Posttreatment of a Brewery Wastewater Using a Sequencing Batch Reactor

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ABSTRACT: This study concerns the application of a sequencing batch reactor (SBR) for the posttreatment of an effluent rejected by an upflow anaerobic sludge blanket (UASB) reactor operating in a brewery. The goal was to achieve the required wastewater quality for discharge to surface water. The primary target was the removal of nitrogen compounds, but chemical oxygen demand and suspended solids were also concerns. Phosphorus concentration and protozoan population were also monitored during SBR operation. Two different strategies were tested: an operation based on an aerobic–anoxic sequence and another based on applying a predenitrification step, that is, an anoxic–aerobic–anoxic sequence. Ammonium (NH_4–N) removal was achieved in all assays. Nitrification efficiency reached 97%, and the maximum observed rate was 0.175 kg NH_4–N/kg volatile suspended solids·d. A denitrification process was detected during the aerated periods, despite a dissolved oxygen concentration in the bulk liquid of 2.8 to 3.7 mg O_2/L. However, denitrification was suppressed when the bulk liquid oxygen concentration was increased to 7 mg O_2/L. The carbon-to-nitrogen ratio of the UASB effluent was too low and hindered the postdenitrification phase. This fact was confirmed by complete nitrate removal when an acetate supplement was added. On the other hand, the insertion of a primary anoxic phase in the reaction cycle was the best treatment strategy, leading to nitrogen values within the legal framework. The protozoan population showed significant changes in response to the aerobic–anoxic conditions. However, periodic nonaerated conditions were not detrimental to aerobic protozoa, which recovered as soon as oxygen was again available. *Water Environ. Res.*, **73**, 45 (2001).

KEYWORDS: sequencing batch reactor, brewery wastewater, anaerobic pretreatment, nitrification, denitrification, protozoa.

Introduction

The effluent discharged by breweries is recognized as a significant environmental problem because of the considerable organic load created by the brewing process. Unicer SA is the most important brewer in Portugal, and wastewater discharge from the factory located at Leça do Bailio represents an average organic load corresponding to 100 000 population equivalents. Such a large value is the result of daily flows between 1500 and 2500 m^3/d and chemical oxygen demand (COD) values typically between 1500 and 3000 mg/L. In 1996, to overcome such a problem, Unicer SA started the full-scale operation of an upflow anaerobic sludge blanket (UASB) reactor. The effect of anaerobic treatment was positive in terms of organic matter elimination. A COD removal of 70 to 80% was obtained.

Despite such efficiency, the final COD concentration was still greater than the threshold value prescribed by Portuguese legislation for wastewater discharge to surface water. Ammonium nitrogen (NH_4–N) concentration also exceeded the legal framework. On the other hand, because of the anaerobic digestion process, the concentration of dissolved organic carbon (DOC) in the UASB effluent was low (average DOC-to-nitrogen and soluble COD-to-nitrogen ratios of 0.8 and 2.5, respectively), which forecasted difficulties for the denitrification process. However, the total COD was high, typically between 400 and 2000 mg/L, raising the possibility of using such a carbon source in the denitrification step, after hydrolysis. The primary cause for nondissolved COD values was the washout of anaerobic granules from the UASB reactor that occurred during this study.

Biological nitrogen removal may be performed with various technologies. Among them, sequencing batch reactors (SBRs) demonstrated a significant potential for such a purpose (Irvine and Ketchum, 1982). The SBR performs multiple biological processes in only one tank, supporting a complex ecosystem with substrate competition and predator–prey interactions. The predator role of protozoa in activated-sludge plants is known in continuous systems (Curds and Cockburn, 1970), and some studies have been carried out on the long-term succession of microbial communities in SBR systems (Cybis and Horan, 1995). However, no information exists about population changes in the short term, that is, throughout each aerobic–anoxic phase.

The goal of this research was to study the feasibility of using SBR technology for the posttreatment of an effluent from an UASB reactor located in a brewery. The aim was to provide a base for upgrading the Unicer SA treatment system. The primary target was nitrogen removal, but suspended solids and COD levels also had to be reduced in accordance with the legal framework. With that in mind, two different SBR strategies of nitrification–denitrification were studied. One was based on an aerobic–anoxic sequence, and the other used a predenitrification step, that is, an anoxic–aerobic–anoxic sequence. In both tests, SBR performance and biological kinetics were evaluated. Protozoan population was monitored during some cycles of operation, and the fate of phosphorus concentration was also analyzed.

Materials and Methods

Experimental Setup. The cylindrical reactor was made of acrylic, with a maximum working volume of 3.3 L and an internal diameter of 144 mm. The biomass inoculum was a grab sample collected in a municipal activated-sludge plant of the extended aeration type (approximately 89% of the inoculum), supplemented with an inoculum of *Alcaligenes denitrificans* (10%) and nitrifying microorganisms (1%), both obtained from two laboratory-scale reactors. The average mixed liquor volatile suspended solids (MLVSS) concentration during the experimental assays was 1690 mg/L.

Solids sampling represented the only biomass wastage carried out during the experimental work. Therefore, the solids retention time was long, estimated at 37 days. This is a typical value for nitrification systems because of the low growth rates of such bacteria. In addition, this wastage rate compensated the low

Table 1—Brewery wastewater composition after UASB pretreatment.

Parameter	Range of values	MPV[a]
pH	7.5–8.0	6–9
Total COD, mg/L	400–2000	150
Soluble COD, mg/L	<470	—
Ammonium nitrogen, mg/L	23–87	7.8
Nitrite nitrogen, mg/L	0–1.2	0.5
Nitrate nitrogen, mg/L	0–3	11.3
Dissolved organic carbon, mg/L	60–83	—
Soluble phosphorus, mg/L	8–20	10
Total suspended solids, mg/L	320–1440	60

[a] Maximum permitted value for wastewater discharge in surface water, according to the Portuguese legislation.

growth rate of heterotrophic organisms observed in the present study because of carbon limitations.

The SBR was operated in the typical sequence of fill, react, settle, and draw. A mechanical stirrer was used to provide mixing during aerated and nonaerated reaction periods. An air diffuser placed at the bottom of the reactor provided the oxygen supply. The time-based SBR schedule was controlled by a data acquisition and control system (Labtech Notebook, LTC, 1996, Landover, Massachusetts) that was also used to regulate the dissolved oxygen (DO) by on–off control of the airflow. The DO concentration was measured continuously in the reactor using a YSI 5750 oxygen probe connected to a transmitter. Continuous monitoring of pH and oxidation–reduction potential (ORP) was carried out with a *YSI* 5000 meter (Yellow Springs, Ohio) using a combined ORP electrode Pt4805-S7/120. The recorded values were not referenced to hydrogen. The SBR was operated at 30 °C, which is the wastewater temperature at the full-scale UASB reactor in the factory. This temperature was maintained by means of a controlled water *jacket*.

Wastewater Composition. Average composition of the UASB effluent collected at UNICER brewery is shown in Table 1.

Analytical Methods. When a quasi-steady state was observed (i.e., repeated cyclic behavior), analyses over routine cycles were performed. All analyses were carried out as described in *Standard Methods* (APHA et al., 1989), with the following additional characteristics.

Nitrogen compounds were measured by colorimetric methods after sample filtration through a membrane with a porosity of 0.22 μm. A preliminary distillation step was introduced for NH_4–N determination to eliminate interferences and increase the precision of the method. A boric acid solution was used as an absorbent.

The soluble phosphorus was assessed by the ascorbic acid method, after filtration (0.45 μm).

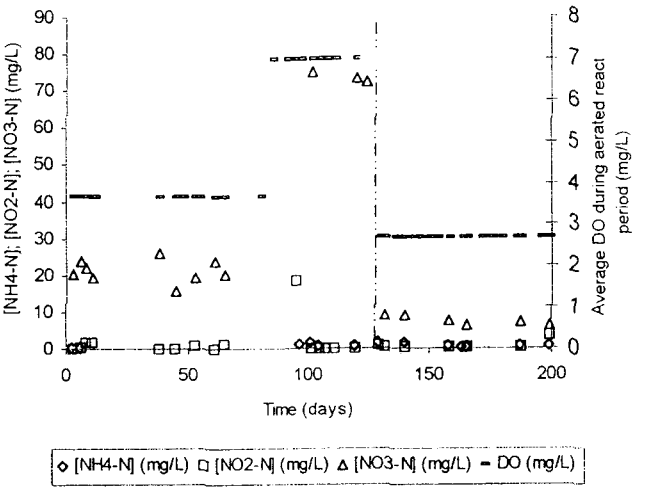

Figure 1—Long-term NH_4–N, NO_2–N, and NO_3–N effluent concentrations and respective average DO levels in the aerated phase.

The DOC was evaluated, after filtration (0.45 μm), using a total organic carbon analyzer OIC 700 (OI Corporation, College Station, Texas).

The sludge volume index (SVI) was calculated using a 1-L Imhoff cone and 30 minutes of settling time.

The biomass was examined by scanning electron microscopy after ethanol washing and air drying.

Protozoa were identified and enumerated as described by Madoni (1994).

Experimental Conditions. Two operating strategies were tested during this work. Their primary features are summarized in Table 2. The settle period was 1 hour, and the fill and draw periods lasted 30 minutes each.

Apparent Nitrification and Denitrification Rates. The apparent nitrification rate was calculated considering the NH_4–N depletion during the reaction time. The apparent denitrification rate was evaluated measuring the removal rates of NH_4–N, nitrite (NO_2–N), and nitrate (NO_3–N), neglecting the nitrogen consumed in cell synthesis and the NH_4–N produced from organically bound nitrogen.

Results

General Reactor Operation. Figure 1 presents the concentration of nitrogen compounds in the effluent after biological treatment. The results were obtained using two SBR operation strategies, as depicted in Table 2. Strategy 1 was characterized by the use of an aerated period, followed by an anoxic phase (postdenitrification). This strategy comprised a first run at a bulk liquid DO

Table 2—Operating conditions of the SBR.

Parameter	Strategy 1	Strategy 2
Reaction sequence	Aerobic (12 h)/anoxic (12 h)	Anoxic (2 h)/aerobic (4 h)/anoxic (2 h)
Total cycle time, h	26	10
Working volume, L	2.9	1.7
Hold-up ratio, %	60	30
Hydraulic retention time, d	1.9	1.2
Nitrogen concentration after fill, mg/L	30–45	20–28

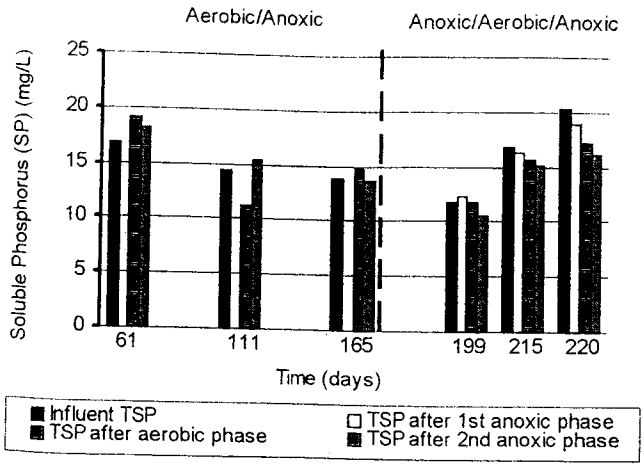

Figure 2—Phosphorus concentration during SBR operation (TSP = total soluble phosphorus).

concentration of 3.7 mg O_2/L in the aerated phase and a second run at a DO concentration of 7 mg O_2/L. Strategy 2 involved a predenitrification step and, thereafter, the aerated (DO = 2.8 mg O_2/L) and anoxic phases.

Figure 1 shows that strategy 1 was unsuccessful in terms of the NO_3–N value prescribed for discharge to surface water (11 mg NO_3–N/L). Indeed, the effluent NO_3–N concentration was greater than 20 mg NO_3–N/L. On the contrary, strategy 2 resulted in nitrogen effluent concentrations within the legal limits, fewer than 8 mg NO_3–N/L. Nevertheless, in both strategies, startup operation was performed in a short time, and no significant changes in efficiency during the experimental time were observed.

The fate of phosphorus during the experimental study was assessed and is presented in Figure 2. As can be seen, contrary to the initial forecast, the phosphorus concentration in the influent was greater than the legal threshold (10 mg/L) in many samples. With regard to strategy 1, changes in the soluble phosphorus concentration in the liquid phase during operation were minor. Biological phosphorus uptake increased during strategy 2, with the anoxic–aerobic–anoxic sequence. Under such conditions, the soluble phosphorus present in the discharge (values between 10.5 and 16.5 mg/L), despite still being greater than the 10-mg/L maximum permitted value limit, decreased 6 to 15% when compared to the values obtained after the filling phase. However, these values are far from those reported with enhanced biological phosphorus removal.

An improvement of the biological floc settleability was noticed during SBR operation. Soon after startup, flocs became larger, and the SVI decreased from 200 to 115 mL/g. A concomitant decrease of the total suspended solids (TSS) concentration in the treated effluent was observed, attaining only 30 mg/L at the end of the experimental period.

Strategy 1: Aerobic–Anoxic Sequence. During strategy 1, the average volumetric nitrogen load was 0.04 kg N/m·d, and the specific load was 0.024 kg N/kg VSS·d, which are relatively low values (e.g., Munch et al. [1996] applied a load of 0.096 kg N/kg VSS·d in their SBR study).

In Figure 3, the behavior of nitrogen compounds and the oxidation–reduction potential (ORP) and DO profiles during a typical 26-hour reaction cycle are shown (experiment of day 111). The results displayed indicate that the extended aeration time along each cycle allowed the full development of nitrifying bacteria. Complete nitrification took place, with NH_4–N and NO_2–N removed from the anaerobically pretreated effluent. After the fill period and at the beginning of the aerobic phase, NH_4–N concentration in the wastewater was 30 mg/L, but only 0.7 mg/L at the end of the anoxic phase.

In terms of overall nitrogen removal, the efficiency was low, and as much as 50% of the NO_3–N formed during the aerobic period remained after the anoxic phase. However, a nitrogen balance in the liquid phase showed that the NO_3–N concentration at the end of the aerobic phase was approximately 50% lower (15 to 20 mg/L) than that theoretically expected according to reaction stoichiometry. Biomass yield is insufficient to account for this discrepancy. Consequently, the data indicate the occurrence of a significant denitrification process during the aerated phase.

A conventional explanation for such a phenomenon relies on oxygen limitations within microbial flocs. Van Loosdrecht and Heijnen (1993) indicate that oxygen limitation exists in biofilms at depths greater than 100 μm. Such environments may provide oxygen-free conditions for heterotrophic denitrifying bacteria activity. However, recent studies indicate that some species perform denitrification even in the presence of oxygen (Lukow and Diekman, 1997, and Patureau et al., 1998). Patureau et al. (1998) operated an SBR inoculated with the aerobic denitrifying *Microvirgula aerodenitrificans* in a consortium with nitrifying bacteria at a DO concentration of 7.2 mg/L. An aerobic denitrification process in an activated-sludge SBR was also observed by Munch et al. (1996).

Such hypotheses were tested by setting a high DO concentration (7 mg/L) during the aerated period. Figure 4 shows a typical cycle using such high DO concentrations in the bulk liquid. As can be seen, denitrification did not occur during the aerobic period when an oxygen concentration of 7 mg O_2/L in the bulk liquid phase was imposed. It seems, then, that there was oxygen limitation when the DO concentration was 3.7 mg O_2/L.

Furthermore, Figures 3 and 4 show that nitrate consumption did not occur in the subsequent nonaerated period. Carbon limitations caused by the low carbon-to-nitrogen (C:N) ratio of the UASB effluent were considered to hamper denitrification, even if a slight increase of soluble COD released by granules hydrolysis during the reaction period is noticed (Figure 4). Acetate was added at the

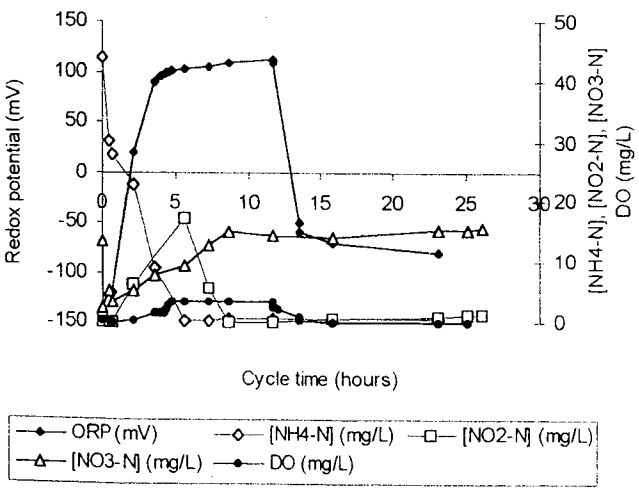

Figure 3—Values of ORP and DO and NH_4–N, NO_2–N, and NO_3–N concentrations.

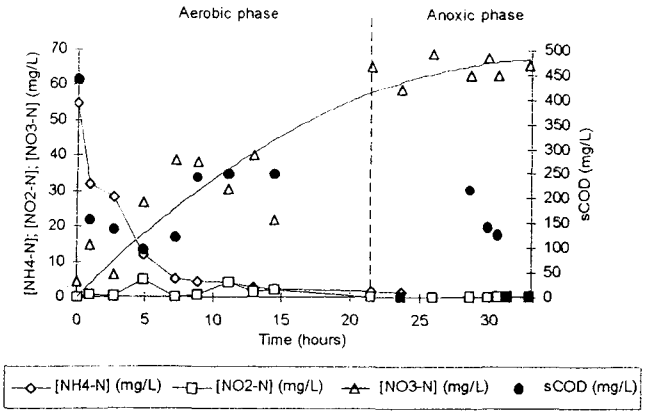

Figure 4—Concentrations of NH_4–N, NO_2–N, NO_3–N, and soluble COD during the reaction period (DO concentration in the aerated phase was 7 mg/L).

end of the aerated phase to evaluate this hypothesis. The C:N that was provided by the acetate spike was 13 g C/g N, and its effect is presented in Figure 5.

As can be seen, complete nitrate removal was recorded when acetate was added. Such a result confirmed that an insufficient carbon supply limited the denitrification process when the real UASB effluent was used.

Strategy 2: Anoxic–Aerobic–Anoxic Sequence. During strategy 1, carbon limitations in the denitrification process were identified. Therefore, to avoid the costs of external carbon supplementation or the splitting of primary wastewater flow, another strategy was tested. This second test used a predenitrification step consisting of 2 hours of an anoxic phase, followed by an aeration time of 4 hours and a second nonaerated period with a duration of 2 hours. This run was carried out applying a greater nitrogen load of 0.086 kg N/m·d, corresponding to a specific load of 0.051 kg N/kg VSS·d.

Figure 6 represents the experimental results along a typical SBR cycle during strategy 2. The results show that a denitrification process occurred during the first nonaerated period. The overall efficiency of nitrogen removal was 37%, and the maximum denitrification rate, 0.038 kg N/kg VSS·d, was observed during this phase. It is worth noting that, despite the absence of aeration, a significant fraction of NH_4–N (approximately 50%) was removed as was a significant fraction of soluble organic matter. Soluble COD concentration decreased from 108 mg O_2/L to a value of fewer than 50 mg O_2/L (which was the detection limit of the COD analytical method). Afterward, NH_4–N was completely removed during the aerobic phase, while simultaneous denitrification took place, as already observed during strategy 1. Overall nitrogen removal efficiency of that period was 43%. As expected, the final anoxic phase was again inefficient. Nevertheless, the NO_3–N concentration in the effluent was low (7.4 mg/L), enabling the wastewater to be discharged to surface water.

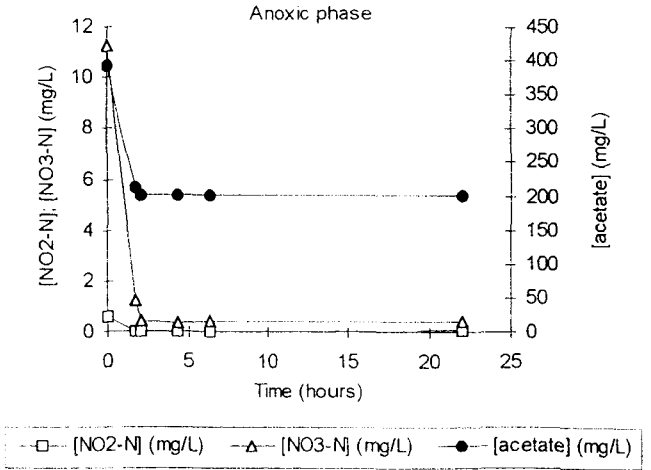

Figure 5—Effect of acetate supplementation on denitrification process (anoxic phase).

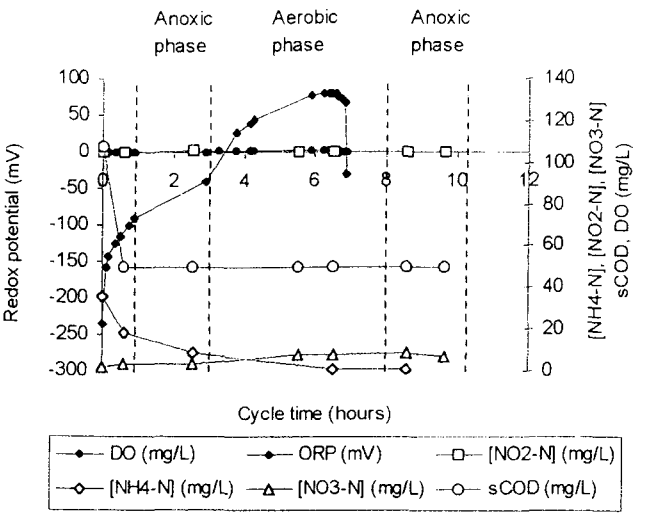

Figure 6—Variation of ORP values, DO, soluble COD, and nitrogen compound concentrations in the bulk liquid during a 10-hour cycle (day 187).

Figure 6 also shows the ORP increase during the first nonaerated phase (from −150 mV after the fill sequence to −50 mV) resulting from the formation of nitrogen oxides. As the aeration started, the ORP further increased to positive values as a result of the presence of DO in the bulk liquid and because of nitrate formation. Once aeration is stopped, the ORP rapidly decreases to negative values, which characterize the second anoxic period.

Nitrification and Denitrification Rates. Experimental data regarding NH_4–N oxidation were correlated testing first-, half-, and zero-order kinetics. During strategy 1, the best results were obtained using a zero-order reaction model when DO concentration was 3.7 mg O_2/L (Figure 3) and half-order kinetics when DO concentration was 7 mg O_2/L (Figure 4). Zero- and half-order reaction constants were 4.76 h^{-1} and 1.2 $mg^{1/2}/L^{1/2}$·h. Correlation coefficients were 0.91 and 0.96, respectively. Experimental results concerning ammonium oxidation during strategy 2 (DO = 2.8 mg O_2/L) are well fitted by a first-order model. The first-order reaction constant was 0.42 h^{-1} (correlation coefficient of 0.89).

Despite the different operational strategies that were tested, maximum observed nitrification and denitrification rates were recorded during the first period of the SBR cycles. When using the aerobic–anoxic sequence, the maximum value for the denitrification rate is obtained when the nitrification rate is zero, after all NH_4–N was converted to NO_3–N. On the other hand, when using the anoxic–aerobic–anoxic sequence, observed profiles of the ni-

Table 3—Range of maximum observed nitrification and denitrification rates.

Operational conditions	Maximum observed nitrification rate, kg NH_4–N/kg VSS·d	Maximum observed denitrification rate, kg N/kg VSS·d
Strategy 1 (DO during the aerobic period = 3.7 mg/L)	0.128	0.165
Strategy 1 (DO during the aerobic period = 7 mg/L)	0.164–0.175	—
Strategy 2 (DO during the aerobic period = 2.8 mg/L)	0.044–0.057	0.038

trification and denitrification rates were similar, and the maximum value for both rates was obtained at the beginning of the first anoxic phase and gradually decreased throughout the reaction period. Different patterns of nitrification and denitrification during strategy 1 compared to strategy 2 can be related to different aeration conditions and hold-up ratios. Strategy 1 led to a greater NH_4–N concentration after the fill period, 30 to 45 mg/L, which might temporarily inhibit the denitrification process (Encina and Rojas, 1996).

The maximum observed nitrification and denitrification rates obtained according to the different operational conditions are presented in Table 3. Despite the lower nitrification and denitrification rates, strategy 2 led to the best results in terms of expected nitrogen removal from the UASB effluent. The maximum observed nitrogen removal rate obtained (0.038 kg N/kg VSS·d) is 30% lower than that reported by others in SBR systems (Choi and Oa, 1996, and Munch et al., 1996). Nevertheless, this value lies in the range of 0.03 to 0.11 kg N/kg VSS·d, reported by Metcalf and Eddy (1991) for specific denitrification rates in systems using wastewater as a carbon source.

Protozoan Observations. To assess the fate of the protozoan population throughout a cycle, mixed liquor samples were observed using an optical microscope. Based on their morphology, size, and mobility, protozoa were identified and enumerated as described by Madoni (1994).

Observations revealed a high density of protozoan organisms, including free-swimming, crawling, and attached ciliates and flagellated protozoa. Some rotifers were also detected, but not in significant numbers. The most significant protozoan species observed during SBR operation were *Vorticella* sp., *Epistylis* sp., *Vaginicola* sp. (stalked ciliated protozoa), *Uronema* sp., *Colpidium* sp. (free-swimming ciliated protozoa), *Aspidisca* sp., *Chilodonella* sp. (crawling ciliated protozoa), and *Peranema* sp. (flagellated protozoa).

The protozoan density was high during the aerated period. Values of 15×10^3 organisms/mL were reached, indicating good reactor performance. Crawling and attached ciliates were the dominant protozoa. With the decrease of the DO concentration throughout the nonaerated phase, these two groups of microfauna showed a reduction of 40%, approximately. The appearance of free-swimming ciliates and flagellated protozoa could then be observed. When aerobic conditions were reestablished, crawling and attached ciliates easily recovered, indicating that cyclic aerobic–anoxic conditions had no detrimental effect on protozoan population. Some cyst formation was observed when the transition from aerated to nonaerated conditions occurred. This phenomenon is likely the biological response of protozoa to unfavorable environmental conditions.

Discussion

General Performance. Overall experimental results for nitrogen, COD, and suspended solids removal from the pretreated wastewater demonstrated that the most appropriate strategy to achieve legal discharge compliance was the anoxic–aerobic–anoxic sequence, with a DO concentration of 2.8 mg/L in the aerated period and a hold-up ratio of 30% (strategy 2). Under such conditions, the nitrate concentration in the effluent was fewer than 8 mg NO_3–N/L. Thus, this strategy optimizes energy requirements for aeration, with an appropriate effluent quality for discharge to surface water.

Experimental data showed that the nitrification rate depends on bulk liquid oxygen concentration. An increase in DO concentration from 2.8 to 3.7 mg O_2/L promoted a rate increase of 65%. However, a further increase to 7 mg O_2/L induced only a small difference in nitrification rate. The data suggest a transition from a first-order reaction at lower oxygen concentrations (2.8 and 3.7 mg O_2/L) to a half-order reaction at greater concentrations. Similar results were also obtained by Lazarova et al. (1998) in a circulating floating bed reactor. These authors concluded that the transition value from a first-order reaction to a half-order reaction was attained at 4.5 mg O_2/L.

Concurrent nitrification and denitrification were detected during the aerated phase at DO concentrations of 2.8 and 3.7 mg/L (Figures 6 and 3). On the other hand, denitrification was inhibited during the aerated period when the bulk liquid DO level was raised to a concentration of 7 mg/L (Figure 4). Under such conditions, aerobic conditions should occur along the longitudinal floc profile. Accordingly, a DO concentration in the bulk liquid between 2.8 and 3.7 mg O_2/L could be insufficient to create an aerobic environment within the biological flocs, leading to oxygen limitations. Therefore, denitrification seems to be provided by heterotrophs located in inner anoxic niches, not by aerobic denitrifiers. This is also in accordance with the results of Lazarova et al. (1998) regarding nitrification kinetics, which indicate that oxygen mass transfer may be, in some cases, the limiting step controlling the nitrification rate.

Denitrification during the final anoxic phase (after the aerobic period) was practically null in all runs. The C:N in the UASB effluent was low (an average value of 0.8), and carbon requirements for complete nitrogen removal were not satisfied (the stoichiometric C:N, using an easily degradable carbon source such as acetate, is 1.25 [Encina and Rojas, 1996]). Results obtained when acetate was used to increase the C:N confirmed that the soluble carbon source was limiting denitrification. It was expected that, during this second anoxic stage, soluble organic carbon could be made available to denitrifying bacteria as a consequence of solubilization and hydrolysis of biomass granules washed from the UASB reactor.

However, despite a total COD removal of between 85 and 97% and the release of some soluble carbon (Figure 4), no postdenitrification was observed, and all organic matter was consumed in the aerated phase. Nevertheless, the solubilization of this particulate matter did occur; a decrease in the suspended solids concentration

was observed, and the legal threshold for solids discharge in surface waters (60 mg TSS/L) could be accomplished (the high TSS concentration in the UASB effluent was caused by the presence of biomass granules washed from the anaerobic reactor). However, no accumulation of soluble carbon could be detected, which means that the consumption rate was equivalent to the releasing one. Therefore, postdenitrifying activity was inhibited.

As shown in Figure 6, simultaneous nitrification–denitrification was also recorded during the first period of nonaerated conditions in strategy 2. Ammonium oxidation under an oxygen deficit has been already reported using *Alcaligenes* sp. in the treatment of anaerobic effluent (Krull, 1976). Ammonium removal may have been possible by using the nitrate remaining from the previous cycle as the electron acceptor. A fraction of NH_4–N also could have been oxidized, consuming the oxygen that was introduced to the system during the filling process (0.2 to 0.5 mg O_2/L).

The hold-up ratio (i.e., ratio of untreated to treated wastewater) affected nitrification and denitrification performance. A lower volumetric replacement (30% in strategy 2, contrary to 60% in strategy 1) corresponded to low initial concentrations of NH_4–N in the reactor after the filling period (20 to 28 mg N/L). Thus, this type of operation leads to lower levels of NO_3–N in the discharge. The nitrification rate was enhanced when the SBR was operated with a greater volumetric replacement strategy, suggesting that ammonium oxidation rates are concentration dependent.

The ORP was kept within the range of +100 to −200 mV in response to oxygen concentration along each cycle. The ORP provides information about the process regime and can be used to control the duration of the denitrification phase (Demoulin et al., 1995). However, the typical breakpoint, the "nitrate knee," that appears in the ORP curve at nitrate concentrations close to zero could not be observed (Figures 3 and 6). Because of the simultaneous nitrification–denitrification and carbon limitations, NO_3–N was always present, even if at low concentrations, throughout the entire operating cycle.

Phosphorus. As noted in the Results section, soluble phosphorus was not consumed significantly throughout each cycle of operation. There are different reasons indicated in the literature for such an outcome. First, the reaction period was not initialized with an anaerobic phase, which should have been the case to provide the release of previously stored phosphates and the storage of organic matter because this is the driving force for biological phosphorus uptake in the subsequent SBR oxic stage (Garzón-Zúñiga and González-Martínez, 1996). However, the experimental work only comprised anoxic cyclic conditions (i.e., chemically bound oxygen species in the form of NO_3–N are still present) and not anaerobic ones.

Second, carbon requirements for phosphorus removal are greater than those for denitrification, and the ratio between the soluble COD (sCOD) and phosphorus was less than 10 (sCOD:P ratios of 26 are recommended, according to Smolders et al., 1995). Thus, the competitive kinetic gain of denitrifiers over phosphate-accumulating bacteria suggests that the available organic carbon was preferably used as the electron donor in the NO_3–N reduction (Barker and Dold, 1996).

Finally, the long solids retention time and, thus, low sludge production rate (Smolders et al., 1997) were not favorable to the biological phosphorus removal process, resulting in a low amount of active mass (which contains phosphorus) within the mixed liquor suspended solids. Regarding temperature influence on biological phosphorus removal, contrasting results are presented in the literature. The 30 °C temperature is not considered to be the optimal temperature for the biological phosphorus removal process, according to Baetens et al. (1998). They state that the maximum aerobic phosphorus uptake typically occurs in the interval between 15 and 20 °C. On the other hand, increased phosphorus uptake rates with increased temperatures, in the temperature range of 5 to approximately 30 °C, were reported by Shapiro et al. (1967), Boughton et al. (1971), Spatzierer et al. (1985), Mamais and Jenkins (1992), and Brdjanovic et al. (1997). Therefore, the relatively high 30 °C temperature was not the key factor affecting the efficiency of the biological phosphorus removal process.

Protozoa. The aerobic or anoxic state of the cycle had a significant effect on the composition of the protozoan population. During the aerated period, the number of free-swimming ciliates was extremely low because they were replaced by attached ciliates because of the competition for dispersed bacteria. This situation occurs because free-swimming and attached ciliates feed on bacteria dispersed in the liquid but, because the biomass in the reactor was primarily composed of floc-forming bacteria, free-swimming ciliates were not able to grow. According to Curds (1971), attached ciliates are filter feeders that are more efficient than the free-swimming forms feeding on suspended bacteria.

The appearance of free-swimming ciliates and flagellated protozoa when aeration stops indicates the existence of low DO concentrations in the bulk liquid phase. These protozoan groups survive better under an oxygen deficiency than other microfauna (Madoni, 1994). Therefore, observations of protozoa are a valuable tool for evaluating reactor status and performance in cyclic processes only under long reaction times within each cycle. Otherwise, changes in microfauna composition within short time frames will not be noticeable. The development of cysts might be the reason for the fluctuations in active protozoan numbers. This is a well-known physiological strategy for addressing unfavorable environmental conditions, namely, oxygen deficits.

Conclusions

The removal of nitrogen from a brewery wastewater that had previously undergone anaerobic treatment and contained a low C:N was attained without an external carbon source or flow splitting. To achieve legal discharge compliance at reduced costs, the most appropriate operation strategy is an anoxic–aerobic sequence (because no denitrification was observed during the second anoxic phase as a result of carbon limitations), with a DO concentration of 2.8 mg/L in the aerated period and a hold-up ratio of 30%. The maximum observed nitrogen removal rate under these operational conditions was 0.038 kg N/kg VSS·d.

A high level of nitrification performance, with a removal efficiency of NH_4–N as great as 97%, using either an aerobic–anoxic or anoxic–aerobic–anoxic reactor sequence, was observed. The maximum observed nitrification rate, 0.175 kg NH_4–N/kg VSS·d, was obtained when the reactor was operated under an aerobic–anoxic sequence, with an average DO concentration in the aerated period of 7 mg/L and a volumetric replacement of 60% (strategy 1), which led to a higher initial nitrogen concentration (30 to 45 mg N/L).

A nitrogen balance deficit in the liquid phase of approximately 50 to 60% indicated that a stable process of denitrification under DO concentrations of 3.7 mg/L can be achieved. Simultaneous nitrification and denitrification have shown to be significant for

nitrogen removal. However, the unfavorable C:N of the anaerobically pretreated wastewater hindered complete NO_3–N removal.

The dominance of a functional subgroup of protozoans was related to the oxygen presence during the SBR reaction cycle. It was also found that a cyclic but short absence of oxygen was not detrimental to the protozoan population.

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