Case Study

Full-scale sequencing batch reactor (SBR) for domestic wastewater: Performance and diversity of microbial communities

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Highlights

- Real operational conditions (low dissolved oxygen) of a full-scale SBR.
- Characterization of microbial diversity in a full-scale SBR to improve operational conditions by molecular techniques.
- The greater activity found by DPAO microorganism (DPAO/PAO was 70%) not necessarily leads to phosphorous removal.

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Abstract

This work describes the performance and microbial diversity in a sequencing batch reactor of a decentralized full-scale system for urban wastewater treatment under limited aeration. The removal efficiency was: 83% for soluble chemical oxygen demand (SCOD), 60% for N–NH4+ , 70% for total suspended solids (TSS) and 80% for volatile suspended solids (VSS). The biomass concentration had a maximum value around 8.7 gVSS L−1 for organic load rate of 0.6 gCOD L−1 d−1. The food/microorganism ratios showed average of 0.2 gCOD/gVSSd. The sludge bacterial flocs were formed an irregular arrangement with organisms attached such as Euglypha sp. and pedunculate ciliates. It was observed the presence of Bacteria domains including Nitrosomonas spp., Nitrobacter spp., Nitrosospira and C. ''Accumulibacter'' cluster. The DPAO activity was 70%. Denaturing gradient gel electrophoresis showed changes in ribotype number over biological treatment time among the groups observed being some are linked to nutrient removal. The reactor showed viability to treat domestic wastewater.

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1. Introduction

Domestic wastewater contains considerable amounts of compounds including nutrients and organic matter and can intensify water eutrophication if not adequately treated before final disposal. Today, the sequencing batch reactor (SBR) is perhaps the most promising and viable of proposed activated sludge modifications made for the removal of organic carbon and nutrients. In a relatively short period, it has become an increasingly popular treatment for domestic and industrial wastewaters and known as an effective biological treatment system due to its simplicity and flexibility of operation (Artan and Orhon, 2005). Their operation is well documented (Wilderer et al., 2001; Zeng et al., 2011). However, most of these studies were carried out in a laboratory and on a pilot scale with only a few full-scale studies were performed (Cabezas et al., 2009; Zhang et al., 2011).

The SBR has been applied in decentralized systems, particularly those located in rapidly growing regions such as the south of Brazil. These areas are facing increasingly rigorous standards for effluent discharge due to limited regional environmental capacities of receiving water (Costa et al., 2008). On the other hand, SBRs are often applied to biological nutrient removal processes, frequently with a sequence of anaerobic/anoxic/aerobic conditions for carbon, nitrogen and phosphorus removal (Littleton et al., 2003).

One method of nitrogen removal during wastewater treatment is through the two-step oxidation of ammonia to nitrate via microbial-mediated nitrification. Ammonia oxidation to nitrate occurs in the first stage by the action of two chemolithotrophic bacteria groups: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (Dionisi et al., 2002). The process is often combined with denitrification (reduction of nitrate/nitrite to nitrogen-N2), which is carried out by anoxic heterotrophic bacteria such as Bacillus, Alcaligenes, Pseudomonas, Methylobacterium (Dytczak et al., 2008).

The heterotrophic nitrification and aerobic denitrification processes can occur simultaneously (simultaneous nitrification and denitrification, SND) in a single reactor under reduced aeration. This is due to the occurrence of anoxic/aerobic micro zones inside the floc, or the presence of anoxic/aerobic macro zones within the
biological reactor but also can be due to the presence of new types of microorganisms (Ju et al., 2007). Furthermore, some nitrifying bacterial groups such as Nitrosomonas europaea and N. eutropha as well as Pseudomonas aeruginosa, also perform aerobic denitrification under conditions of low dissolved oxygen concentrations (Chen et al., 2003).

Biological phosphorus removal depends on the uptake of excessive phosphorus by phosphorus-accumulating organisms (PAOs) and this is usually achieved by alternating anaerobic, aerobic and sometimes anoxic conditions (those without oxygen but containing nitrate as an electron acceptor) with biodegradable organic carbon available under anaerobic conditions (Wu et al., 2009). Although some uncertainty remains regarding PAO metabolism, it is widely accepted that PAOs gain a competitive advantage over other organisms through their ability to anaerobically take up volatile fatty acids (particularly acetic acid) and store them as PHAs (particularly poly-β-hydroxybutyrate or PHB) for later processing in anoxic or aerobic conditions (Schuler and Xiao, 2008).

The wastewater purification in SBRs can contain a quite diverse microbial community. The question of how the diversity and the dynamics of the communities contribute to SBR stability is an uncertain and evolving issue. The knowledge of the microbial ecology involved in wastewater purification becomes relevant for the control and efficiency of treatment since it allows for the evaluation of the microorganisms interaction with the environment and other organisms.

A combination of polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) based 16S rDNA analysis associated with fluorescence in situ hybridization (FISH) results are widely used to describe bacterial community composition and, sometimes, to describe the physiological state of cells. These methods are frequently used for molecular fingerprinting and allow the characterization of the assemblage diversity by subsequent DNA sequencing (Pholchan et al., 2010).

A few studies were conducted on full-scale SBR in many cases when operational difficulties appeared due to complexity of control. This paper presents real operational conditions of a full-scale SBR when operational difficulties appeared due to complexity of control. This paper presents real operational conditions of a full-scale SBR.

2. Methods

2.1. Sequencing batch reactor and operational conditions

A full-scale sequencing batch reactor (SBR), fed with domestic wastewater, was used for this work. This reactor is installed in a residential condominum, located in the city of Florianópolis, in the state of Santa Catarina, south of Brazil (27°33′58″ S–48°30′58″ W). The total volume was 247 m³ with an average working volume of 155 m³. The reactor was operated for 180 days under anoxic-aerobic conditions and each cycle had an average duration of 8 h, included feeding for 1 h, aeration for 3 h, settling for 3 h and effluent withdrawal for 1 h. Throughout the operational period, the mean dissolved oxygen (DO) concentration was 0.3 mg L⁻¹. The sludge retention time (SRT) in SBR was about 107 days. The applied loads into the SBR are shown in Table 1. The ratio between C:N:P was 17:6:1.

2.2. Physicochemical analysis

Ammonium (NH₄⁺–N), nitrite (NO₂–N), nitrate (NO₃⁻–N), total kjeldahl nitrogen (TKN), total nitrogen (TN), soluble chemical oxygen demand (SCOD), phosphate (PO₄³⁻–P), alkalinity (mg CaCO₃ L⁻¹), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to APHA (2005). The temperature, pH and DO were measured in situ using a multiparameter probe (YSI 6600 V2).

2.3. Microscope identification

Bacteria were identified to the genera level using an optical microscope (Olympus – BX41), an inverted microscope (Bioval – XDS-1). Scanning electron microscope (SEM) was made according Kunkel (2008). The slides were examined with a Philips XL30 microscope and a golden cover was made by Sputter Coater Bal-Tec SCD 005.

2.4. Phosphorus uptake batch tests

Activated sludge samples obtained from the SBR were analyzed in batch tests in order to determine the phosphorus uptake and release rates of the PAOs and DPAOs present as per Monclús et al., 2010. Mixed liquor (2 L) containing active biomass from the SBR was incubated anaerobically in the presence of sodium acetate (0.2 g L⁻¹ of CH₃COONa) for 3.5 h. Subsequently the incubations of anaerobic sludge were divided into two parts. One part of the sludge was exposed to aerobic conditions, and the other was exposed to anoxic conditions by the addition of nitrate to a final concentration of 20 mg N–NO₃⁻ L⁻¹. The phosphate uptake rates (PUR) were estimated from the linear regression of phosphorus concentrations.

The ratio of anoxic PUR to aerobic PUR (anoxic/aerobic PUR ratio) was used as an index reflecting the fraction of DPAOs.

2.5. Bacteria identification by molecular methods

2.5.1. DNA extraction and PCR amplification

Samples were collected monthly from SBR during aeration phases and subsequently, DNA extractions, sequencing and phylogenetic analysis were performed. DNA extractions were completed using QIAamp DNA stool mini kit (QIAGEN), following the manufacturer’s recommendations. The extracted DNA was evaluated on 1% (wt/vol) agarose gel and stored at −20 °C until further use. Around 10 ng of genomic DNA was used as a template for PCR amplification and the universal primers used for the bacterial domain of the 16S rRNA gene are as follows: 358F (containing 51-bp GC clamp) – 5′ CGC CCG CGG CGG GCC GGC GGG GGA CGA CGG GGC GCC TAC GGG CAG CAG CAG C 3′ + 517R – 5′ ATACGCCGGTCTGGCAG 3′. The DNA was amplified by PCR as described by Viancelli et al. (2011). PCR products of 16S rRNA were quantified in a 0.8% (wt/vol) agarose gel.

2.5.2. Denaturing gradient gel electrophoresis (DGGE)

DGGE was performed using the Bio-RadDCode system (Bio-Rad, Richmond, USA). For 16S rRNA, electrophoresis was run in 1-mm-thick gels containing 8% polyacrylamide and the denaturing gradients of the gels varied from 25% to 75%. Gels were submerged in

<table>
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<tr>
<td>NH₄⁺–N</td>
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<td>TKN</td>
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<td>PO₄³⁻–P</td>
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1 × TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA, pH 7.4) and electrophoresis of 16S rRNA PCR products lasted for 5 h at a constant voltage of 130 V and a temperature of 60 °C. After electrophoresis, the gels were stained in the dark for 20 min in a 5-mL 1 × TAE solution containing ethidium bromide nucleic acid stain. Gels were visualized in a transilluminator and the gel images were captured with the Kodak molecular imaging software v.5.0.0.90. Individual 16S rRNA bands from DGGE gels were excised using sterile tips, eluted in sterile deionized water, and stored overnight at 4 °C. The same PCR programs as previously described were used for DNA re-amplification with non-GC-clamped primers, in which 10 µL of the DNA from the DGGE band was used as template. ACT-Gene molecular analysis (Brazil) carried out DNA sequencing analysis. Sequence determination was performed in an automatic sequencer ABI-PRISM 3100 genetic analyzer. The quality of DNA sequences was checked and the overlapping fragments were assembled using BioEdit 7.0.5. The phylogenetic relationships between these sequences were determined with MEGA 5.0 and neighbor-joining trees were constructed from Kimura-2 parameters and bootstrap values using 250 replications. Cluster analysis, displayed as a dendrogram, was done using Gel Compar II version 6.5 with binary data (presence/absence of bands) and bootstrap calculations over 1000 iterations.

2.5.3. Fluorescence in situ hybridization (FISH) analysis

FISH analyses were performed essentially as described by Amann et al. (1990). Samples were fixed in 4% paraformaldehyde-phosphate-buffered saline and placed on 0.1% gelatin and 0.01% KCr (SO₄)₂ gelatin-coated glass slides. For bacteria identification, probes were used according to Table in Supplementary material and details on oligonucleotide probes are available at probeBase. All microbial cells were detected by staining with 1% 4,6-diamidino-2-phenylindol (DAPI). The slides were examined with an Olympus BX41 microscope. All samples were analyzed against DAPI (considered 100%).

3. Results and discussion

3.1. Organic matter and nutrients removal

The reactor showed satisfactory removal efficiency for the analyzed parameters, producing an effluent that fits the quality standards established at Brazilian legislation. Throughout all monitoring period, the reactor has operated at low dissolved oxygen concentrations with an average of 0.3 mg L⁻¹, that has not affected, however, their performance.

Organic matter has a fundamental influence on biological community composition and at the trophic base that sustains the ecosystem in the reactor. The average removal of SCOD was 83% as shown in Fig. 1A. The removal efficiency was below the average on days 22, 63 and 113, with values of 72%, 65% and 66%, respectively. Although the efficiency for these days are low compared to other days, the removal showed satisfactory performance when observed with the applied Organic Loading Rate (OLR). Low SCOD concentration was founded in day 32 and 99, with influent values of 125 and 102 and effluent values of 6 and 22 mg L⁻¹ (Fig. 1B).

Some studies using SBR (Artan and Orhon, 2005; Denecke et al., 2012) presented COD removal efficiency above 90%, showing that SBR has an increased ability to remove organic carbon and a better resistance against variable loadings, which suggests this reactor is able to guarantee process stability. The removal efficiency of TSS and VSS were 70% and 80%, respectively.

Total kjeldahl nitrogen (TKN) removal efficiency was around 60% but levels changed due to influent fluctuations. Basically, almost all TKN was found as NH₄⁺ (Fig. 2A and B). During day 43, a higher TKN influent concentration (102 mg L⁻¹) was noted and
the removal efficiency was 75%. The average ammonium nitrogen removal was 61% and concentrations were 45.9 and 17.6 mg L\(^{-1}\) for the influent and effluent, respectively (Fig. 2B).

The average total nitrogen (TKN + NO\(_x\)-N) removal was 50% which was affected by low oxygen concentrations. According to Sliekers et al. (2005), low DO concentrations inhibit autotrophic nitrifying bacteria development, which leaves the role of oxidizing ammonium to the nitrifying heterotrophs.

The alkalinity was not a limiting factor for nitrification since the consumption of alkalinity in the reactor did not exceed 7.14 mg CaCO\(_3\)/mg N–NH\(_4\)+oxidized, according to Van Haandel and Marais (1999). The pH was maintained within a range of neutrality (mean of 6.7), without ammonium volatilization according to the same authors.

In the present study, the C/N ratio was 3 g-COD/g-N. Bassin et al. (2012) used C/N ratios ranging from 1.14 to 2.29 in a sequencing batch reactor (laboratory scale) and obtained efficiency above 95% for ammonium removal. The C/N ratio of domestic wastewaters is, however, often lower than these prescribed values, and nitrogen removal is limited by the lack of available organic carbon source (Ryu and Lee, 2009). For Sun et al. (2010), the shortcut nitrification followed by denitrification of NO\(_2\) reduced approximately 40% of carbon requirements, 25% of the oxygen supply, and resulted in 40% less biomass production compared to oxidation and denitrification of NO\(_3\). The reductions in the COD requirement by this pathway make nitrogen removal from low C/N ratio domestic wastewater feasible.

Phosphate concentration did not have a clear trend probably due to the changes in the inlet phosphate concentrations (Fig. 3A). Low concentrations were found when the withdrawal period was made. On day 85, the concentrations of influent and effluent were the same, indicating that removal had not occurred for this period. This probably happened because DO concentrations were low, therefore, inhibiting PAO activity. However, for most monitoring days, it was observed that the effluent concentration was always less than the influent showing an average removal efficiency of 50%.

### 3.2. Biomass properties

The values of TSS for mixed liquor varied between 1.2 g L\(^{-1}\) (day 36) and 10 g L\(^{-1}\) (day 57) (Fig. 4A). The VSS/TSS ratio during the whole operating period was about 70%. The maximum value (8.7 g VSS L\(^{-1}\)) was obtained when the OLR was 0.6 g COD L\(^{-1}\) d\(^{-1}\) and an average sludge retention time occurred for 77 days until the end of operation. On day 22, due to a withdrawal of excess sludge, the concentration of TSS and VSS effluent was higher. This resulted in a low concentration of solids and other compounds analyzed in the reactor. The food/microorganisms (F/M) ratio increased with decreasing VSS concentration, ranging between 0.07 and 0.48 g COD/g VSS d (Fig. 4B) and average of 0.2 g COD/g VSS d, which is below the standard range of 0.25–0.50 g COD/g VSS d (van Haandel and Marais, 1999).

### 3.3. Microbial communities analysis

Optical microscopy analysis showed a highly concentrated sludge formed by compact flocs that were composed of suspended matter and particulates rich in organic matter and had little presence of filamentous bacteria, which does not interfere with sedimentation.

The zooplankton was composed of ciliated protozoa in greater frequency (attached and crawling ciliates) and naked amoebae and/or testate and rotifers were observed for a longer period of time. Among the recurring groups in this system, it is cited: Euglypha sp., Arcella sp., Vorticella sp., Epistylis sp., Rotatoria sp., Tocophyra sp. and numerous crawling ciliates. Zooflagellates were present in a few samples and its occurrence indicates unstable

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**Fig. 3.** (A) P-PO\(_4\)\(^{3-}\) concentration in the influent and effluent; (B) Phosphorous uptake during anaerobic/aerobic batch assay with the activated sludge from the SBR (VSS = 2.5 and 2.8 g L\(^{-1}\), respectively).

**Fig. 4.** (A) Solids concentration in the reactor and effluent; (B) ratio F/M (gCOD/gVSS d).
operation conditions, poorly aerated sludge, overload in the discharge form, or early station operation (Warren et al., 2010).

The metazoans (ciliates and amoebae) are a heterogeneous group of organisms, which are attached to flocs or are free-living and dispersed among the particles of activated sludge. These organisms play an important role in this system since they feed mainly on bacteria, as well as organic substances and other small organisms. Through their feeding behavior, they maintain bacterial population densities and rejuvenate the bacteria population by predation. They also contribute to the flocculation process by being responsible for an improvement in the quality of the effluent (Zhou et al., 2008). The naked amoebae have higher growth rates when compared to ciliates and rotifers. The ciliates are faster and can get food with greater ease while the rotifers are the slowest.

The scanning electron microscope (SEM) images were made to enhance the understanding of sludge microbial composition. SEM imaging indicated bacterial flocs were well formed with most cells in the form of cocci that form an irregular arrangement. The analysis also identifies zooplanktonic organisms attached to sludge such as pedunculate ciliates and Euglypha tuberculata. According to Canler et al. (1999), the presence of Euglypha tuberculata indicates an operating system with or without nitrification, where there is low load or extended or sufficient aeration. The densities of these organisms decrease when the water quality deteriorates. The importance of ciliates in the purifying process in wastewater treatment is because they feed off dispersed bacteria and eliminate them. Thus, the presence of ciliates can also be taken as indicators of good effluent quality (Samaras et al., 2009).

3.4. FISH analysis and PAO/DPAO activity

The FISH technique was applied to identify some bacterial genera involved in nitrogen and phosphorus metabolism occurring in the reactor. A large amount (85% of DAPI-stained cells) of the bacterial domain were observed, which indicates high metabolic activity of bacteria present in biological treatment systems. Mostly colonial shaped cocci and a few filamentous bacteria formed the bacterial community. The signals obtained from all oligonucleotide probes were strong and indicated high cellular rRNA contents. Nitrifying bacteria were found in different percentages including 25% for beta subclass of Proteobacteria (15% of the DAPI-stained cells for Nitrosomonas spp.), 10% for alpha subclass Proteobacteria (5% of DAPI-stained cells for Nitrobacter spp.) and 20% for Nitrosospira spp. According to Denecke et al. (2012), although the concentration of nitrite in the reactor was particularly low and nitrite oxidizers of the genus Nitrosospira, were uncultured, the concentration of nitrite may be sufficient for the growth of Nitrosospira. Members of the genera Nitrosomomas and Nitrosospira are the dominant nitrifiers in activated sludge plants, and only a small ratio of Nitrobacter spp. exists in wastewater treatment plants.

The presence of sulfate-reducing bacteria (SRB) groups can be explained by previous research, which showed that dissimilatory sulfate reduction can occur in the presence of O2. This challenges the conventional view that sulfate reduction is a strictly anaerobic process (Canfield and DesMarais, 1991). Desulfovibrionaceae was found in rare quantities (less than 5% of DAPI-stained cells).

Members of polyphosphate-accumulating organisms (Candidatus “Accumulibacter” cluster) were found (25% of DAPI-stained cells). The development of poly-P accumulating organisms (PAOs) was favored in SBRs due to their competitive advantages over non-poly-P accumulating microorganisms to survive during starvation periods. This is characteristic of SBR, which presents low F/M ratios, such as the studied reactor.

Fig. 3B summarizes the evolution of PAO/DPAO activity. The test showed that under anaerobic conditions phosphorous release occurs to the media (PAO and DPAO) and in aerobic phase (PAO) the uptake was bigger than the anoxic phase (DPAO). The percent of PAO activity (DPAO/PAO ratio) was 70% (Table 2). Monclús et al. (2010) found the ratio DPAO/PAO P uptake rate, after 150 days (13.6 mg P g−1 VSS h−1), was relatively constant throughout the experimental operation at 41%.

Therefore DPAOs values found in this study were higher than expected, which may justify the low levels of nitrates found in the effluent, because nitrate produced during the nitrification process has been simultaneously consumed by this group of organisms.

This fact coupled with low denitrifying organisms occurrence, as discussed in the FISH analysis, suggests that much of the nitrate removal was due to the action of DPAOs. This information may help in operating procedures since it is enough to feed the reactor once per cycle, to supply substrate for denitrifying biomass.

3.5. DGGE based sequencing and phylogenetic analysis

The microbial community composition of the SBR was investigated through PCR-DGGE analysis. Fig. 5 shows the typical DGGE

![Fig. 5. DGGE gel banding profile of microbial community of the sequencing batch reactor over the days. The cluster analysis showing the similarities between different DGGE fingerprints is displayed graphically as a dendrogram.](image)
fingerprints of the reactor monitored over days. In general, DGGE analysis showed that there were changes in band profile over biological treatment time as well as variations in band intensity (e.g., samples 15–150, band 1).

Only the first bands (1, 2 and 4) were present in all lanes, showing variation in their intensity. Band 1 showed increased intensity for lanes 15–90 while it decreased in lanes 115–150. The other bands that follow (3 and 5–16) appear at different times and are not necessarily present throughout the monitored period, which indicates changes in the microbial composition of the reactor. Therefore, we suggest that the influent significantly affects the microbial composition of the reactor. The viability of a full-scale SBR to treat domestic wastewater, with variation of the applied loads and low C/N ratio (3 g-COD/g-N) was studied. It was verified the significant changes of microbial composition in band profile over time due to considerable variations in the influent quality. This reinforces the idea that SBR can adapt to significant changes in nutrient concentrations through gradual acclimation of biomass, which ensures process stability even in a system where F/M ratios are low. Furthermore, the low aeration over time in the reactor could explain the greater activity found by DPAO microorganism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.01.027.

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1 Uncultured bacterium clone F1Q32TO04EN7LK 16S ribosomal RNA gene
2 Uncultured Chlorobi bacterium clone OMBR76 16S ribosomal RNA gene
3 Uncultured soil bacterium clone S084 16S ribosomal RNA gene, partial sequence
4 Uncultured beta proteobacterium SBR1011
5 Uncultured bacterium clone N1.2_4797 16S ribosomal RNA gene
6 Uncultured bacterium clone J3_2_777 16S ribosomal RNA gene
7 Uncultured bacterium clone F1Q32T005GHG5F 16S ribosomal RNA gene
8 Uncultured bacterium clone F1Q32T004EN7L1K 16S ribosomal RNA gene

Table 3 NCBI BLAST search results of sequences from DGGE bands.
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