Treatment of High Strength Distillery Wastewater (Cherry Stillage) by Integrated Aerobic Biological Oxidation and Ozonation

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The performance of integrated aerobic digestion and ozonation for the treatment of high strength distillery wastewater (i.e., cherry stillage) is reported. Experiments were conducted in laboratory batch systems operating in draw and fill mode. For the biological step, activated sludge from a municipal wastewater treatment facility was used as inoculum, showing a high degree of activity to distillery wastewater. Thus, BOD and COD overall conversions of 95% and 82% were achieved, respectively. However, polyphenol content and absorbance at 254 nm (A_{254}) could not be reduced more than 35% and 15%, respectively, by means of single biological oxidation. By considering COD as substrate, the aerobic digestion process followed a Contois' model kinetics, from which the maximum specific growth rate of microorganisms (μ_{max}) and the inhibition factor, β, were then evaluated at different conditions of temperature and pH. In the combined process, the effect of a post-ozonation stage was studied. The main goals achieved by the ozonation step were the removal of polyphenols and A_{254}. Therefore, ozonation was shown to be an appropriate technology to aid aerobic biological oxidation in the treatment of cherry stillage.

Introduction

Nowadays, to obtain spirits as a way of agricultural sources exploitation, the use of fruits and other substances as feedstock for distillation processes is being increasingly common. The manufacturing processes basically consist of mashing, fermenting, and distilling the raw materials after the initial cleaning and grinding steps. Despite the valuable product obtained, this type of industry has a drawback associated with the discharge of large volumes of highly polluted wastewater, namely, spent mash or stillage. For instance, a typical ethanol distillery generates between 10 and 15 L of wastewater per liter of alcohol obtained (*1*). Moreover, distillery wastewater is generally acidic (pH between 3 and 5) and contains considerable organic load and solids. Separation of solids can be accomplished by mechanical methods. These solids may be used either as a source of energy by means of anaerobic digestion or released to soil as a fertilizer (*2*). Concerning the treatment of the liquid waste, a number of processes have been investigated, including aerobic and anaerobic classic methods, trickling filters, lagoons, combustion, chemical oxidation, etc. (*1*, *2*). However, at small distilleries located in urban areas, delivery to a central wastewater treatment plant after the payment of the appropriate charge to the local authority to cover the cost incurred is the preferred option to deal with liquid wastes. In this case, only dilution and neutralization is required. Acceptance of distillery wastewater at the sewage works, however, implies some drawbacks. Thus, production of an effluent with the required standards requires the combination of biological treatment with chemical advanced wastewater treatments aimed at removing refractory organic materials (*3*). For the biological treatment at the sewage works, the activated sludge process is considered to be the most cost-effective way to remove organic materials from wastewater (*4*). In addition, ozone is currently becoming a classic oxidizing agent for advanced treatment of wastewater (*5*). As a result, the integrated activated sludge−ozone system has been successfully applied in the treatment of a number of wastewaters, including domestic and wine-distillery wastewater (*6*, *7*).

The present work deals with batch treatment of cherry stillage, a high strength distillery wastewater, by an integrated aerobic biological oxidation and post-ozonation system. The paper is primarily focused on the kinetics of the biological stage as well as on the overall process performance.

Materials and Methods

Feed Wastewater. Distillery wastewater was collected from a processing plant located at Valdastillas (Cáceres province, Spain). Raw wastewater contained a fraction of solids that were settled down and separated from the liquid phase. Because of its high organic load, the distillery wastewater was diluted with tap water to simulate the concentration of a typical industrial effluent entering a wastewater treatment plant. The main features of the wastewater after solids separation and after dilution are given in Table 1. In some cases, the pH of the final wastewater was modified up to neutral conditions by adding sodium hydroxide.

Inoculum. Biomass from the activated sludge system operating at the sewage works of Badajoz (Spain) was used to start up the aerobic digestion process. Microorganisms of the inoculum were first acclimated to distillery wastewater in a digester operating in fill and draw mode by increasing the influent strength step by

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10.1021/bp010021c CCC: $20.00
Published on Web 05/02/2001

Table 1. Main Features of the Wastewater Used in This Work

parameter	after solids separation	after dilution[e]
COD (kg/m^3)	145−180	2.4
BOD (kg/m^3)	100−140	1.9
TOC (kg/m^3)	70−90	0.9
total phenols[a] (kg/m^3)	1.0−2.0	0.022
A_{254}[b]	0.6−0.8[c]	0.9
pH	3.2−3.6	3.8
total acidity[d] (kg/m^3)	13−16	

[a] As gallic acid. [b] Measured with 1-cm path length quartz cell. [c] Sample diluted 100 times for measurement. [d] As $CaCO_3$. [e] Mean values after ca. 1:80 dilution.

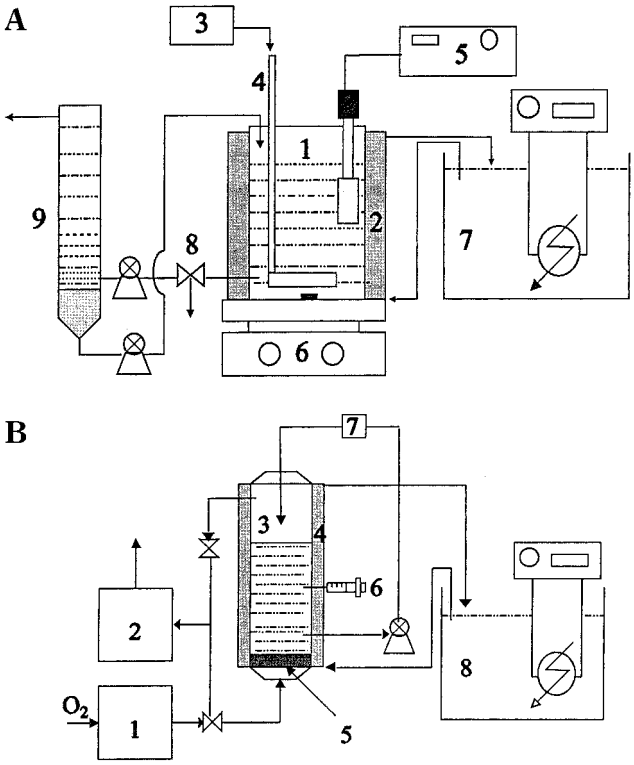

Figure 1. Schematic of the experimental setup for (A) aerobic biological oxidation and (B) ozonation. A: (1) digestor; (2) thermostatic jacket; (3) air pump; (4) air difusser; (5) oxygen meter; (6) magnetic stirrer; (7) thermostatic bath; (8) sample line; (9) settler. B: (1) ozone generator; (2) ozone analyzer; (3) bubble column; (4) thermostatic jacket; (5) gas difusser; (6) sample point; (7) pH controller; (8) thermostatic bath.

step. During the acclimation stage, exhausted wastewater and excess of sludge were removed daily. Afterward, fresh wastewater was fed into the reactor. Throughout this period, influent and effluent COD, as well as volatile suspended solids (VSS) production were followed every day. After 1 week, the maximum biological activity was reached (maxima overall COD removal and aerobic microorganism growth rates), and therefore the acclimation was considered to be attained.

Experimental Setup and Procedures. Schematic of the experimental systems for aerobic digestion and ozonation are depicted in Figure 1. The biological system consisted of a 4-L perfectly mixed tank, an aeration system, feed and effluent reservoirs, and a clarifier. To start a typical experiment, previously acclimated sludge was mixed with feeding wastewater to produce 2.5 L of a suspension of a given VSS concentration. An airflow rate of 100 L/h was continuously supplied to the reaction system to ensure a dissolved oxygen concentration above 2.5 mg/L (i.e., aerobic conditions). The reaction temperature was kept constant by means of a thermoregulated bath. Good mixing conditions were achieved using a magnetic stirrer. Then, after 30 min of start-up, a 40-mL sample was taken out of the reactor at determined intervals of time, centrifuged, and kept to analyze liquid and solid phases for COD and VSS, respectively. Once the experiment was considered completed, the treated wastewater was separated from the sludge in the clarifier and replaced by the same volume of fresh wastewater from the feed reservoir when required. The sludge was returned to the digester with the aid of a peristaltic pump. In required cases, excess of sludge was drained off from the clarifier in order to get a convenient concentration of VSS in the digester for the next run.

Table 2. Experimental Conditions Applied for the Aerobic Degradation of Wastewater

parameter	range	standard value[a]
initial VSS (g/L)	0.3−2.0	1.6
initial COD (g/L)	1.0−7.2	2.4
initial pH	3.6−7	7.0
reaction temperature (°C)	10−35	20

[a] Constant value for the study of the influence of other operating variable.

The ozonation system consisted of an ozone generator (Sander 307.1) and a glass bubble column (i.d. 9 cm; length 45 cm) used as a contactor. This reactor was loaded with 1.5 L of the water effluent from the biological oxidation that were continuously recirculated to provide appropriate mixing conditions. Ozone was produced from air and supplied to the bottom of the column through a diffuser plate (pore diameter 16−40 μm). The reaction temperature was kept constant by means of a thermostatic reactor jacket. Wastewater samples were periodically withdrawn to analyze COD, TOC, and dissolved ozone.

Final effluents from both the biological and ozonation systems were analyzed for pH, COD, BOD, TOC, IC, A_{254} and total phenols.

Analyses. Most of wastewater analyses were carried out according to standard methods (*8*). TOC and IC were determined with a Dohrmann DC-190 analyzer, and A_{254} was measured with a Hitachi 2000 spectrophotometer using a 1-cm path length quartz cell. Total phenols were analyzed by the Folin Ciocalteau method using gallic acid as the standard (*9*). Total microorganism concentration in suspension was considered to be related to the concentration of VSS, which were analyzed according to standard methods (*8*). Ozone in water was analyzed by the indigo method (*10*), while in the gas phase it was measured by means of an Anseros Ozomat GM109 analyzer.

Results and Discussion

Aerobic Degradation. Aerobic degradation of distillery wastewater was carried out under a variety of operating conditions. Thus, the effect of initial substrate and cell concentrations, reaction temperature, and medium pH were studied. Table 2 gives the experimental conditions applied in this work.

Influence of Cell and Initial Substrate Concentrations. As seen in Figure 2, the cell growth curve for experiments carried out at different initial VSS concentrations with the rest of the operating variables at constant values (see Table 2 for standard conditions) showed the typical exponential and stationary phases for a substrate limiting growth system. No lag phase was

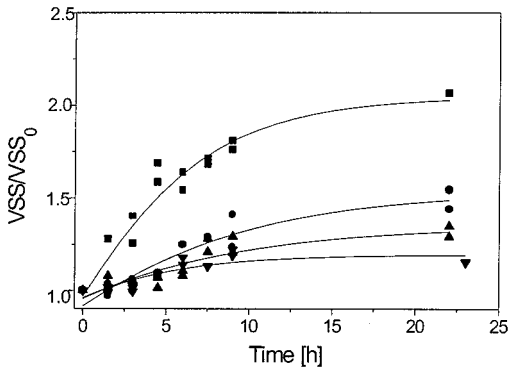

Figure 2. Evolution of VSS during aerobic degradation of distillery wastewater. Conditions: $T = 20$ °C; initial COD= 2.4 g/L; initial pH = 7; initial VSS: ■ 0.3 g/L; ● 0.8 g/L; ▲ 1.6 g/L; ▼ 2.0 g/L.

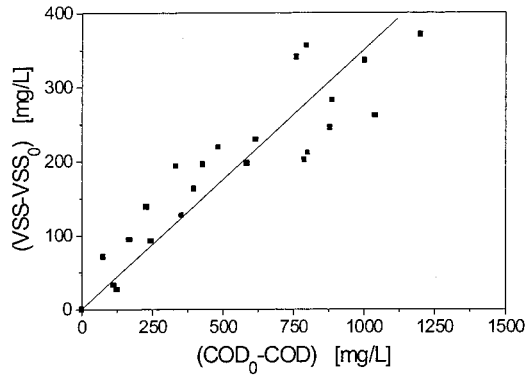

Figure 3. Determination of the heterotrophic growth yield for the aerobic degradation of distillery wastewater according to eq 1. Conditions: $T = 20$ °C; Initial pH = 7; initial COD = 1.0−3.6 g/L; initial VSS = 0.8−2.0 g/L.

observed at the beginning of the experiments because of the use of microorganisms acclimated to the wastewater environment. The cell mass growth was intimately related to substrate degradation. Thus, regardless of the initial microorganisms load, for the VSS range studied, aerobic treatment was able to remove most of the organic matter, the overall decreases of BOD and COD being about 95% and 82%, respectively. As expected in an aerobic biological oxidation method, TOC and COD varied similarly (overall TOC removal was about 78%), which means that original biodegradable organic compounds were completely oxidized to CO_2. The reaction brought about an increase in IC up to 50 mg/L and pH up to 8, making a buffered wastewater due to the accumulation of carbonates. In contrast to the high BOD, COD, and TOC removals, the biodegradation of phenols was significantly lower. Thus, no more than 35% of total phenols were removed after 24 h of biotreatment at the standard conditions of Table 2. In addition to the low degradation of total phenols, the aerobic culture was not able to reduce A_{254} to a high extent (overall removal < 15%). Similar results were observed in experiments completed at different initial COD.

For the purpose of kinetic study and in accord with the most common practice (*4*), COD was considered to represent the multicomponent substrate (S) while the cell concentration (X) was evaluated by the concentration of VSS. Since the degradation proceeded with cell mass growth and substrate degradation, a yield coefficient $Y_{X/S}$ could be obtained from eq 1 applied to the exponential cell growth phase:

$$(X - X_0) = Y_{X/S}(S_0 - S) \tag{1}$$

Figure 3 shows a plot of $(X - X_0)$ against $(S_0 - S)$ for experiments conducted at different initial VSS and COD. The evaluation of $Y_{X/S}$ was carried out by least-squares regression analysis of the entire experimental data of Figure 3. By doing this, $Y_{X/S}$ was found to be 0.35 ± 0.02 g VSS/g COD. Because of the large volume of sample necessary to obtain accurate VSS measurements, in some kinetic study experiments the analysis of VSS was avoided and this parameter was determined indirectly by applying eq 1 to actual COD measures and initial conditions. The model of Monod is quite useful to describe the kinetics of the exponential growth phase of microorganisms in most of biological processes. Therefore, to evaluate the kinetics of the system, the Monod equation (eq 2) was first tried to fit experimental results:

$$\mu = \frac{1}{X}\frac{dX}{dt} = \mu_m \frac{S}{K_S + S} \tag{2}$$

where μ_m is the maximum specific growth rate of microorganisms, and K_S is the saturation constant defined as the value of the limiting substrate concentration where the specific growth rate, μ, has half its maximum value. Unfortunately, in the particular instance of this work, the Monod equation did not give acceptable fits of experimental data. This was likely due to the existence of inhibition effects that the Monod equation is not able to deal with. Then, a number of other substrate-limited growth models reported in the literature (*11*) were tested. Among them the Contois model resulted in the best agreement with sets of experimental data obtained, as will be shown later. This agrees with other investigations dealing with aerobic systems treating distillery wastewater of different nature (*7*, *12*). According to the Contois model, the relationship between the specific substrate growth rate of microorganisms (μ) and the substrate concentration in a batch process limited by the amount of available substrate can be expressed as

$$\mu = \frac{1}{X}\frac{dX}{dt} = \mu_m \frac{S}{\beta X + S} \tag{3}$$

where β is a kinetic parameter related to the inhibition of the process due to overpopulation of microorganisms. Taking into account the linear relationship between substrate consumption and cell growth (i.e., eq 1) eq 3 becomes

$$\mu = \frac{Y_{X/S}}{X_0 + Y_{X/S}(S_0 - S)}\frac{dS}{dt} = \mu_m \frac{S}{\beta[X_0 + Y_{X/S}(S_0 - S)] + S} \tag{4}$$

If the growth model considered is correct, then the experimental substrate concentration and time should fit eq 4. As can be seen from Figure 4 the experimental results agreed the best with a Contois expression where μ_m and β were 0.058 h^{-1} and 0.74 g COD/g VSS, respectively, at 20 °C and pH 7.

Influence of the Reaction Temperature. Aerobic biological oxidation methods for the treatment of industrial wastewater treatment should be able to treat wastewater in a wide range of temperature, depending on the local climate. Tolerance of temperature changes

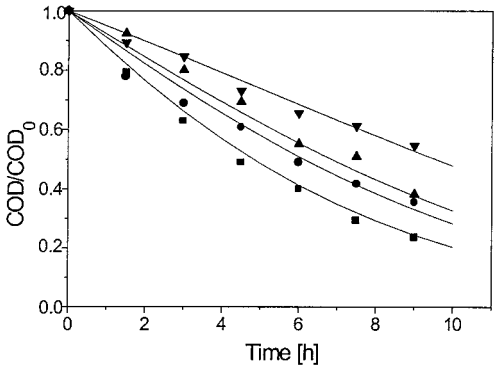

Figure 4. Experimental and simulated evolution of COD/COD_0 during aerobic degradation of distillery wastewater. Conditions: $T = 20$ °C; Initial pH = 7. Symbols (experimental data): ■ $COD_0 = 2.03$ g/L, $VSS_0 = 1.85$ g/L; ● $COD_0 = 2.20$ g/L, $VSS_0 = 1.70$ g/L; ▲ $COD_0 = 2.14$ g/L, $VSS_0 = 1.60$ g/L; ▼ $COD_0 = 2.28$ g/L, $VSS_0 = 0.88$ g/L. Lines: simulation according to the Contois model with $Y_{X/S} = 0.35$; $\mu_{max} = 0.058$ h^{-1}; and $\beta = 0.74$ gCOD/g VSS.

Table 3. Calculated Values for the Heterotrophic Growth Yield ($Y_{X/S}$), the Maximum Specific Growth Rate of Microorganisms (μ_{max}), and the Inhibition Parameter (β)[a]

exptl conditions		kinetic parameters		
T (°C)	pH	$Y_{X/S}$ (g VSS/g COD)	μ_{max} (h^{-1})	β (g COD/g VSS)
13	7	0.38	0.036	0.69
20	7	0.35	0.058	0.74
25	7	0.35	0.065	0.87
30	7	0.41	0.074	0.78
35	7	0.43	0.089	0.75
20	4[b]			
20	4[c]	0.31	0.054	0.75

[a] Conditions: $COD_0 = 1-3.4$ g/L; $VSS0 = 0.3-2.0$. [b] Microorganisms nonacclimated to acidic pH. [c] Microorganisms acclimated to acidic pH. Acclimation time: 1 week.

and acceptable treatment efficiency at such possible temperature are prerequisites to successful implementation of this technology at the industrial scale. Therefore, knowledge of kinetic parameters at different temperature must be assessed before the design stage. As expected, the temperature was found to favor the substrate degradation rate in the biological oxidation of cherry stillage in the range 13−35 °C. Table 3 shows the results of average $Y_{X/S}$, μ_{max}, and β obtained from the kinetic study of various experiments performed at different temperature. From this table, it can be seen that $Y_{X/S}$ and β were practically independent of the reaction temperature. Contrarily, μ_{max} was observed to increase with temperature. An activation energy of 28.4 kJ/mol was found for the maximum specific cell growth rate from an Arrhenius-type plot of experimental data at different temperatures (not shown).

Influence of pH and Acclimation of Microorganisms. Since distillery wastewater has an acidic pH, wastewater neutralization requires the addition of high amounts of alkali and therefore negatively influences the cost of the treatment process. In this work, some attempts were made to treat distillery wastewater by biological oxidation at the actual wastewater pH. Thus, a series of experiments were conducted in parallel at acidic (i.e., pH 4) and neutral conditions. Figure 5 shows that the COD removal rate for the process at pH 7 was much higher than that for the oxidation at pH 4 with nonacclimated microorganisms. In addition, the cell growth for this latter experiment was negligible, which confirms the low

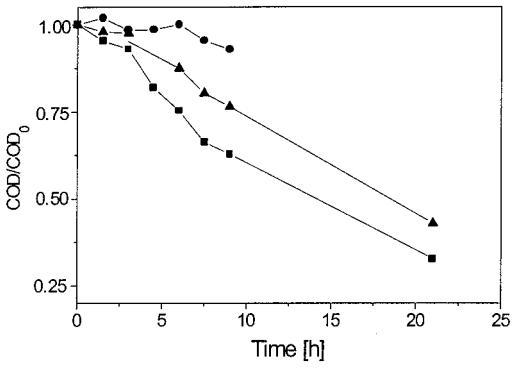

Figure 5. Evolution of normalized residual COD during the aerobic degradation of distillery wastewater at acidic and neutral conditions. Conditions: $T = 20$ °C; initial COD = 2.4 g/L; initial VSS = 0.4 g/L. Symbols: ■ initial pH = 7, acclimated microorganisms; ● initial pH = 4, nonacclimated microorganisms; ▲ initial pH = 4, acclimated microorganisms.

activity of microorganisms at acidic conditions. However, after a period of microorganism acclimation to the acidic environment, the biological activity started to be important. Thus, as shown in Figure 5, after 1 week of acclimation the COD removal rate at pH 4 was close to that achieved when acclimated microorganisms at pH 7 were used. Also, Table 3 shows the kinetics parameters evaluated at different pH conditions. Data from experiments at pH 4 with nonacclimated microorganisms failed when fitting to the Contois model, while $Y_{X/S}$, μ_{max}, and β were similar for pH 7 and pH 4 with acclimated microorganisms. In addition to a high degradation rate, other advantages of the biological oxidation of acidic distillery wastewater with acclimated culture were the increase of the effluent pH and good settling characteristics of the sludge. As a result of the formation of carbonates, the wastewater pH changed from 4 to 7.5 during the biological oxidation, making the pH suitable for the discharge of the effluent without further neutralization. Regarding sludge settleability, the sludge volume index (SVI) was maintained in a value lower than 100 mL/g when treating acidic wastewater with acclimated microorganisms. This is a very important fact because the performance of such an aerobic biological oxidation process (e.g., activated sludge) is highly affected by the settleability of the sludge that must be separated in a clarifier after the biological oxidation (*13*).

Post-Ozonation Stage. The coupling of biological oxidation and ozonation was tested for the treatment of cherry stillage. Since no important inhibition due to initial wastewater composition was observed during the biological oxidation of cherry stillage, the treatment based upon the integration of ozonation followed by activated sludge could be ruled out as a process that would noticeably improve the effluent quality compared to that obtained by single activated sludge treatment. However, as shown before, the ability of single biological oxidation to remove polyphenols and other UV-absorbing compounds was very limited. Therefore, the activated sludge−ozonation combination was expected to provide benefits over the conventional biological process (*3*). Thus, the impact of an ozonation stage on the pollution indicators (i.e., COD, TOC, IC, polyphenol, and A_{254}) of water effluent from the biological oxidation was investigated. For doing this, the activated sludge process was first applied to distillery wastewater at optimum conditions to reduce COD up to about 80%; thereafter, effluents thus produced were subjected to ozonation at different ozone doses (i.e., mass of ozone fed per volume of wastewater treated).

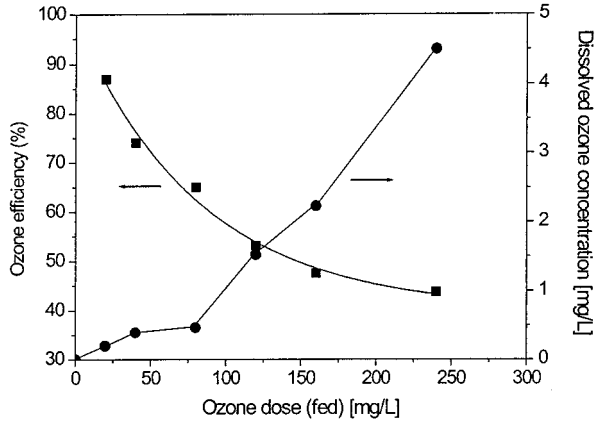

Figure 6. Evolution of ozone mass-transfer efficiency and dissolved ozone concentration with the ozone dose fed to a system treating distillery wastewater effluent from biological oxidation. Conditions: T = 20 °C; initial COD = 250−280 mg/L; initial TOC = 60−75 mg/L; initial IC = 43−50 mg/L; initial pH = 7.5−8.4. Symbols: ■ ozone mass transfer efficiency; ● ozone concentration in solution.

Table 4. Removal Efficiency (%) and pH Change of Distillery Wastewater during a Post-ozonation Stage[a]

ozone dose (mg/L)							
fed	consumed	COD	TOC	IC	total phenols	A_{254}	pH
20	17.4	1.0	0.8	9.4	53.7	34.7	7.6
40	29.7	6.8				47.8	7.6
60	39.0	9.8		10.2	69.3	52.2	7.4
120	63.8	26.5	5.1	43.6		57.1	7.3
240	105.1	34.5	15.0	50.7	79.5	59.9	7.0

[a] Initial wastewater parameters are those typically found after efficient biological oxidation: COD = 264 mg/L; TOC = 65 mg/L; IC = 53 mg/L; total phenols = 10.5 mg/L; A_{254} = 0.82; pH = 7.7.

Figure 6 presents experimental results of the evolution of the ozone mass-transfer efficiency and ozone dissolution with the ozone dose, shedding light on the dynamics of ozone absorption. As observed, the ozone efficiency decreased with the ozone dose while more ozone became dissolved into water. From Figure 6, conclusions on the kinetic regime of ozone absorption can be drawn (*14*). At low ozone dose, ozone was not found in solution, which means that the absorption of the oxidant gas proceeded in a fast regime and reactions might occur in either the gas−liquid interface or the liquid side film. As the ozone dose was increased the kinetic regime of ozone absorption changed from fast to moderate or even slow, with reactions taking place in the bulk of wastewater. Table 4 shows the changes of a few wastewater features achieved by the post-ozonation. As seen in this Table, both COD and TOC final conversions were rather low, and consequently extremely high ozone doses would be required to significantly decrease the values of these parameters. However, polyphenols and A_{254} were satisfactorily removed with relatively low ozone consumption. Another important fact is the decrease observed in pH, likely as a consequence of the acidic products given rise by ozonation. At this point it is worth noting the low decrease observed in pH even at high ozone dose, likely due to the buffer effect because of dissolved carbonates (i.e., initial IC of about 50 mg/L).

The results obtained are consistent with previous research (*6*), and they can be explained on the basis of ozonation mechanisms, which are schematically shown in Figure 7. Ozone can act as a strong oxidizing species both by direct reactions of molecular ozone with organic matter or indirectly through reactions of hydroxyl free

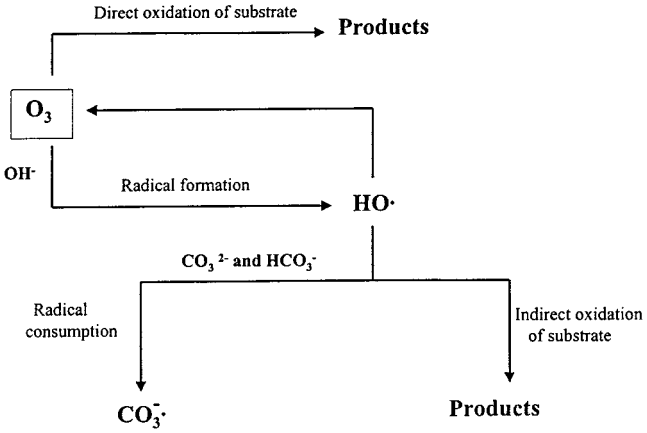

Figure 7. Schematic of reaction pathways for ozonation of aqueous systems.

radicals, HO•, formed from an ozone self-decomposition cycle catalyzed by the hydroxide ion. While molecular ozone is a strong though relatively selective oxidant (i.e, to remove unsaturated and aromatic compounds), the hydroxyl radical is a much stronger and nonselective oxidant species. Accordingly, regarding distillery wastewater composition, direct ozone reactions would lead to removal of polyphenols and other aromatics and unsaturated compounds that absorb UV-254 nm radiation. On the other hand, oxidation by hydroxyl radical should bring about wastewater mineralization and, therefore, important COD and TOC reductions. From the results shown in Table 4, it can be inferred that in the case study molecular ozone reaction occurred to a high extent (i.e., elimination of polyphenols and A_{254}) but hydroxyl radical oxidation was ineffective for low ozone dose. This is likely a consequence of the fast kinetic regime of ozone absorption that develops under that condition. Since molecular ozone hardly reaches the wastewater bulk (i.e., low dissolved ozone), it cannot react with the hydroxide ion to produce appreciable concentrations of HO•. Furthermore, even if HO• were formed the presence of high concentration of carbonates, which are known to be hydroxyl radical scavengers (*15*), would avoid the wastewater mineralization since hydroxyl radicals would be primarily consumed by reacting with carbonates without important COD removal. However, when the ozone dose supplied to the system is high, the kinetic regime of ozone absorption passes from fast to moderate and eventually to slow. In these regimes ozone diffuses to the liquid bulk. Under this circumstance, according to the pathways of Figure 7, a number of competitive reactions may take place. For ozone doses higher than 60 mg/L, it can be assumed that the acidic products from ozonation are neutralized by the carbonates in solution, whose concentration in turn decreases considerably (IC carbon concentration decreased up to below 30 mg/L). This allowed the concentration of hydroxyl radicals to be high enough to yield wastewater mineralization to some extent, as suggested by the greater COD and TOC removals.

Conclusions

Integrated aerobic biological oxidation and ozonation has been shown to be an efficient process to treat distillery wastewater (cherry stillage). The most important fraction of pollutants is eliminated by biological oxidation (i.e., BOD and COD removals higher than 95% and 80%, respectively) to make economically acceptable the combined treatment. Acclimation of microorganisms to the acidic environment (actual pH of distillery waste-

water is about 4) was successful, and high efficiency was observed in the depurative process using this acclimated bioculture to treat nonneutralized distillery wastewater. However, the biological process has been shown unable to effectively remove polyphenols and other UV-absorbing compounds. In this sense, ozonation has been proved as an efficient treatment with the purpose of removing the latter biorefractory compounds. Nevertheless, the high alkalinity accumulated in the wastewater due to biological oxidation of organic matter made ozonation a noneffective treatment to further decrease TOC and COD.

Acknowledgment

The authors wish to express their thanks to the CICYT of Spain and the European Commission for the financial support of this investigation under grant 1FD97-0087.

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Accepted for publication February 15, 2001.

BP010021C