Investigation of full-scale step-fed SBR under low dissolved oxygen: performance and microbial community response

Heloísa Fernandes, Regina Vasconcellos Antonio and Rejane Helena Ribeiro da Costa

ABSTRACT

A decentralized full-scale sequencing batch reactor (SBR) system for treating wastewater was operated to assess their feasibility and the response of bacterial population dynamic and nutrient removal performance. The reactor was operated under low dissolved oxygen (DO) concentration (0.3–0.7 mgL^{-1}) and an average applied organic load of 0.5 g COD L^{-1} d^{-1} (COD: chemical oxygen demand). Removal efficiencies were higher than 70% for both soluble chemical oxygen demand and ammonium, with average effluent concentration of 51 ± 15 mg COD L^{-1} and 16.0 mg NH_4^+ L^{-1}. The mixed liquor volatile suspended solids/total suspended solids ratio was 0.9, and the average food/microorganism ratio was 0.3 g COD g VSS^{-1} d^{-1} (VSS: volatile suspended solids). The active biomass was composed of 94.9% heterotrophic and 5.1% autotrophic organisms. The most frequently identified were chemoorganoheterotrophic organisms affiliated with Bacteroidetes and Firmicutes, some of them with the capacity to denitrify and grow under low DO concentration. Temperature and sludge withdrawal were important factors in determining nitrification and phosphorus removal rates. The SBR was viable for domestic wastewater treatment and showed that the microbial community greatly influenced its performance. This work can also provide valuable insights into further applications in systems operated under low DO condition.

Key words | active biomass, decentralized system, molecular techniques, sequencing batch reactor, treatment, wastewater

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INTRODUCTION

Several developing countries have adopted new strategies for managing and conserving natural resources due to their rapid population growth and economic development in the past few years. Many of these communities have no wastewater collection or sewage systems, and, consequently, require effective decentralized treatment systems.

Decentralized wastewater management covers the collection, treatment, and disposal or reuse of treated wastewater at or close to the source of generation (USEPA 2010; Suriyachan *et al.* 2012). Significant attention has been focused on these systems, and some advantages, such as their reduced capital investment, low pumping costs, and minimum energy consumption have already been linked to their recurrent use (Devi & Dahiya 2008). Furthermore, for scattered buildings and residential areas without a centralized system, on-site treatment of domestic sewage in a decentralized system not only would avoid dealing with costly centralized systems, but it allows the sewage owner to reuse the treated effluents (Jorsaraei *et al.* 2014).

Several options are available for on-site wastewater treatment (e.g., wetland, activated sludge, biofilter), and one of these alternatives, the sequencing batch reactor (SBR), is a viable decentralized system, particularly for rapidly growing regions. Additionally, SBR can be easily engineered to provide

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highly effective nitrogen and phosphorus removal (Artan & Orhon 2005). The basic operating phases of SBRs are well-documented and consist of a cyclic operation of the following steps: filling, reaction, settling, drawing, and idle (Shaw *et al.* 2009). However, the effectiveness of the decentralized approach depends on establishing a management program that assures regular system inspection and maintenance.

Due to nutrient discharge regulations, SBR systems have been modified to achieve nutrient removal by adjusting the reaction cycle steps. Accumulated experience has also indicated that the intrinsic operation flexibility of SBRs allows them to act as an alternative to continuous-flow systems for nutrient removal. As stated above, in addition, SBRs can be easily engineered to provide highly effective nitrogen and phosphorus removal (Wilderer *et al.* 2001).

In the last decade, studies have also shown that decreasing the amount of oxygen required for nitrogen removal resulted in economic savings. If low dissolved oxygen (DO) concentration is applied, both nitrification and denitrification can occur under identical operating conditions by simultaneous nitrification and denitrification (SND). The efficiency of SND is dependent on three factors: the oxygen concentration (<2 mg L^{-1}), the floc size, and the availability of sufficient organic carbon substrate (Third *et al.* 2005). Low DO concentration (<2 mgL^{-1}) and large flocs are essential for optimal SND (Pochana & Keller 1999).

SBR/SND is an attractive alternative technology for N-removal due to its potential to eliminate the need for separate tanks as required in conventional treatment plants, resulting in a simplified and smaller plant design. Under these circumstances, SBR/SND has emerged as an efficient process (Third *et al.* 2005; Jia *et al.* 2012).

However, evaluating microorganisms' interaction with the environment and other organisms is still important for understanding the active mechanism behind the SND treatment process (Hesham *et al.* 2011).

Molecular biology tools have helped to elucidate this microbial ecology through the analysis of their 16S rRNA and rDNA using fluorescence *in situ* hybridization (FISH), polymerase chain reaction (PCR) amplification, and denaturing gradient gel electrophoresis (DGGE) for wastewater treatment systems (Zhu & Chen 2011; Aydin *et al.* 2015). These methods allow the characterization of microbial diversity via molecular fingerprinting and DNA sequencing without the need to cultivate the different microbial strains. PCR-DGGE is cheap and fast when compared to other methods and can be used to compare microbial community structure changes at different system stages. Additionally, FISH is highly effective for detecting specific bacteria and analyzing complex microbial communities and also offers the advantage of microscopically observing the physical relationships among different cells (Rittmann 2002; Seviour 2010).

The purpose of this study was to investigate the performance of a decentralized full-scale step-fed SBR fed with domestic wastewater and operated under low DO. The step-fed system allows a good management of the reactor for nitrogen and phosphorous removal, while the low DO enables saving energy costs. In parallel, molecular techniques were used to evaluate nutrient removal and identify bacterial community structures throughout the process, in order to determine the influence of microbial communities' changes on the system efficiency and also how these different factors could improve the effluent quality.

MATERIALS AND METHODS

Full-scale treatment system

The full-scale treatment system was designed to support 440 people, with a flow of 119 L/capita.d, and consisted of an equalization tank (29.8 m^3), a secondary treatment (SBR), and a disinfection contact tank (13.5 m^3). The system was fully automated.

Step-fed sequencing batch reactor

The reactor has a total volume of 76.7 m^3 with an average working volume of 62 m^3. There were large variations in the characteristics and volume of the influent stored in the equalization tank due to rush hour, seasonality, and the flow of wastewater. Different cycles were monitored weekly for 192 days and cycle profiles of chemical oxygen demand (COD), ammonia, nitrite, and nitrate are provided. During the monitored period, the average cycle duration was 8 hours which included step-fed (2 or 3×15 min), anoxic reaction (3×1 h), oxic reaction (3×1 h), settling (1 h), and effluent withdrawal

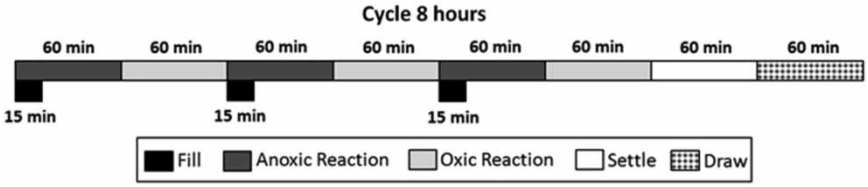

Figure 1 | Operational scheme of SBR cycle.

(1 h) (Figure 1). The total filling volume per cycle was 29 m^3. The sludge retention time (SRT) was not controlled and sludge discharge occurred when the reactor's mixed liquor volatile suspended solids (MLVSS) concentration reached 5 g L^{-1} (days 44 and 85). The sludge was disposed of into a controlled landfill.

Sampling and monitoring

Samples of raw wastewater from the equalization tank were collected at the central point at 30 cm depth, weekly, before the start of each cycle period. To monitor the microbial profile, reactor samples were collected from mixed liquor. At the end of the settle period, a sample representing the treated effluent was taken at the surface 5 min before the effluent draw. For each point sampled, temperature, pH, and DO were measured *in situ* using a multiparameter probe (YSI 6600V2). In order to evaluate the efficiency of the reactor, physicochemical analyses of soluble and total chemical oxygen demand (SCOD and TCOD), ammonium (NH_4^+-N), total Kjeldahl nitrogen (TKN), total and volatile suspended solids (TSS and VSS), and alkalinity were conducted according to APHA *et al.* (2005). Also, nitrite (NO_2-N), nitrate (NO_3^--N), and phosphate (PO_4^{3-}-P) were determined by ionic chromatography (DIONEX DX-120). Sludge volume index (SVI) was determined according to Metcalf & Eddy (2003).

Microorganism identification

The genera of Protozoa and Metazoa were identified using an optical microscope (Olympus BX41) and an inverted microscope (Bioval XDS-1). Taxonomic identifications were determined as described by Canler *et al.* (1999).

Oxygen uptake rate and active biomass determination

The oxygen uptake rate (OUR) was measured according to Ochoa *et al.* (2002), in suspended biomass, using a closed respirometric cell. Three different conditions were used: (1) endogenous OUR, (2) oxygen consumption during nitrification of ammonia without a carbon source, and (3) exogenous respiration with a carbon source in the presence of Allylthiourea (nitrification inhibitor). The active heterotrophic (gX_H–$CODL^{-1}$) and autotrophic biomasses (gX_A–$CODL^{-1}$) were calculated according to Activated Sludge Model No.1 (ASM1) (Henze *et al.* 1987) using Equations (1) and (2), respectively. The kinetic parameters are constant, as described in the model: $\mu_{Hmax} = 6\ d^{-1}$; $\mu_{Amax} = 0.8\ d^{-1}$; $Y_H = 0.67$ g cell COD formed (g COD oxidized)$^{-1}$, and $Y_A = 0.24$ g cell COD formed (g N oxidized)$^{-1}$. The assays were realized at room temperature and the temperature correction factor was applied to the values of kinetic parameters (Ochoa *et al.* 2002).

$$X_H = \frac{1}{\mu_{H\max}} \frac{Y_H}{1 - Y_H} (OUR)_{H\max} \quad (1)$$

$$X_A = \frac{1}{\mu_{A\max}} \frac{Y_A}{4,57 - Y_A} (OUR)_{A\max} \quad (2)$$

Bacteria identification by molecular methods

DNA extraction and PCR amplification

Samples were collected every 2 weeks from the SBR. DNA extractions, sequencing, and phylogenetic analysis were subsequently performed. DNA extractions were completed using the QIAamp DNA Stool Mini Kit (QIAGEN),

according to the manufacturer's recommendations. The quality of extracted DNA was evaluated on 1% (wt/vol) agarose gels and stored at −20 °C until further use. Around 10 ng of genomic DNA was used as a template for PCR amplification. The universal primers used for amplification of the bacterial 16S rRNA gene are as follows: 358F (containing a 51-bp GC clamp) and 517R. The DNA was amplified by PCR as described by Viancelli *et al.* (2011). PCR products of 16S rRNA were quantified on 0.8% (wt/vol) agarose gels.

Denaturing gradient gel electrophoresis

DGGE was performed according to Fernandes *et al.* (2013) in a parallel study using another SBR reactor. The gels were visualized with a transilluminator, and the images were captured with the Kodak Molecular Imaging Software, v.5.0.0.90. Individual bands from DGGE gels were excised using sterile tips, eluted in sterile deionized water, and stored overnight at 4 °C. The same PCR programs described above were used for DNA re-amplification but with non-GC-clamped primers, and 10 μL of DNA from the DGGE band was used as templates. ACTGene Molecular Analysis (Brazil) performed the DNA sequencing analysis. Sequence determination was conducted using an automatic sequencer ABI-PRISM 3100 Genetic Analyzer. The quality of DNA sequences was checked, and overlapping fragments were assembled using BioEdit 7.0.5. DGGE fingerprints were automatically scored by the presence or absence of co-migrating bands, independent of intensity using GelCompar II, 6.5. Pairwise community similarities were quantified using the Dice index which ranges from 0 (no common bands) to 1 (identical band patterns). Cluster analyses displayed as a dendrogram were performed using the complete linkage method with arithmetic averages (UPGMA). Binary sequences were generated for individual DGGE lanes by determining the number and position of bands compared to the total number of band positions detected.

Fluorescence *in situ* hybridization analysis

FISH analyses were performed as described by Amann *et al.* (1990). The probes used for bacterial identification were EUB_{mix} (most bacteria Planctomycetales and Verrucomicrobiales), PAO_{mix} (*Candidatus* 'Accumulibacter' cluster), THIO (*Thiobacillus*), DSV 407 (*Desulfovibrionaceae*), Ntspa (Nitrospirae), NSO 190 (ammonia oxidizers β-Proteobacteria), NEU (*Nitrosomonas* sp.), and NIT 3 (*Nitrobacter* sp.). Probe details are available at probeBase. Microbial cells were detected by staining with 1% 4,6-diamidino-2-phenylindol (DAPI). Slides were then examined using an Olympus BX41 microscope, and all samples were analyzed against the DAPI staining which was considered 100% of overall population.

RESULTS AND DISCUSSION

Organic matter and nutrient removal

The applied loads in the SBR were 1.14 ± 0.96 g TCOD $L^{-1}d^{-1}$ and 0.07 ± 0.01 g NH_4^+-N $L^{-1}d^{-1}$. The ratio C (TCOD):N (TKN):P (TP) was 150:12:1.7. Throughout the operational period, the mean DO concentrations during anoxic and oxic reactions were 0.1 mg L^{-1} and 0.7 mg L^{-1}, respectively.

The reactor showed satisfactory removal efficiency for the analyzed parameters even when operated at low DO concentrations. The influent SCOD underwent significant variations throughout the monitored cycles. The average SCOD removal was $76 \pm 14\%$ (Figure 2(a)), with effluent average value of 51 ± 15 mg L^{-1} in accordance with Brazilian legislation resolution CONAMA 430/11, which recommends 120 mg BOD L^{-1} (BOD: biochemical oxygen demand). Low effluent SCOD concentrations (<75 mgL^{-1}) were also obtained in other studies using SBR reactors (Artan & Orhon 2005; Costa *et al.* 2013; Chen *et al.* 2015). The average influent pH was 7.2 ± 0.4 and the effluent pH was 6.9 ± 0.5 (Figure 2(b)).

TKN removal efficiency was around 50% (Figure 2(c)) and effluent TKN levels were <55 mgL^{-1} and these values are influenced by the influent TSS variations. Almost all TKN was found as NH_4^+. The average ammonium nitrogen removal was 70% and concentrations were 52.6 mgL^{-1} and 16.0 mgL^{-1} for the influent and effluent, respectively (Figure 2(d)). According to Sliekers *et al.* (2005), low DO concentrations inhibit autotrophic nitrifying bacteria development.

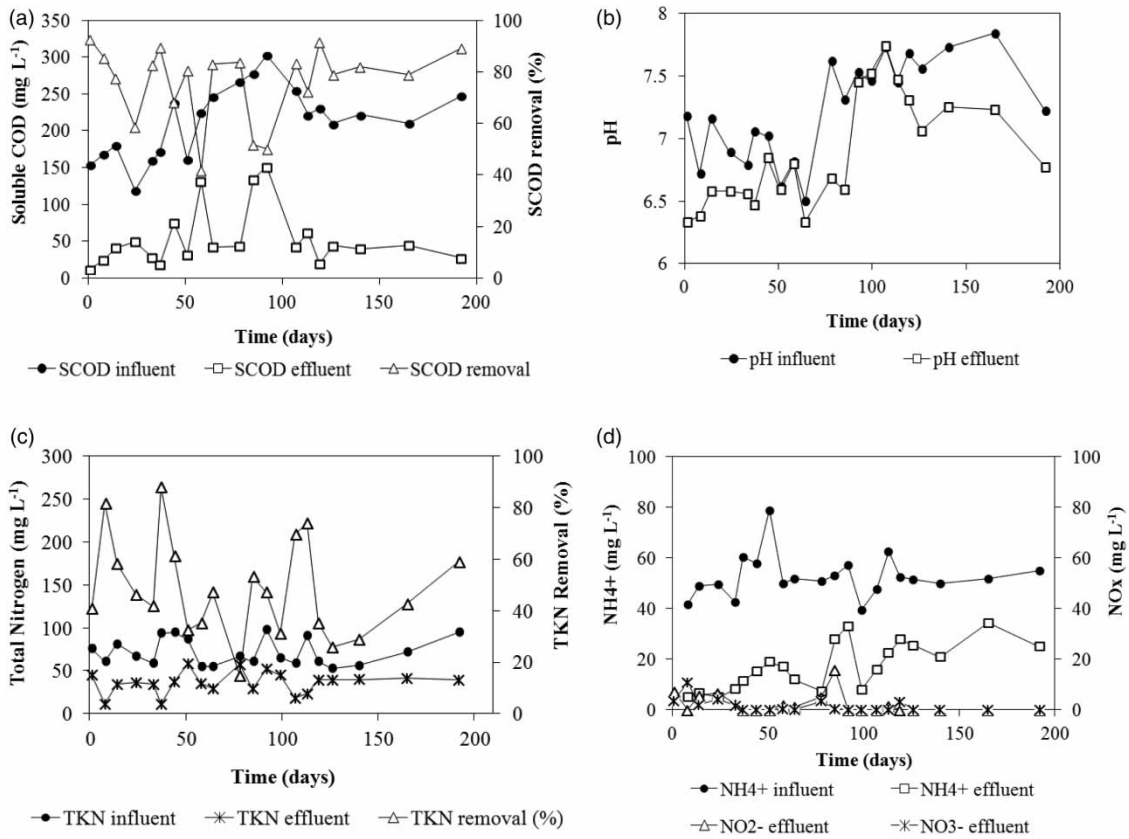

Figure 2 | (a) SCOD concentration and removal efficiency; (b) pH influent and effluent values; (c) TKN removal; and (d) different nitrogen species concentration in the SBR.

Figure 3 shows the profile during the cycle for approximately 8 hours. From Figure 3(a), it can be seen that DO in the reactor was low over the entire cycle and ranged between 0.36 and 0.11 mgL^{-1}. The profile of N-nitrogen concentration shows that the effluent ammonia nitrogen concentration was decreased from an average of 58 $mg\ L^{-1}$ in the influent to 12 mgL^{-1} in the effluent. Values of nitrite concentrations were not observed indicating that the nitrite formed was simultaneously nitrified to nitrate. Hence, the overall nitrite levels were found to be very low or almost negligible. The nitrate exhibited a low range with the average maximum values obtained of 5 mgL^{-1} at the end of the aerobic phase, indicating simultaneous aerobic denitrification to gaseous nitrogen end products. This is an indication of total nitrogen removal occurring in the SBR, with high removal of carbon (SCOD), as shown in Figure 3(b).

It is also observed that the pH values in the reactor were below 7 (Figure 3(b)), indicating that there was no removal of ammonia through volatilization. During the cycles, the pH decreased to 6.4 ± 0.2, indicating consumption of the alkalinity by the nitrification occurrence in oxic phases. During anoxic phases, the recovery of alkalinity was observed, suggesting the SND process occurrence, in accordance with Wang *et al.* (2005) and Ju *et al.* (2007).

It is important to emphasize that the low effluent nitrite (NO_2-N) and nitrate (NO_3^--N) concentration throughout the monitored period can be associated with the SND process.

For complete SND, the rate of ammonium oxidation should approximately equal the rate of denitrification. As autotrophic nitrification is generally slow in comparison to heterotrophic metabolism, SND requires a slowly degradable organic substrate to provide reducing power for denitrification during the nitrification process. Slowly degradable COD is often intrinsically available in wastewater (Third *et al.* 2005). The reactor operated in step-fed sequentially supplies the carbon required for denitrification.

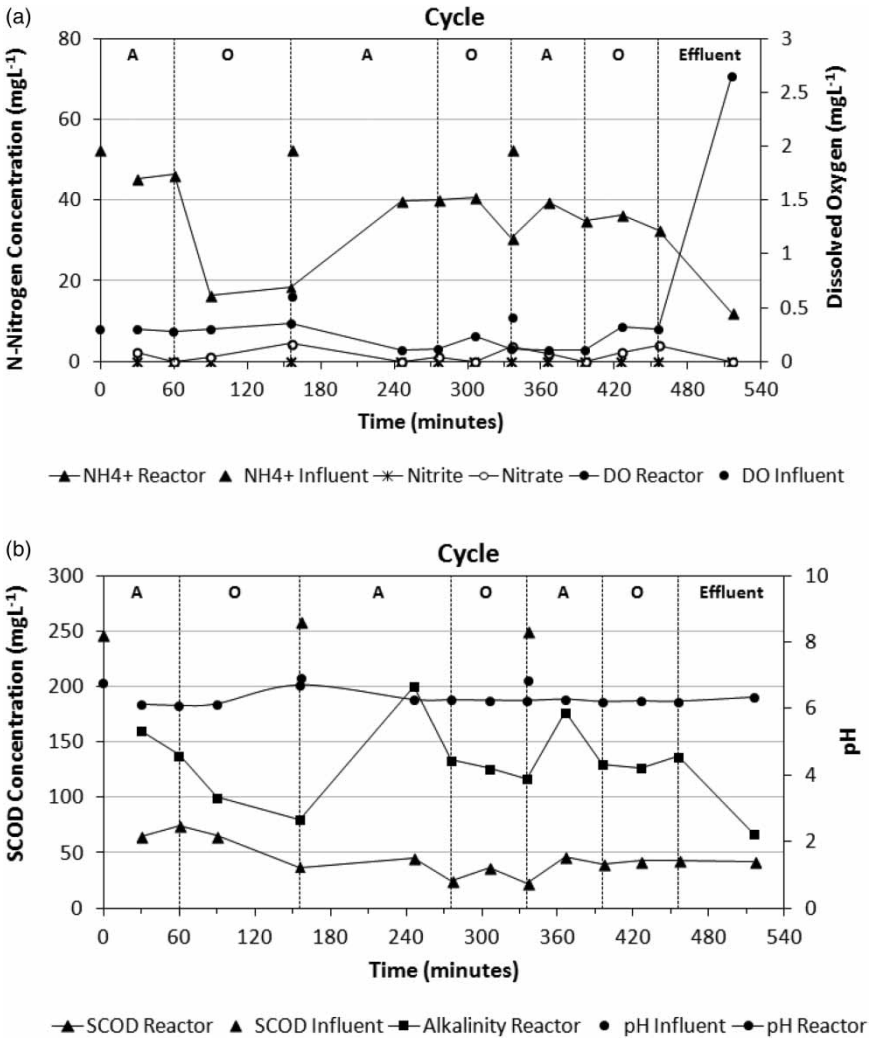

Figure 3 | (a) Profiles of N-nitrogen and DO concentration of the SBR along the cycle; (b) profiles of SCOD, alkalinity, and pH in the SBR during the cycle. 'A' represents the anoxic period and 'O' represents the oxic period.

This condition provides organic substrates to some of the heterotrophic bacteria, which use these substrates as sources of carbon and energy for denitrification.

Phosphorus concentrations are presented in Figure 4(a). Low influent concentrations were found at the start of monitoring until day 44, after which the concentrations

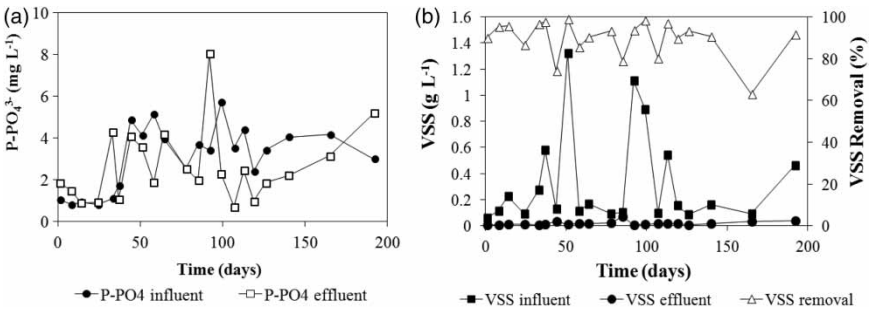

Figure 4 | (a) $P\text{-}PO_4^{3-}$ concentration and (b) VSS concentration and removal in the SBR.

increased. Biological phosphorus removal was observed only after day 99, when the ratio C:P was 60.5. During this period, the average PO_4^{3-}-P removal efficiency was 59%. According to Puig (2008), the biological phosphorus removal is negatively affected when the C:P is lower than 55. Moreover, there was a decrease in temperature after day 100 of about 7 °C (Figure 5(a)), which favors phosphorus accumulating organisms (PAOs) (Panswad *et al.* 2003; Lopez-Vazquez *et al.* 2009).

Panswad *et al.* (2003) found the highest concentration of phosphorus in the sludge by PAOs at 20 °C, decreasing with the temperature rise. According to the authors, the temperature increase favors the concentration of the glycogen accumulating organisms (GAOs) group over the PAO group. Lopez-Vazquez *et al.* (2009) concluded that PAOs at a temperature of 20 °C are dominant, while above this temperature, the GAOs tend to have greater importance. On the other hand, the influent pH also has a great importance for the

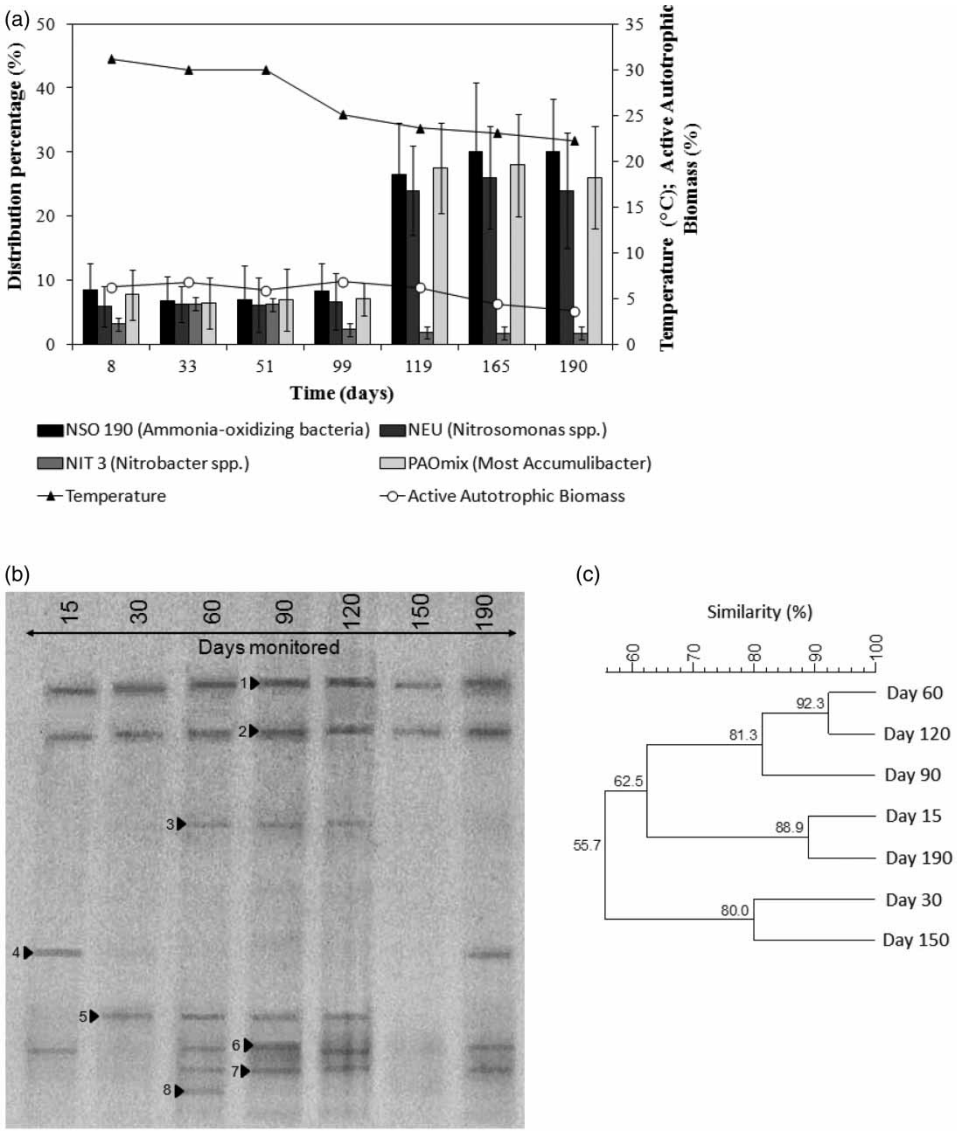

Figure 5 | (a) Bacterial distribution ratios as analyzed by FISH and its correlation with temperature (°C) and active autotrophic biomass (%) throughout the monitored period; (b) DGGE band profiles of amplified 16S rDNA fragments using the total genomic DNA extracted from mixed liquor samples of the SBR, over the days; and (c) cluster analysis showing the similarities between different DGGE fingerprints.

biological phosphorus removal. Converti *et al.* (1995) have highlighted that the biological phosphorus removal system becomes stable with pH above 7.6. It can be seen in Figure 2(b) that after day 90 the mean influent pH increased from 7.0 to 7.8.

Biomass characterization

The food/microorganisms (F/M) average ratio was 0.3 g COD (g VSSd)$^{-1}$. This value falls within the standard range of 0.25–0.50 g COD (g VSSd)$^{-1}$ (Metcalf & Eddy 2003). The VSS removal efficiency was about 91% (Figure 4(b)). The values for mixed liquor total suspended solids (MLTSS) varied between 1.0 and 9.8 gL^{-1}.

The MLVSS concentration ranged between 0.5 and 8.5 gL^{-1} and resulted in high and variable SRT (81 ± 64 days). The MLVSS/MLTSS ratio over the whole period was 0.9. Sludge disposal was only performed on days 44 and 85 when the MLVSS was 5.69 gL^{-1} and 8.5 gL^{-1}, respectively. The settling sludge properties expressed by the values of SVI showed an average of 50 ± 5 mLg^{-1}, which according to Metcalf & Eddy (2003) is considered a good/very good settling sludge. The settling characteristics of the mixed liquor solids were not affected by the low DO reactor concentration.

Microbial diversity and bacterial phylogenetic analyses

The recurring protozoa were crawling ciliates: Hypotriches (*Aspidisca* sp.) and Holotriches. Ciliate protozoa dominated the protozoan community, but a few ciliates (*Vorticella convallaria*) were also attached to flocs. Flagellate protozoa were present in a few samples. This group indicates unstable operating conditions, poorly aerated sludge, and discharge overload (Warren *et al.* 2010).

The average specific OUR for nitrifying and heterotrophic biomasses were 3.7 mg O_2 g VSS^{-1}h^{-1} and 19.5 mg O_2 g VSS^{-1}h^{-1}, respectively. The active heterotrophic biomass concentration (X_H) was 123.6 mg TCOD L^{-1} (94.9% of the global system biomass) while the active autotrophic biomass (X_A) was 5.1 mg TCOD L^{-1} (5.1% of the global system biomass), indicating that the reactor was composed mainly of heterotrophic organisms. This low percentage of autotrophic results from the influent BOD/TKN ratio, which was between 5 and 10 g BOD/g TKN. To Rittmann & McCarty (2001), in this range of values, the nitrifiers normally constitute <20% of the active biomass and are a smaller fraction of the VSS.

FISH analysis showed a large number of bacteria domains (90% of DAPI-stained cells). The bacterial community comprised mostly cocci with colonial morphology, some bacilli, and few filamentous bacteria. Figure 5(a) shows the bacterial distribution ratios of the reactor's biomass and their correlation with reactor temperature and active autotrophic biomass X_A (%). Until day 99, the distribution of each analyzed bacterium group was low, representing only 5–9% of the DAPI-stained cells. However, after day 99, there was an increase in the concentration of ammonia-oxidizing bacteria (AOB) (24% of *Nitrosomonas* genera) and PAOs (27%). A number of studies mentioned by Okabe *et al.* (2011), have demonstrated that *Nitrosomonas* is the most abundant AOB in the biomass obtained from a full-scale municipal wastewater treatment plant (WWTP), an aerated activated sludge bioreactor, a SBR, industrial and municipal waste. The authors stated that this genus predominance was due to its relatively higher growth rate than other AOB.

Although AOB concentration increased after 99 days, NH_4^+-N removal during this period was 53.6% (Figure 2(d)). In the previous period, however, the average NH_4^+-N removal reached 74.9%. The increase of AOB after 99 days can be justified by the operational process, because excess sludge withdrawal (MLVSS > 5 gL^{-1}) occurred after day 85, which may have stimulated microbial growth in the reactor by removing some sort of inhibiting substance. Moreover, the average temperature decreased approximately 7 °C during this period (23.9 °C), and resulted in negative influence on nitrifiers' activity. Tourna *et al.* (2008) reported a decrease of nitrifying activity in soil samples of 37% when the temperature dropped from 30 to 25 °C. Therefore, although the nitrifying population in the reactor was high, the activity of this population was low and the nitrogen removal efficiency was not improved. This behavior was confirmed by measuring respirometric activity (5.1 mg TCOD L^{-1}), once the autotrophic active biomass reached stability for the entire monitored period (Figure 5 (a)).

The presence of *Nitrobacter* remained low throughout the monitored period (below 5% of DAPI-stained cells),

and *Nitrospira* rarely appeared (average $\leq$3%), being noticed only in some periods of this study (data not shown).

PAOs (*Candidatus* 'Accumulibacter' cluster) were found at low concentrations (average 7% of DAPI-stained cells) until day 99 and at higher concentrations thereafter (average 27% of DAPI-stained cells). PAOs are favored in SBRs because they possess competitive advantages over non-poly-P accumulating microorganisms and are able to survive during food shortage periods. The considerable increase of PAOs during the monitored period was concurrent with the increase in phosphorus removal (average of 50%) during days 99–165. As discussed previously, the conditions of pH (7.6) and low temperature (23.5 °C) contributed to the effective biological phosphorus removal process.

Denitrifying organisms (some *Thiobacillus* and *Desulfovibrionaceae*) were not detected in this study. Some PAOs are also capable of performing denitrification (Kong *et al.* 2004), suggesting that the denitrifying processes that occurred in this reactor may be due to the action of this group.

Changes in the bacterial community composition of the SBR were investigated using PCR-DGGE. Figure 5(b) shows the typical gel banding of the reactor's DGGE fingerprints throughout the monitored period. Each band corresponds approximately to a dominant microbial community. In general, DGGE analysis showed changes in the band profile and band intensity (e.g., days 15–190, band 1) throughout the biological treatment.

DGGE bands 1 and 2 were detected in all profiles but with varying intensity. Intensity of both bands increased between days 30 and 120 and decreased on day 150. The other bands (bands 3–8) appeared at different times and were not always present throughout the monitored period, indicating changes in the reactor's microbial composition. Bands 3, 5, 6, and 7 appeared during days 60–90, and bands 4 and 6 appeared in lanes corresponding to days 15 and 140. Band 7 was also found at day 7, and band 8 was exclusive to day 60. These differences suggest some variance in the microbial community composition during this period, which may be affected by influent variation, since each sample was taken from the same place in the reactor.

A total of eight different bright bands from the DGGE profile were excised (Figure 5(b)). The total DNA was the same length, but different sequences could be excised and then sequenced. Each sequence was submitted to a BLAST search, and the results are shown in Table 1. The most commonly identified microorganisms were taxonomically classified as Bacteroidetes, and band 4 was affiliated to Firmicutes. These organisms are usually observed in activate sludge processes and are extremely diverse. Firmicutes are chemoorganoheterotrophic

Table 1 | NCBI BLAST search results of sequences from DGGE bands

Band no.	Most related sequence	% Similarity	Source	Acession no.
1	Uncultured bacterium clone 11 16S ribosomal RNA gene	99	Nitrifying aerobic granules produced in SBRs	DQ673352.1
2	Uncultured bacterium isolate DGGE gel band X15 16S rRNA gene	98	Polyhydroxyalkanoates (PHA) producing wastewater treatment bioreactor	GU395573.1
3	Uncultured bacterium gene for 16S rRNA, clone 0729	97	Wastewater treatment under reduced oxygen supply procedure of a food processing factory	AB286448.1
4	Uncultured Firmicutes bacterium isolate DGGE gel band 16S rRNA	99	Wastewater treatment using bioelectrochemical system	JX548548.1
5	Uncultured Bacteroidetes bacterium isolate DGGE gel band 16S rRNA	98	Activated sludge process using ozone-treated sludge for sludge reduction	FJ750465.1
6	Uncultured bacterium clone C036 16S rRNA gene	98	Bioaugmentation with *Pseudomonas* sp. strain MHP41 in agricultural soils	FJ561582.1
7	Uncultured bacterium clone Pohang_WWTP 16S rRNA gene	97	Activated sludge process analyzed by pyrosequencing	HQ517990.1
8	Uncultured bacterial clone F1Q32TO05BRWN 16SrRNA	98	Bacterial community of a full-scale fixed biofilm activated sludge system	GU503329.1

organisms that includes *Bacillus* spp. and *Trichococcus flocculiformis*. The first are aerobic with capacity to denitrify. Bacteroidetes are chemoorganoheterotrophic organisms, and factors determining their presence are largely unknown, but low DO may be one of them. Their ecophysiology suggests they use mainly sugars in their metabolism (Seviour *et al.* 2010).

All bands were closely matched to uncultured organisms similar to microorganisms previously found in different wastewater treatment plants. Among these, there were organisms related to fixed biofilm reactors, SBRs using aerobic granules, and systems with reduced oxygen supply (Table 1). This fact suggested that the SBR used in this study formed microzones of different oxygen concentrations during the treatment process and the little DO variation occurred during the oxic–anoxic phase, resulting in the development and activity of diverse microbial groups. These microbial groups include those that are capable of performing SND by using organic substrates as sources of carbon and energy for the aerobic conversion of ammonium to nitrogen gas (Ju *et al.* 2007; Jia *et al.* 2012).

Cluster analysis was used to show the percentage of similarity between different DGGE profiles (Figure 5(c)). This comparison method showed that, between day 60 and 120, the community assemblage present in SBR exhibited maximum similarity. 92.3% similarity was observed during this period, followed by an 88.9% similarity between days 60 and 120. The minimum similarity occurred in the second and penultimate monitored periods with 80% similarity between days 30 and 190. High variation and diversity in the microbial community was observed between days 60 and 90, and low diversity and high similarity percentage (80%) was observed on days 30 and 150.

Hai *et al.* (2014) using canonical correspondence analysis in one study with two activated sludge bioreactors (pilot and laboratory scale) showed that wastewater characteristics, operational parameters (temperature, SRT, hydraulic retention time (HRT), MLSS), and bioreactor scale could independently explain 20.3%, 19.9%, and 3.6% of the variation of bacterial communities, respectively. In the present work the temperature and SRT were the most important variables that influenced the microbial community structures.

CONCLUSIONS

The step-fed SBR showed viability for treating domestic wastewater, even under low DO concentration (0.7 mgO_2L^{-1}), with variation in the applied loads and with a high C:N ratio (12 g-COD/g-N). The reactor showed removal efficiencies above 70% for both organic matter and ammonium. The average effluent concentrations of SCOD, NH_4^+-N, and TKN were 51 mgL^{-1}, 16 mgL^{-1}, and 55 mgL^{-1}, respectively, which is in accordance with the National Wastewater Discharge Standards of Brazil. Low temperature (23.5 °C) proved to be an important factor in determining biological phosphorous removal rates and showed negative influence on nitrification. PCR-DGGE verified significant changes in the bacterial community composition, which was due to environmental (temperature) and operational factors (SRT). Additionally, most of the identified bacterial sequences belonged to bacterial groups found in activated sludge systems. These results reinforce the idea that SBR under low DO can adapt to significant changes in nutrient concentration by gradually acclimating its biomass. The proposed SBR operational condition was shown to be an alternative technology to establish new decentralized wastewater treatment systems. The information obtained in this research could be used for future studies, including isolation of specific microorganisms, phylogenetic analysis of some important groups of microorganisms in wastewater treatment, and targeting novel microorganisms in SBR systems.

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