Removal of organic carbon, nitrogen and phosphorus in sequential batch reactors integrating the aerobic/anaerobic processes

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Abstract

The performance of a bench-scale apparatus composed of two sequential batch reactors (SBR), forming an anaerobic/aerobic treatment system, was evaluated as to its potential use for biological removal of organic matter and nutrients. Both the reactors with 12.5 L of useful volume each were operated for the batch cycles of 12 hours receiving 8.0 L of substrate in each cycle. The first reactor (SBRAn) fed with synthetic substrate simulating domestic sewage was meant to remove the largest fraction of carbonic matter and to promote the substrate ammonification. The second reactor (SBRAe) was operated under alternating aerobic and anoxic conditions to establish conditions for achieving nitrification; denitrification and biological phosphate removal in the same batch cycle. Sodium acetate was used as an external carbon source for phosphate removal. Processes monitoring included the analyses of: COD, NTK-N, NH4^+ -N, NO2^- N, NO3^- N, PO4^-P, alkalinity, volatile acids, pH, redox potential, total, fixed and volatile solids. Under the operating conditions imposed, the system exhibited high performance in removing organic matter (COD), nitrogen and phosphorus, producing effluent with COD, NTK-N and PO4^-P concentrations lower than 50.0 mg/L, 4.0 mg/L and 1.0 mg/L, respectively.

Keywords: Biological wastewater treatment; biological phosphorus removal; denitrification; nitrification; SBR reactor

Introduction

The biological wastewater treatment technology is experiencing remarkable development, mainly due to progress in the knowledge of anaerobic processes, leading to the enlargement in the availability of diverse treatment system options. There are many new possibilities, they include combined anaerobic/aerobic systems, in which the anaerobic unit removes significant fraction of the organic matter, and the pre-treatment proceeds at incomplete secondary level.

Although anaerobic process has been increasingly used for organic matter removal, its effluent is rich in ammonia nitrogen and does not fit the established quality patterns for the industrialized countries. This is particularly true for COD, suspended solids, nitrogen, phosphorus and sulfate (Garuti et al. 1992; Duncan et al. 1999). For this reason, the use of an aerobic unit following the anaerobic reactor is often required to promote the complementary carbon oxidation and nitrification (Sousa and Foresti, 1996). Denitrification and biological removal of phosphorus can also be achieved, allowing the discharge quality patterns to be reached (Bernardes and Klapwijk, 1994).

The oxidation of the ammonia nitrogen to nitrite and nitrate occurs in the presence of dissolved oxygen, that is, in an aerobic environment. The two main groups of microorganisms responsible for the sequential oxidation of ammonia nitrogen are the bacteria *Nitrossomonas* that oxidize the ammonia to nitrite and *Nitrobacter* that complete the nitrification process oxidizing nitrite to nitrate. The concentration of dissolved oxygen (DO), the pH, the temperature and the cellular retention time (CRT) are important parameters in that
process. The maximum nitrification rate occurs at DO concentrations higher than 2 mg/L, temperature in the range of 25 to 35°C, and pH between 7.5 and 9.0 (Surampalli et al., 1997).

In denitrification, nitrate is biologically reduced under anoxic conditions in the presence of a carbon source, while the combined oxygen serves as the terminal hydrogen acceptor for microbial respiration. The process occurs in several steps; nitrate is first reduced to nitrite; then intermediary nitrogen oxides (NO and N₂O) are formed; and finally the gaseous product N₂. Environmental factors, such as pH, temperature and DO concentration have influence on the denitrification rate. DO concentrations higher than 1.0 mg/L are known to inhibit the denitrification process. Temperatures in the range of 10 to 30°C, and pH between 6.5 and 8.0 are the other main environmental conditions that favor the process (Surampalli et al., 1997). Therefore, nitrification and denitrification are processes occurring at different environmental conditions that can be achieved, for example, in a system composed of aerobic and anaerobic reactors sequentially disposed.

Biological phosphorus removal can be achieved essentially by imposing operating conditions that make it possible for the biomass to assimilate more phosphorus than necessary for its normal metabolism. This can happen when the biomass is subjected alternately to aerobic and anaerobic conditions. Under anaerobic conditions, the microorganisms will first liberate phosphate to the liquid phase and soon after under aerobic conditions they absorb more phosphate than the active biomass usually does.

The survival mechanism peculiar to some bacteria like *Acinetobacter* derives from their capacity to store phosphorus in the polyphosphate form (poli-p) and carbon in the form of poli-b-hydroxiburate (PHB). Under anaerobic conditions, the phosphate stored as poli-p is liberated due to the need of bacteria *Acinetobacter* to use quickly the biodegradable substrates like PHB, thus increasing the orthophosphate concentration in the liquid. In the aerobic phase, due to the low concentration of quickly biodegradable substrate, the organisms that have stored PHB begin to use it as a source of carbon and energy. Part of the energy is used to absorb phosphate, storing it inside the cells as poli-p, with consequent decrease of the phosphorus concentration in the liquid phase (Surampalli et al., 1997). In the anaerobic phase, phosphate is liberated in the presence of an easily degradable carbon source (Sedlak, 1991), in the absence of DO and oxidized nitrogen. In the aerobic phase, the “luxury phosphate up-take” is maximized in concentrations of DO higher than 2.0 mg/L (Surampalli et al., 1997).

An alternative to the conventional anaerobic/aerobic process of phosphorus removal is the anaerobic/anoxic process. It is based on the ability of some denitrifying organisms to store high amounts of polyphosphate as they have a metabolism similar to the phosphorus accumulating bacteria growing in conventional anaerobic/aerobic system. Therefore, the removal of nitrogen and phosphorus can proceed simultaneously. According to Kuba et al. (1996) such denitrifying phosphorus removal bacteria accumulate easily in anaerobic/anoxic SBR.

The use of combined systems composed of SBR reactors seems to be a viable option for obtaining organic matter oxidation, nitrification, denitrification and biological phosphorus removal. Due the characteristics of the SBR reactor, its operation can be adjusted to obtain aerobic, anoxic and anaerobic periods inside the standard cycles (Droste and Massé, 1995; Surampalli et al., 1997).

This present paper presents and discusses the performance of an anaerobic/aerobic system composed of two sequential batch reactors (SBR) in series treating synthetic substrate simulating domestic sewage. Special attention is given to the behavior of the second SBR reactor in removing nitrogen and phosphorus in the same cycle.
Methods
The research was carried out using two sequential batch reactors in series, one anaerobic (SBRAn) and the other intermittently aerobic (SBRAe), forming an anaerobic/aerobic system, as shown in Figure 1.

The two bench-reactors were built using Plexiglas tube with internal diameter of 14.5 cm, total height of 100 cm and useful height of 84 cm. The resulting total volume was 16.5 L with useful volume of 14 L. They were operated in series and fed by gravity with 8.0 L/cycle. Each cycle comprised the sequence of four different phases: filling, reaction, sedimentation and discharge. In the reaction phase in the SBRAn, the intermittent recycling of the produced gas to its bottom provided mixing. The SBRAe had its reaction phase divided into three phases: aerobic with mixing provided by aeration; anoxic, with mixing provided by liquid recycling; and aerobic similar to the first phase.

The SBRAn was meant to remove the largest fraction of organic matter (measured as COD) and to promote ammonification. The SBRAe was intended to proceed to the nutrient removal through nitrification of the anaerobic effluent, denitrification and biological removal of phosphate. An external carbon source, sodium acetate at the concentration of 500 mg/L, was added at the beginning of the anoxic phase for denitrification and biological phosphorus removal in the SBRAn. The acetate concentration was stoichiometrically calculated to permit phosphorus removal, using data from the literature.

The system was operated for 41 days with 84 cycles of 12 hours, at the temperature of 28±1°C. The duration of the phases is presented in Table 1. The operation of the system with respect to phase cycling and mixing in each reactor was controlled by a microcomputer.

The substrate was prepared daily using tap water, meat extract, starch, cellulose, sucrose, edible oil, detergent and micronutrient solution. The resultant COD of 800 ± 100 mg/L was achieved with the quantities of the constituents presented in Table 2. Sodium acetate was added to the anoxic phase for denitrification and biological phosphorus removal.

Figure 1 Scheme of the experimental installation

Table 1 Duration of different phases of the operational cycles

<table>
<thead>
<tr>
<th>Phase of the cycle</th>
<th>RSBRAn</th>
<th>RSBAe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filling</td>
<td>0.25 hours</td>
<td>0.25 hours</td>
</tr>
<tr>
<td>Reaction</td>
<td>9.5 hours</td>
<td>3.0 hours aerobic - continuous aeration</td>
</tr>
<tr>
<td>Intermittent mixing</td>
<td>2 min/15 min</td>
<td>3.5 hours aerobic - continuous aeration</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>2.0 hours</td>
<td>2.0 hours</td>
</tr>
<tr>
<td>Discharge</td>
<td>0.25 hours</td>
<td>0.25 hours</td>
</tr>
</tbody>
</table>
bicarbonate was also added at the concentration of 200 mg/L to increase the substrate buffer capacity. Such a synthetic substrate has been used to simulate domestic sewage in lab-scale treatment systems (Sousa and Foresti, 1996). The substrate composition is shown in Table 2.

The anaerobic reactor was inoculated with 4.0 L of granulated sludge presenting good sedimentation characteristics, taken from an UASB reactor installed at a paper factory. The aerobic reactor was not inoculated. Before the beginning of the research, the system was operated under cycles of 24 hours and reaction phase of 20 hours for adaptation of the anaerobic biomass and growth of the aerobic sludge. In this period, the SBRAe received the effluent of the SBRAn and 0.5 L of the synthetic substrate to fasten the aerobic sludge growth.

System monitoring included the determination of pH and redox potential, dissolved oxygen (OD), chemical oxygen demand of filtered (COD_f) and non-filtered samples (COD), total volatile acids (TVA), total alkalinity (Alk), total solids (TS), fixed solids (FS) and volatile solids (VS), total phosphate (PO_4^{3-}-P), total nitrogen (NTK-N), ammonia nitrogen (NH_4^{+}-N), nitrite (NO_2^{-}-N) and nitrate (NO_3^{-}-N). All the analysis were according to the “Standard Methods for the Examination of Water and Wastewater” (APHA, 1995). Besides the main physical-chemical parameters, assays were done to estimate the number of nitrifying and denitrifying microorganisms, and the biomass was examined by optical microscope under common light and fluorescence. The number of nitrifying and denitrifying microorganisms were estimated by the most probable number (MPN) technique described by Tiedje (1982) and Gianotti et al. (1997), respectively.

The MPN assays were done at the beginning and at the end of the experimental work. The nitrifying microorganisms were incubated in a selective medium for autotrophic growth described by Smith and Belser (1982). The denitrifying microorganisms were incubated in generic nutrient broth (“Difco”) and 0.5 mM of NaNO_3 (Gianotti et al., 1997).

Results and discussion
The results from the 84 cycles of operation showed quite a stable behavior of the system with global COD removal efficiency of 94 ± 3%, for filtered and non-filtered samples. Approximately 70% of organic matter expressed as COD was removed in the RSBAn. Part of the remaining organic fraction was removed in the RSBAe that presented COD removal efficiency of 80 ± 10% and 70 ± 7% for non-filtered and filtered samples, respectively. The higher COD removal efficiency for non-filtered samples in SBRAe was a consequence of the VS retention and degradation inside it. The average VS concentration in the SBRAn effluent was 210 ± 20 mg/L, decreasing to 45 ± 15 in the SBRAe effluent.

Table 3 presents the average values of the main monitored parameters of the synthetic substrate and effluents of the sequential batch reactors, calculated from the results obtained during the operation of the system.

Table 2 Composition of the synthetic sanitary sewer

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% of COD</th>
<th>Source</th>
<th>Quantity added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>50%</td>
<td>Meat extract, 100%</td>
<td>1.87 mL/L</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>40%</td>
<td>Sucrose, 20%</td>
<td>57.6 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Commercial starch, 60%</td>
<td>179.2 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellulose, 20%</td>
<td>65.5 mg/L</td>
</tr>
<tr>
<td>Lipids</td>
<td>10%</td>
<td>Soybean oil, 100% (emulsified with 3 detergent drops/L)</td>
<td>0.09 mL/L</td>
</tr>
<tr>
<td>Mineral salts</td>
<td>-</td>
<td>Solution of NaCl with 50.0 g/L</td>
<td>5.0 mL/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution of MgCl_2 . 6H_2O with 1.4 g/L</td>
<td>5.0 mL/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution of CaCl_2 . 2H_2O with 0.9 g/L</td>
<td>5.0 mL/L</td>
</tr>
</tbody>
</table>
The average conversion of organic nitrogen to ammonia nitrogen in SBRAn was 75%, resulting in the increase of alkalinity due to the ammonification process (3.38 g.CaCO₃/g.NH₄⁺-N produced). The influent and effluent alkalinity concentrations of SBRAn were 170 ± 30 mg.CaCO₃/L and 285 ± 25 mg.CaCO₃/L, respectively. This reactor operated at redox potential values close to –309 mV, measured at the end of the reaction phase, and average VS concentration of 28.8 g/L in the settled sludge.

Figure 2 exhibits a typical temporal profile of COD and NH₄⁺-N in SBRAn. It can be verified that the largest fraction of COD removal and ammonia formation occurred during the first six hours of reaction.

The SBRAe was operated at the average TS concentration of 4,800 mg/L in the mixed liquor and of VS between 2,800 and 3,800 mg/L. The ratio COD/TVS was about 0.05 at the beginning of the first aerobic phase; 0.053 at the beginning of the anoxic phase when sodium acetate was added; and 0.028 at the beginning of the second aerobic phase. Sludge discard occurred during the sedimentation phases, whenever the height of the settled sludge reached 30 cm and the TS concentration was approximately 4,800 mg/L.

When the concentration of TS in the mixed liquor surpassed 5,000 mg/L, the DO concentration dropped close to 1.0 mg/L and the nitrification process was affected. However, as soon as the sludge discharge was done and OD concentration achieved values close to...
4.4 mg/L, ammonia was again completely oxidized to nitrate. Denitrification and biological removal of phosphate were also very high, with average efficiency of nitrogen and phosphate removal of 90% and 96%, respectively. For 75% of the cycles analyzed, effluents presented total phosphate, NTK-N and NO$_3^-$-N concentration lower than 1.0 mg/L, 4.5 mg/L and 0.03 mg/L, respectively. Moreover, the presence of NH$_4^+$-N and NO$_3^-$-N was not detected.

Temporal profiles obtained in SBRAe showed that ammonia was completely oxidized to nitrate along the three hours of the first aerobic phase, provoking the decrease of the alkalinity due to nitrification (5.07 g CaCO$_3$/g NH$_4^+$-N oxidized). In the anoxic phase, denitrification occurred immediately in the first two hours resulting in alkalinity increase (6.3 g CaCO$_3$/g NO$_3^-$-N), as can be observed in Figure 3.

At the beginning of the anoxic phase, 500 mg/L of sodium acetate was added as external carbon source for denitrification and biological phosphate removal. Figure 4 shows the temporal profiles of COD, phosphate and VA in SBRAe. Typical values of redox potential at the end of each phase were of +166 mV in the first aerobic phase, −153 mV in the anoxic phase and +177 mV in the second aerobic phase.

Figure 4 shows that phosphate is liberated just after the addition of acetate at the beginning of the anoxic phase, and assimilated at the second aerobic phase. It is also assimilated in the first aerobic phase, after receiving the anaerobic effluent, attaining a concentration as low as 1.6 mg/L in the first hour. This quick phosphate removal from the liquid can be attributed to the previous storage of biodegradable substrate in the anoxic stages and to the low concentration of easily biodegradable substrate in the first aerobic phase. Once the easily biodegradable substrate was not available in the SBRAn effluent, the organisms began to degrade the stored substrate by using it as carbon and energy source, storing...
phosphate in cells. Once released in the anoxic phase, phosphate was again taken up in the second aerobic phase.

The estimated numbers of nitrifying and denitrifying microorganisms resulting from MPN assays, at the beginning and end of the experiment, are presented in Table 4.

The data of enumeration of NMP of ammonia oxidizer and nitrite oxidizers microorganisms show that this population increased, with nitrite oxidizers outnumbering ammonia oxidizers by a factor ranging from 2.96 initially to 4.91 at the end final of the experiment. It can be concluded that nitrite oxidizer was stimulated during the experimental time. The MPN of denitrifying microorganisms decreased from the beginning to the end of the experiment. However, this decrease did not affect the denitrification process.

In order to verify the predominant morphotypes in the SBRAe, a microbiological evaluation of the biomass was carried out. The observations in common optical microscopy showed the presence of fixed ciliates and free swimming protozoa. These organisms appear in reactors operating at very low organic matter concentration in the bulk liquid, thus indicating good purification conditions. The presence of *Aspidisca* and amoeba similar to *Arcella*, both indicators of good nitrification conditions, was also verified.

The prevalence of cocci, short rod, short and curved rods, and cells resembling *Methanosarcina sp* was observed in sludge flocks of SBRAe. The presence of fluorescent methanogenic cells was unexpected since these organisms depend on anaerobic environmental conditions for growth and maintenance. Possibly they were washed out from the SBRAn and adapted to the environment in SBRAe, due to the alternated aerobic/anoxic phases. Figure 5 shows photos of common microscopy and under fluorescence for these microorganisms.

### Table 4 Results of the NMP rehearsals

<table>
<thead>
<tr>
<th></th>
<th>Ammonium oxidizers</th>
<th>Nitrite oxidizers</th>
<th>Denitrifying</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beginning</strong></td>
<td>Selective medium</td>
<td>5.4 x 10^5 cel/mL</td>
<td>Nutrient broth</td>
</tr>
<tr>
<td></td>
<td>1.6 x 10^6 cel/mL</td>
<td>2.5 x 10^6 cel/mL</td>
<td></td>
</tr>
<tr>
<td><strong>Final</strong></td>
<td>Selective medium</td>
<td>5.4 x 10^6 cel/mL</td>
<td>3.5 x 10^5 cel/mL</td>
</tr>
</tbody>
</table>

**Figure 5** Morphotypes observed in the SBRAe: (a) Fixed ciliates; (b) Amoeba similar to Arcella; (c) Amoeba similar to Arcella and Fixed ciliate; (d) Methanosarcina-like cell; (e) Methanosarcina-like cell under fluorescence microscopy; (f) short rods
Conclusions
The system composed of SBRAn/SBRAe exhibited excellent performance in the removal of COD (94%), nitrogen (96%) and phosphorus (90%). The SBRAn presented a performance comparable to other anaerobic reactors treating domestic sewage in terms of average COD removal efficiency, attaining 70%. Complete nitrification of the anaerobic effluents in the SBRAe was obtained in the first aerobic phase of the reaction period. Denitrification and phosphorus removal occurred in RSBAe operated under alternate aerobic and anoxic conditions in the reaction phase. Both the processes occurred only when sodium acetate was added at the beginning of the anoxic phase.

Acknowledgements
The authors are grateful to FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo for the financial support given, and to CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for the scholarship given to the first author.

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