, are the main control keys of an ASP plant:

$$f/m = \frac{BOD_0}{Xt_h} \quad (13)$$

Previous works have shown that $f/m$ ratios between 0.25 and 0.6 g BOD/(g MLVSS day) are appropriate for domestic sewage treatment (22). Therefore, the process modeling presented here has been limited to this operational $f/m$ range. Table 2 summarizes the input concentration data, the performance of the pre-ozonation step, and the equation numbers used for the ASP simulation.

<table>
<thead>
<tr>
<th>input wastewater concentrations [mg/L]</th>
<th>pre-ozonation$^a$ pollution removal$^b$ [mg/L]</th>
<th>simulation of ASP parameter to estimate</th>
<th>eq used</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD 274</td>
<td>COD 46</td>
<td>BOD 4</td>
<td></td>
</tr>
<tr>
<td>BOD 158</td>
<td>BOD -5</td>
<td>COD 5</td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$-N 35</td>
<td>NH$_4^+$-N 4</td>
<td>$\Delta X$ 9</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Assuming an ozone dose of 40 mg of ozone per liter of treated wastewater. $^b$ Average from four repeated experiments.

Results of the variation of the effluent normalized BOD, COD, and [NH$_4^+$-N] with the $f/m$ ratio are shown in Figure 5. As seen from this figure, regardless of the process employed, these three parameters increased with the increasing $f/m$ ratio, resulting in a decrease in the system performance. Likewise, for a given $f/m$ ratio the conversion achieved with the integrated process was higher than that reached with single ASP, especially for COD and NH$_4^+$-N. Therefore, the results obtained indicate a clear improvement of nitrification and the biological removal of initially recalcitrant substances when ozone was used as pretreatment agent. Figure 6 shows the effect of $f/m$ on the biomass production. It is stated that high $f/m$ implies a fast growth of microorganisms and, consequently, a high excess of sludge within the process as seen in Figure 6. Moreover, when comparing single ASP and ozone-ASP systems, it is observed that the amount of sludge produced in the combined process was higher, particularly at high $f/m$ ratio.

Finally, Figure 7 shows the variation of the apparent specific oxygen uptake rate and the oxygen requirement for nitrification in the integrated process was much higher as a result of the improvement of the nitrifying growth conditions after wastewater pre-ozonation. As a result, the calculated apparent oxygen
uptake rate of the combined ozone-ASP was significantly higher than that corresponding to the single ASP in which a low or negligible nitrification was achieved.

**Conclusions**

In light of the kinetic determinations and simulation results, the integrated ozone-ASP seems conceptually more advantageous than the single ASP for wastewater treatment, especially at removing recalcitrant substances and improving nitrification. Thus, ozonation can improve the COD and ammonia utilization rates at the subsequent ASP treatment. Moreover, ozonation leads to a wastewater with high concentration of readily biodegradable substances. As a consequence, the ASP of pre-ozonated wastewater led to a better quality effluent (lower residual COD and ammonia). For the optimization of the combined process, knowledge of the economics of the system should be incorporated to provide necessary insight into the process selection and operating conditions.

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**References and Notes**