Wine Distillery Wastewater Degradation. 2. Improvement of Aerobic Biodegradation by Means of an Integrated Chemical (Ozone)−Biological Treatment

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Biological degradation of vinasses, generated during alcohol production from wines and pressed grapes, has been studied in four digesters operating in fill and draw mode. Digesters 1 and 2 were fed with nonozonated and ozonated mixtures of vinasses and domestic sewage (1:10 by volume), respectively. Digesters 3 and 4 operated with pure vinasses at acid and neutral pH values, respectively. The effects of pH, temperature, and ozone dose conditions were studied. Preozonation removed inhibitory compounds and improved the growth of nitrifiers. Ozone dose is the key variable to treat a vinasse−domestic sewage effluent effectively with a combined chemical−biological system. Contois's kinetic model has been applied to experimental results, and kinetic parameters related to the maximum specific growth rate of microorganisms, μ_{max}, and inhibitory effects, α, were calculated and compared for nonozonated and ozonated wastewaters.

Keywords: *Wine distillery; biological treatment; biokinetics; ozonation*

INTRODUCTION

Food industry wastewater can usually be treated biologically, in both aerobic and anaerobic reactors (Borup and Ashcroft, 1991). Wastewaters from wine distilleries traditionally have fallen into this category (Sheehan and Greenfield, 1980; Sales et al., 1987a). They have a high organic load (COD = 20−180 g/L), although most of it is easily biodegradable (BOD = 12−50 g/L) (Basu, 1975; Sales et al., 1987a). Anaerobic digestion is the preferred method of treatment because of its advantages: little energy and low nutrient requirements, small quantities of excess sludge, and the ability of anaerobic bacteria to transform organic substances into methane, used as an energy source in the same distillery (Bories et al., 1988; Romero et al., 1993; Borja et al., 1993a). However, in addition to some readily biodegradable matter, vinasses contain some other organic compounds, such as phenols, that are toxic to bacteria and, as a consequence, inhibit the digestion (Johnson and Young, 1983; Fedorak et al., 1984; Borja et al., 1993b). Also, due to the seasonal nature of many of these industries and the absence of microorganisms in vinasses capable of carrying out anaerobic digestion, long incubation periods are required for the start-up stage (Valcárcel et al., 1984; Stronach et al., 1987). Other operational problems are the low growth rate of anaerobic bacteria and the loss of biomass in systems with high hydraulic loading rates. Thus, conventional anaerobic digestion frequently does not achieve a satisfactory purification of vinasses (Borja and Banks, 1994). Therefore, some pretreatment is often made before the waste enters an anaerobic digester (Lele et al., 1989; Torrijos and Moletta, 1997). In some cases direct release of wastewater to a municipal sewer with payment of the appropriate fee results in the lowest cost option. This problem requires more rigorous consideration at the design state than that necessary for a plant treating purely domestic sewage. The final assessment is that the inhibitory and recalcitrant nature of phenolics present in vinasses and sludge bulking problems causes major operational difficulties for aerobic biological treatment.

In previous work, ozonation was demonstrated to be a good technology to improve the biodegradability of vinasses (Beltrán et al., 1999). Nevertheless, further studies were required to attain optimal operation conditions. This paper presents an analysis of the process variables that affect the performance of start-up and operation of an aerobic reactor for treating a mixture of ozonated and nonozonated wine distillery wastewater and domestic sewage. Finally, the paper presents a kinetic discussion of the process purification performance.

MATERIALS AND METHODS

Feed Wastewater. Distillery wastewater was collected from an ethanol-producing wine distillery plant in Villafranca de los Barros (Badajoz province, Spain). This waste has been characterized elsewhere (Beltrán et al., 1999). The original wastewater was diluted with domestic sewage from the municipal plant of Badajoz. The mixture was prepared to obtain a chemical oxygen demand of ∼2250 mg/L, corresponding to a 10:1 by volume ratio of domestic sewage to vinasses.

Inoculum. Biomass from the activated sludge system operating at the sewage works of Almendralejo (Badajoz province, Spain) was used to start the aerobic digestion process. Agricultural wastes from olive process and winery activities seasonally enter this plant in addition to domestic sewage.

Apparatus and Procedure. Aerobic biological oxidation experiments were completed in a 4 L glass digester operating in fill and draw mode. A schematic of the experimental setup is shown in Figure 1. For starting the process, the sludge used

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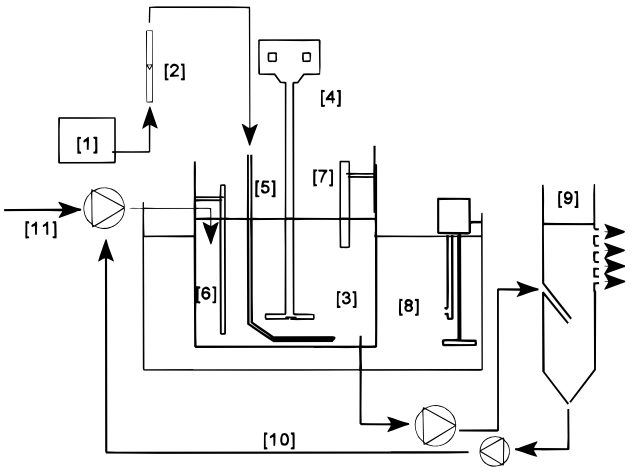

Figure 1. Schematic of experimental apparatus: 1, air supply system; 2, flow meter; 3, aerobic digester; 4, stirrer; 5, air bubble diffuser; 6, sample point; 7, pH and DO control; 8, thermostatic bath; 9, clarifier; 10, sludge return; 11, fresh wastewater.

as inoculum was centrifuged and mixed with distilled water to produce a suspension of ~10 g of volatile suspended solid (VSS) per liter. The digester was filled with 50 mL of this suspension and supplemented with 2450 mL of feed wastewater. Then, at time intervals, the treated wastewater was separated from the sludge in the clarifier and replaced by the same volume of nontreated feed wastewater in the digester. The sludge was returned to the digester by the action of a peristaltic pump. Also, when appropriate, a part of the sludge was drained off after the clarification to maintain a convenient concentration of VSS within the digester. An air flow rate of 100 L/h was continuously supplied at the bottom of the reactor through a diffuser to keep the dissolved oxygen concentration around 3 mg/L. Temperature was also kept constant by placing the digester in a thermostatic bath, and mixing was achieved using a mechanical stirrer. At determined time intervals, 40 mL samples were withdrawn from the reactor, centrifuged, and kept to analyze both liquid and sludge.

For combined ozone−biological oxidation experiments, the feed wastewater was first ozonated as described in the previous paper (Beltrán et al., 1999) and then biologically treated as detailed above.

Analytical Methods. Chemical oxygen demand (COD), total and inorganic carbon (TC and IC, respectively), ultraviolet absorbance (UV_{254}), total Kjeldahl nitrogen (TKN), total phenols, and pH were measured in the liquid following the methods indicated in the previous work (Beltrán et al., 1999) Also, VSS, pH, dissolved oxygen (DO), and sludge volumetric index (SVI) were measured in the mixed liquor, following standard methods (APHA, 1985).

RESULTS AND DISCUSSION

Start-up of the Digester. Microorganisms of inoculum were first acclimated to both nonozonated and ozonated wastewater in digesters 1 and 2 at 20 °C, with a hydraulic retention time (HRT) of 24 h and an initial biomass concentration of 1.1 g/L. Table 1 shows the results obtained from the effluent analysis. The excess of sludge produced by means of microorganism growth was removed daily. Start-up and acclimation of microorganisms were judged to be complete when the organic matter (i.e., COD and TOC) and nutrient (i.e., nitrogen) removal rates reached maximum values and the growth rate of biomass was constant with time. Accordingly, from data listed in Table 1, it appears that the acclimation to nonozonated wastewater was ~1 week faster than that to the ozonated one. In this latter case, >2 weeks was needed to achieve the maximum biological activity. It can be observed that nonacclimated microorganisms after 1 day of treatment allowed high reductions in COD, TKN, and TOC (about 60, 50, and 60%, respectively), regardless of the use of ozone. The short time required here for starting up compared to the longer ones reported by others (Sales et al., 1987b; Romero et al., 1993) may be attributed to the fact that microorganisms were collected from a plant treating wastes with compositions similar to those used in this work.

Table 1. Results of Start-up of Digesters 1 and 2

Digester 1[a]						
	feed	acclimation time, weeks				
parameter	wastewater	0	1	2	3	4
pH	5.4	6.7	7.0	6.8	6.7	6.7
COD, mg/L	2412	897	840	841	830	829
UV_{254}[c]	2.35	2.09	1.97	1.92	1.90	1.92
TKN, mg/L	147	74	64	59	62	57
TOC, mg/L	853	295	271	260	264	258
IC, mg/L	29	112	131	114	129	125
total phenols, mg/L[d]	843					725
ΔVSS, g/L		0.49	0.60	0.62	0.61	0.64
Digester 2[b]						
	feed	acclimation time, weeks				
parameter	wastewater[e]	0	1	2	3	4
pH	5.2	6.4	6.7	6.4	6.1	6.4
COD, mg/L	2170	870	780	620	590	600
UV_{254}[c]	1.62	1.40	1.29	1.38	1.18	1.22
TKN, mg/L	154	71	61	31	31	28
TOC, mg/L	799	321	324	214	218	217
IC, mg/L	21	57	47	62	49	54
total phenols, mg/L[d]	590					438
ΔVSS, g/L		0.34	0.49	0.56	0.58	0.55

[a] Digester 1, single aerobic biological oxidation. [b] Digester 2, biological oxidation after ozonation. [c] Measured with 1 cm path length cell quartz. [d] Expresed as gallic acid. [e] Ozone dose applied during pretreatment = 0.2 g/L.

On the other hand, it is known that original organic compounds are partially oxidized to low molecular weight intermediates rather than completely oxidized to CO_2 when some wastewaters are treated with low ozone doses (Gilbert, 1987). The new organic compounds formed could eventually act as a substrate for microorganisms. Thus, start-up of digester 2 under these conditions was longer due to the lack of biomass acclimation to the new substrate. After the start-up, COD removal efficiency varied from 65.0 to 72.3% for digesters 1 and 2, respectively. Also, TOC and TKN removal efficiencies followed a similar trend, as can be deduced from Table 1. Therefore, it can be inferred that ozonation improved the subsequent aerobic biological treatment. Further experiments were conducted to obtain optimum operating conditions for the combined processes and to investigate the reason for this improvement.

Influence of Variables. A number of environmental factors affect the activity of wastewater microbial populations and the rate of biochemical reactions. Of particular importance are temperature, pH, nutrients, and inhibition by toxic compounds.

Temperature. The effect of the reaction temperature on COD and TOC removals and mass of microorganism produced during aerobic biological oxidation is shown in Figures 2 and 3. As expected, COD and TOC removal rates and VSS growth rate increased with temperature

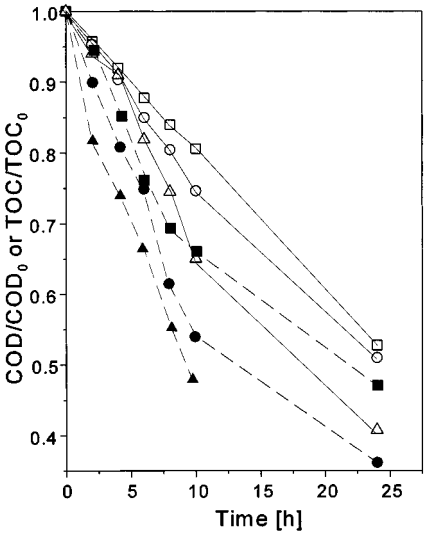

Figure 2. Influence of reaction temperature on COD and TOC removals during wastewater biodegradation: (□, ■) $T = 5$ °C; (○, ●) $T = 20$ °C; (△, ▲) $T = 30$ °C.

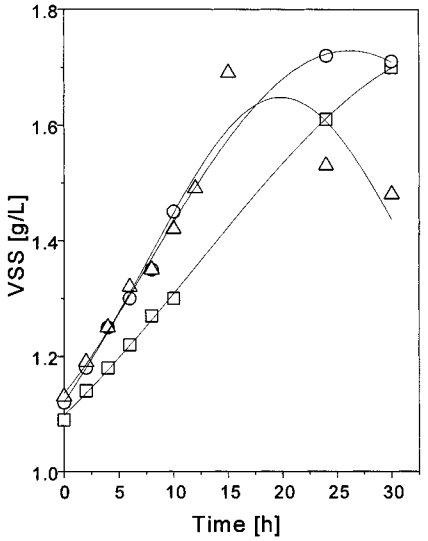

Figure 3. Influence of reaction temperature on VSS growth during wastewater biodegradation: (□) $T = 5$ °C; (○) $T = 20$ °C; (△) $T = 30$ °C.

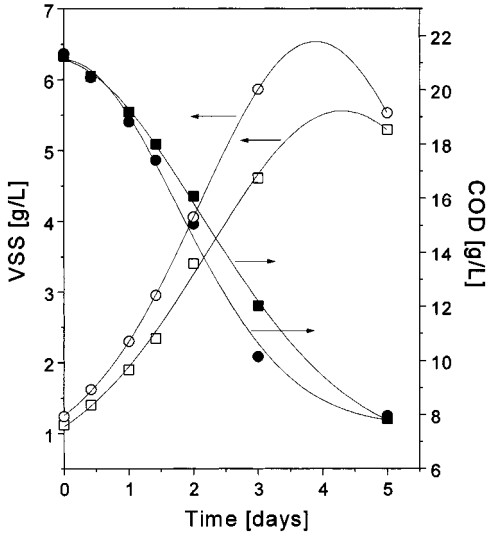

Figure 4. Profiles VSS time and COD time during biodegradation of acidic and neutralized vinasses: (□, ■) $pH_0 = 3.5$; (○, ●) $pH_0 = 7.0$.

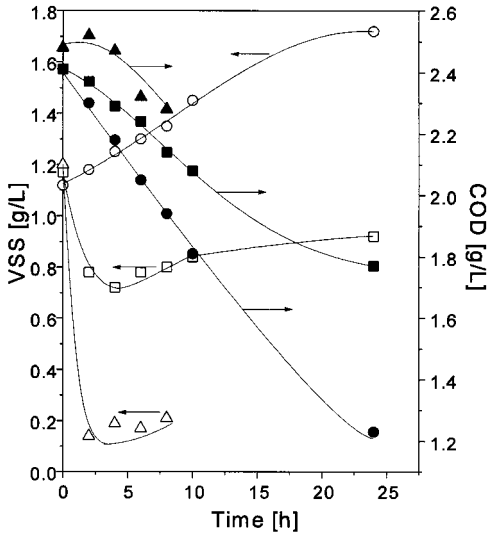

Figure 5. Effect of initial pH on vinasse−domestic sewage degradation rate: (□, ■) $pH_0 = 2.0$; (○, ●) $pH_0 = 5.4$; (△, ▲) $pH_0 = 10$.

in the range studied (5−30 °C). The higher temperatures resulted in increased biological activity, which in turn increased the rate of substrate removal. These variations in reaction rate are undoubtedly due to changes in the reaction rate constants of the biochemical process with temperature. However, in a practical situation, control of temperature to ensure that microbial population growth continues under optimum conditions is unlikely.

pH. Due to the acidic nature of vinasses, pH is one of the most relevant factors affecting the microbiological activity in the biological process. Some authors have reported that vinasses must be neutralized for anaerobic oxidation (Sales et al., 1989; Borja et al., 1993a), and neutralization is recommended for aerobic processing (Sales et al., 1987b). The effect of pH on aerobic biodegradation of nonozonated vinasses was evaluated with two new digesters operating with undiluted wastewater at pH 3.5 (original pH of vinasses) and pH 7.0 (digesters 3 and 4, respectively). After the start-up, longer for digester 3, biological activity and hence COD removal rate were similar in both reactors (see Figure 4). After 5 days of operation, the pH increased from 3.5 to 6.4 in digester 3 and from 7.0 to 8.0 in digester 4. This can be attributed to the oxidation of organic acids to CO_2 and the reaction between the carbon dioxide and basic compounds to form carbonates and bicarbonates. Although the aerobic population became acclimated to acidic vinasses and hence COD could be effectively reduced in digester 3, nitrification did not develop and poor sludge settling was observed compared to results from digester 4 (average SVI in digester 3 was ∼30 mL/g higher than that measured in digester 4).

A series of experiments were also completed by varying the influent pH of nonozonated and ozonated vinasses−domestic wastewater. Extreme pH conditions were achieved by adding NaOH or H_2SO_4 aqueous solutions. Aerobic heterotrophic microbial activity decreased significantly, especially at pH 2, as can be deduced from Figure 5, where the experimental results from digester 1 are presented.

Nitrogen. Nutrients (i.e., nitrogen and phosphorus) play an important role in a biological wastewater process. The generally accepted BOD/N/P mass ratio of

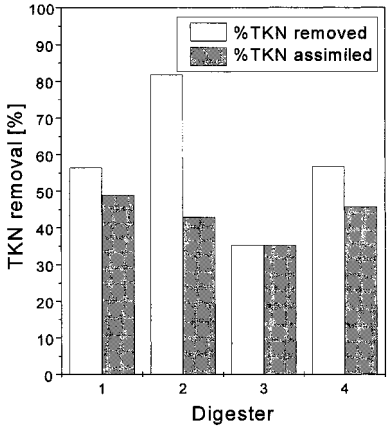

Figure 6. Balance of nitrogen removal during wastewater biodegradation in digesters 1−4. Wastewater feed: digester 1, vinasse−domestic sewage (1:10); digester 2, ozonated vinasse−domestic sewage (1:10); digester 3, vinasses at pH 3.5; digester 4, neutralized vinasses.

100:6:1 will maintain optimal nutrient balance for heterotrophic activity in a conventional activated sludge process (Metcalf and Eddy, 1991). The wastewater used in this work has nitrogen in excess of the microbial requirement. Hence, only part of the nitrogen load can be removed by conventional heterotrophic activity by incorporation into the microbial mass. The residual nitrogen, if discharged into a watercourse, stimulates eutrophication. For this reason, minimal nitrogen concentration in the effluent is desirable. In this work, results of TKN removals were followed from digesters 1−4 operating at 20 °C. The amount of nitrogen oxidized by autotrophic nitrifying bacteria was calculated from a nitrogen balance assuming that 12% of biomass cell composition is nitrogen (Ramalho, 1991). Results are shown in Figure 6. The only significant nitrification occurred with digester 2 (with ozonated wastewater). The role of pH and inhibitory compounds in the nitrification process could explain the results. Nitrification is favored by mildly alkaline conditions (pH 7.2−9.0). Below this range the rate of nitrification decreases, becoming completely inhibited below pH 5 (Wiessman, 1994). Additionally, toxic compounds such as phenols inhibit the growth of nitrifiers. Thus, the rise of pH when vinasses are mixed with domestic sewage and the removal of toxic compounds achieved with preozonation are likely the reasons for the high TKN removal efficiency observed in aerobic degradation of ozonated diluted vinasses in digester 2.

Inhibitory Compounds. Toxic compounds such as phenols, heavy metals, or some pesticides are difficult to biodegrade and are inhibitors of microbial activity. Some authors suggest that polyphenol-like compounds present in vinasses are responsible for the low biodegradation in these wastewaters (Bories, 1982). In this work, total phenols were found to be 0.735 and 0.084 g/L, expressed as gallic acid concentration, in pure vinasses and after dilution with domestic sewage (1:10 by volume), respectively. After 24 h of treatment in digester 1, only 14% of phenols originally present were removed. However, the application of ozone before biological oxidation had important effects on phenol destruction. For example, with an ozone dose of 200 mg/L >30% of phenols were removed and an additional 18% removal was achieved in the subsequent biological step in digester 2. Moreover, as has been discussed before, biological treatment in digester 2 resulted in increases of the COD, TOC, and TKN removals greater than those found in digester 1 (with nonozonated wastewater).

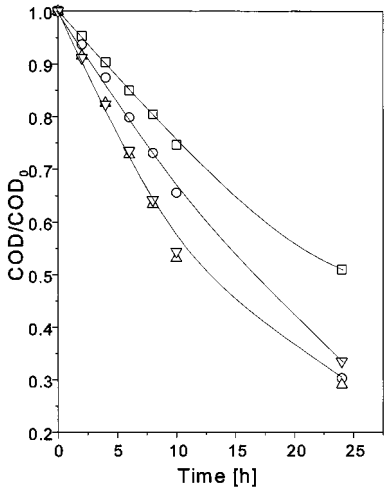

Figure 7. Influence of preozonation on COD removal rate during biodegradation of vinasse−domestic sewage: (□) no ozone dose; (○) 0.1 g/L ozone dose; (△) 0.2 g/L ozone dose; (▽) 0.3 g/L ozone dose.

Ozone Dose. In this work ozonation was used as a pretreatment step to eliminate toxic compounds such as polyphenols and thus improve aerobic biodegradation. As far as ozonation is concerned, the key point is the efficient use of expensive ozone. To elucidate the optimum ozone dose, several ozonation experiments were performed and the effluents then were treated biologically in digester 2. Figure 7 shows the variation of normalized residual COD with time during the biological step. An ozone dose of 200 mg/L was found to be optimum. With more severe ozonation pretreatment, COD reduction rate did not improve.

Kinetic Study. Several kinetic models have been proposed to describe the relationships between substrate utilization and biological growth in biochemical processes (Bailey and Ollis, 1977), Monod's model being one of the most commonly used in aerobic processes (Monod, 1949). However, experimental data of this work did not follow this model. Contois's model was then applied (Contois, 1959). According to this, provided that the loss of viability of the microbial mass is negligible, the specific growth rate of microorganisms is given by

$$\mu = \frac{1}{X}\frac{dX}{dt} = \mu_{max}\frac{S}{\alpha X + S} \quad (1)$$

where μ_{max} is the maximum specific growth rate of microorganisms, S is the rate-limiting substrate concentration, and α is a dimensionless kinetic parameter related to the inhibition of the process. To develop the model here, the organic matter, as measured by COD, was considered to be the rate-limiting substrate and the cell mass was expressed by VSS. The apparent growth yield coefficient, Y, was then defined as follows:

$$Y = -dX/dS = -dVSS/dCOD \quad (2)$$

Assuming that Y is constant throughout the batch experiment, it can be calculated for a finite interval time as

$$Y = (X - X_0)/(S_0 - S) \quad (3)$$

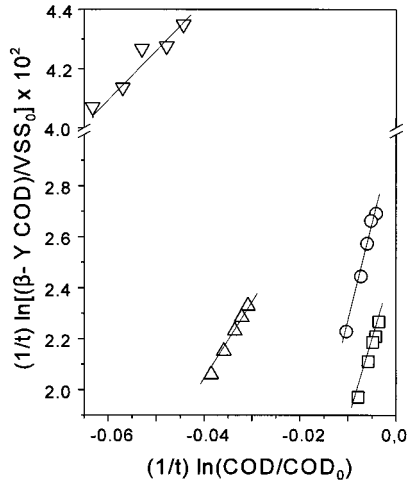

Figure 8. Determination of Contois' model rate constants according to eq 6: (△) digester 1; (▽) digester 2 (ozone dose = 0.2 g/L); (□) digester 3; (○) digester 4.

Table 2. Calculated Values for the Heterotrophic Growth Yield, Maximum Specific Growth Rate of Microorganisms, and Dimensionless Parameter α[a]

digester	ozone dose, mg/L	Y,[b] g of VSS/g of COD	$\mu_{max} \times 10^2$, h^{-1}	α, g of COD/g of VSS
3		0.38	2.47	1.79
4		0.44	3.04	1.79
1		0.52	3.80	0.87
2	100	0.52	4.26	0.65
2	200	0.55	5.00	0.55
2	300	0.49	5.19	0.66

[a] Experimental conditions: T = 20 °C; VSS_0 = 1.2 g/L. [b] Mean value of the run.

where X_0 and S_0 are the VSS concentration and COD at time $t = 0$, respectively, and X and S are the corresponding values at any given time. Equation 1 was then linearized to determine the rate constants μ_{max} and α. Thus, combining eqs 1−3 and rearranging yields the resulting differential equation

$$\mu = \frac{1}{X}\frac{dX}{dt} = \mu_{max}\frac{\beta - X}{\beta + (\alpha Y - 1)X} \quad (4)$$

where β is a constant defined as follows:

$$\beta = YS_0 + X_0 \quad (5)$$

Integration of eq 4 led to

$$\frac{1}{t}\ln\left(\frac{\beta - YS}{X_0}\right) = \mu_{max} + \frac{\alpha Y}{t}\ln\left(\frac{S}{S_0}\right) \quad (6)$$

According to eq 6 a plot of the left-hand side against $(1/t)\ln(S/S_0)$ should lead to a straight line having a slope of αY and with intercept μ_{max}. Figure 8 shows examples of this plot for experiments conducted in digesters 1−4. It can be seen that the model fit the experimental results adequately. Table 2 presents the values of Y, μ_{max}, and α determined by fitting eq 6 to the experimental data by linear regression using the least-squares method. From the results of this table, it appears that Y is dependent on the vinasse concentration and pH but not on the degree of ozonation. However, the ozonation step led to an increase of μ_{max} and a marked decrease of α. This suggests that ozonation enhanced the fraction of readily biodegradable compounds, removed inhibitory effects, and, hence, improved the biological activity, leading to higher oxidation rates and levels of remediation.

ABBREVIATIONS USED

S, substrate concentration, g of COD/L; X, microorganism concentration, g of VSS/L; Y, apparent growth yield coefficient, g of VSS/g of COD; α, kinetic parameter of eq 1, g of COD/g of VSS; β, dimensionless parameter defined by eq 5; μ, specific growth rate of microorganisms, h^{-1}; μ_{max}, maximum specific growth rate of microorganisms, h^{-1}; BOD, biological oxygen demand, mg/L; COD, chemical oxygen demand, mg/L; DO, dissolved oxygen, mg/L; HRT, hydraulic retention time, h; IC, inorganic carbon, mg/L; TC, total carbon, mg/L; TKN, total Kjeldahl nitrogen, mg/L; TOC, total organic carbon, mg/L; SVI, sludge volume index, mL/g; UV_{254}, ultraviolet absorbance at 254 nm; VSS, volatile suspended solids, mg/L.

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