The use of bottle caps as submerged aerated filter medium

Laurence Damasceno de Oliveira, Amir Mohaghegh Motlagh, Ramesh Goel, Beatriz de Souza Missagia, Benício Alves de Abreu Filho and Sandro Rogério Lautenschlager

ABSTRACT

In this study, a submerged aerated filter (SAF) using bottle caps as a support medium was evaluated. The system was fed with effluent from an upflow anaerobic sludge blanket system at ETE 2-South wastewater treatment plant, under different volumetric organic load rates (VOLRs). The population of a particular nitrifying microbial community was assessed by fluorescent *in situ* hybridization with specific oligonucleotide probes. The system showed an average removal of chemical oxygen demand (COD) equal to 76% for VOLRs between 2.6 and 13.6 kg COD m^{-3}_media.day^{-1}. The process of nitrification in conjunction with the removal of organic matter was observed from applying VOLRs lower than 5.5 kg COD m^{-3}_media.day^{-1} resulting in 78% conversion of NH_4^+-N. As the applied organic load was reduced, an increase in the nitrifying bacteria population was observed compared with total 4′-6-diamidino-2-phenylindole (DAPI) stained cells. Generally, SAF using bottle caps as a biological aerated filter medium treating wastewater from an anaerobic system showed promising removal of chemical oxygen demand (COD) and conversion of NH_4^+-N.

Key words | bottle caps, nitrification, submerged aerated filter, support filter medium

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INTRODUCTION

Wastewater treatment in developing countries is still considered as one of the main infrastructure challenges and high investment is needed to implement the technologies that are already developed. This deficiency in wastewater treatment results in excess discharge of nutrients, particularly nitrogen and phosphorus, which results in eutrophication of surface water bodies. Excessive amounts of nutrients cause loss of aquatic habitats, excessive algal blooms and poor water quality of water bodies that serve as water sources for the cities downstream. Thus, combinations of materials and processes that improve nutrient removal at the treatment plant at reduced cost need to be studied. For instance, a biological aerated filter (BAF) is one of the treatment technologies that has advantages including reduced size of the treatment plant, smaller footprint and excellent performance at high organic loads compared with conventional biological processes (Mann *et al.* 1998). In addition, this system obtains high ammonia conversion and efficient removal of suspended solids in a single unit (Stephenson *et al.* 1993; Fdz-Polanco *et al.* 2000). However, the choice of support material is a crucial step in the operation of a BAF to maintain a high amount of biomass and the different microbial populations responsible for the conversion of pollutants found in wastewater (Moore *et al.* 2001). In this regard, a variety of media, such as clay, shale, polyethylene plastics (Rozic *et al.* 2000; Osorio & Hontoria 2002), oyster shells, plastic beads (Liu *et al.* 2010) and scoria (Morgan-Sagastume & Noyola 2008) have been studied. A support medium used in a BAF applicable for wastewater treatment in developing countries

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should also have a low cost, high relative surface area/volume ratio, good mechanical strength, easy acquisition and be suitable for aggregating microorganisms. Thus, the purpose of this study was to evaluate the reuse of caps from polyethylene terephthalate (PET) bottles as an alternative material for media support in a submerged aerated filter (SAF). These caps are constantly discarded in the environment and only a small volume is being recycled. Therefore, the reuse of these caps as a support filter medium has the following environmental goals: encourage reuse, improve removal of nutrients from wastewater, reduce use of energy and raw material used for producing support medium material.

For this purpose, a SAF pilot-scale system was constructed to investigate the removal of carbonaceous matter and nitrification when bottle caps are used as a support medium.

MATERIAL AND METHODS

PET bottle caps cut in half were used as the support medium. To determine the porosity, a 0.03 m^3 cube was used. The cube was filled with caps chosen randomly three times. The volume of water used to fill the cube after filling it with caps was 0.0228 m^3 and the porosity was calculated by Equation (1)

$$\text{Porosity}\,(\%) = 100 \times \frac{\text{water volume to fill cube box without caps}}{\text{water volume to fill cube box with caps}} \quad (1)$$

The surface area for each half cap was 0.00145 m^2 and for filling the 0.03 m^3 cube, 5,234 units of half caps were needed. The specific surface area (SSA) was calculated using Equation (2)

$$\text{SSA} = \frac{(\text{units half caps}) \times (\text{surface area for each half cap})}{\text{cube volume}} \quad (2)$$

The resulting characteristics of the support material are presented in Table 1.

Table 1 | Characteristics of support material

Suport medium	Diameter (cm)	Specific surface area ($m^2\ m^{-3}$)	Porosity (%)
Bottle cap	1.4	253	76

The reactor (Figure 1) had an upflow configuration and was 2.01 m long, 0.98 m wide and 0.32 m deep. The height of the filter bed was 1.56 m with a volume capacity of 0.48 m^3. The air distribution inside the reactor was performed by two PVC tubes of 12.5 mm, diametrically perforated with holes of 2 mm in diameter with a spacing of 10 cm, placed vertically. The airflow was controlled through a registration-type ball to keep the concentration of dissolved oxygen in the range of 5 to 7 mg $O_2\ L^{-1}$.

The dissolved oxygen was monitored by a probe (HACH LDO® Model 2 – Optical Process Dissolved Oxygen Probe) without measuring the air flowrate.

The effluent from the modified upflow anaerobic sludge blanket (UASB) was pumped continuously into the inlet of the SAF with a hydraulic pump (KSB Hydrobloc, model P500T). The reactor was inoculated for 30 days with this effluent. The input flow for the inoculation phase was the same as for phase 2 (Table 2). After the inoculation phase, the input flow was increased and phase 1 started (Table 2). The secondary sedimentation tank was made of steel with a 1.0 m circular section diameter, 1.56 m^3 volume and surface area of 0.62 m^2. The settling tank was being fed from the top of the tank, a pipe fixed in the center of the settling tank effluent ensured the insertion 60 cm below the water level of the settling tank. The wastewater effluent was discharged through 10 holes of 25 mm made diametrically at the top wall unit, where liquid flows to an outer channel installed on the circumference of the settling tank. A 75 mm pipe was installed at the base and the sludge settled in the settling tank was conveyed continuously to the reactor inlet with a recirculation hydraulic pump (KSB Hydrobloc, model P500T). The recirculation was performed to maintain the biomass concentration in the reactor.

The influent wastewater was collected from a modified UASB at ETE 2-South wastewater treatment plant, administered by the Sanitation Company of the State of Paraná (SANEPAR), located in the city of Marialva-PR, which receives domestic wastewater and industrial pretreated wastewater from the southern city of Maringa, PR.

The experimental period consisted of 150 days, which were divided into three phases. At each phase, the feed flow and sludge recirculation flow were changed in order to simulate different volumetric organic loading as shown in Table 2. The wastewater characteristics used to feed the pilot reactor, chemical oxygen demand (COD), biochemical oxygen demand (BOD), NH_4^+-N, total suspended solids (TSS) and temperature, are shown in Table 2. The

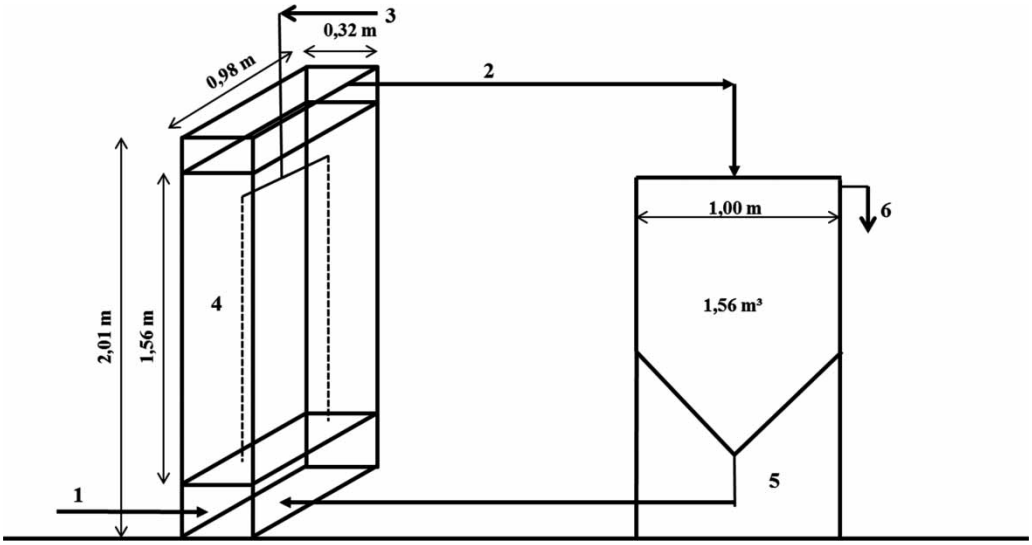

Figure 1 | (1) SAF in, (2) SAF out, (3) air distribution, (4) support medium, (5) recirculation sludge and (6) treated wastewater.

Table 2 | Operating conditions during the three phases

Phase	1	2	3
Input flow ($m^3\,h^{-1}$)	0.5	0.2	0.2
Recirculation flow ($m^3\,h^{-1}$)	0.1	0.1	0.2
Recirculation/Input	0.2	0.5	1
O_2 concentration ($mg\,L^{-1}$)	5–6	6	6
COD ($mg\,L^{-1}$)	414 ± 265.4	415 ± 168.2	196 ± 36.3
BOD ($mg\,L^{-1}$)	214 ± 133	155 ± 61	66 ± 11
VOLR ($kgCOD\,m^{-3}$_media d^{-1})	13.6 ± 8.8	5.5 ± 2.2	2.6 ± 0.5
NH_4^+-N ($mg\,L^{-1}$)	42 ± 4.65	45 ± 6.3	54 ± 8.5
TSS ($mg\,L^{-1}$)	295 ± 273.3	387 ± 305.2	116 ± 57.8
Temperature (°C)	27 ± 1		
Duration (days)	45	60	45

volumetric organic loading rate (VOLR) was calculated using Equation (3)

$$\text{VOLR} = \frac{\text{Flow} \times \text{Concentration}}{\text{Medium Volume}} \quad (3)$$

The samples were collected in plastic containers at one-hour intervals from 8 a.m. to 6 p.m., twice a week at points 1 and 6 (Figure 1). Analyses were performed for total alkalinity, COD, ammonia (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N). These measurements were performed according to the procedures of *Standard Methods for the Examination of Water and Wastewater* (2005).

The support material was sampled at three days, once for each of the three phases. For each of the samples, 45 half caps were collected on the top of the reactor. These samples were placed in sterile 500 mL plastic bottles and kept on ice. Then the biofilm was extracted by shaking and scraping manually and fixed with 4% (w/v) paraformaldehyde solution (Amann *et al*. 1990) and stored in a sodium phosphate buffer (PBS 130 mM NaCl, 7 mM $Na_2H_2PO_4$, pH = 7.2) at −20 °C for *in situ* hybridization analysis. Subsequently, the samples were hybridized with a hybridization buffer according to the concentration of formamide (Table 3) (0.9 M NaCl, 20 mM Tris-HCl pH 7.2, 5 mM EDTA, 0.01% SDS). Then the samples were hybridized in a 46 °C incubator for 2 hours. After the samples were washed with a NaCl solution according to Table 3 (20 mM Tris-HCl pH 7.2, 10 mM EDTA, SDS 0.01), they

Table 3 | FISH oligonucleotide probes used in this study

Probe	Sequence (5′–3′)	Specificity	%FA/NaCl (mM)[a,b]	Reference
EUB338	GCTGCCTCCCGTAGGAGT	Most bacteria	20/225	Amann *et al.* (1990)
NON338	ACTCCTACGGGAGGCAGC	Negative control	20/225	Wallner *et al.* (1993)
NSM156	TATTAGCAACATCTTTCGAT	Cluster *Nitrosomonas*	5/80	Mobarry *et al.* (1996)
NIT3	CCTGTGCTCCATGCTCCG	Genus *Nitrobacter*	40/56	Mobarry *et al.* (1996)
CNIT3	CCTGTGCTCCATGCTCCG	Competitor *Nitrobacter*	–	Mobarry *et al.* (1996)
NTSPA662	GGAATTCCGCTCTCCTCT	Genus *Nitrospira*	35/80	Daims *et al.* (2000)
CNTSPA662	GGAATTCCGCTCTCCTCT	Competitor *Nitrospira*	–	Daims *et al.* (2000)

[a]FA, formamide concentration in the hybridization buffer.
[b]NaCl concentration, NaCl concentration in the wash buffer.

were incubated for 15 min at 46 °C. Then the samples were counter-stained with DAPI (4′,6-diamidino-2-phenylindole) (2 μg mL^{-1} concentration). After this procedure, the samples were washed with consecutive immersions of 50, 80 and 96% ethanol solutions (3 min each) and finally 10 μL of Citifluor and Vectashield as antifading reagent were added. The probes used in this study are given in Table 3.

The sample slides were analyzed under a Zeiss fluorescence microscope (model Axioskop 2 plus) equipped with filters specific for DAPI and rhodamine or Cy3. The absolute number of cells labeled by the probes was determined by counting 10 randomly chosen microscopic fields, which contained not more than 300 cells labeled by the probes and 3,000 cells in total DAPI-stained cells. The percentage of cells hybridized with specific probes was calculated relative to the total number of cells stained with DAPI plus or minus the standard deviation.

RESULTS AND DISCUSSION

The average removal of organic matter expressed in terms of COD was 76% by applying organic loads between 2.6 and 13.6 kg COD m^{-3}_media d^{-1} over three experimental phases (Figure 2). The removal efficiency increased with the reduction of organic loading rate (VOLR) from phase 1 to Phase 2, where the load was reduced from 13.6 to 5.5 kg COD m^{-3}_media d^{-1}.

The concentration of COD in the effluent for phase 1 (VOLR = 13.6 kg COD m^{-3}_media d^{-1}) was 98 ± 31 mg L^{-1} and COD removal was 68 ± 21%. With the reduction of the VOLR to 5.5 kg COD m^{-3}_media d^{-1} (phase 2), the concentrations of COD in the effluent was 77 ± 17 mg L^{-1}, and the removal efficiency was 79 ± 9%.

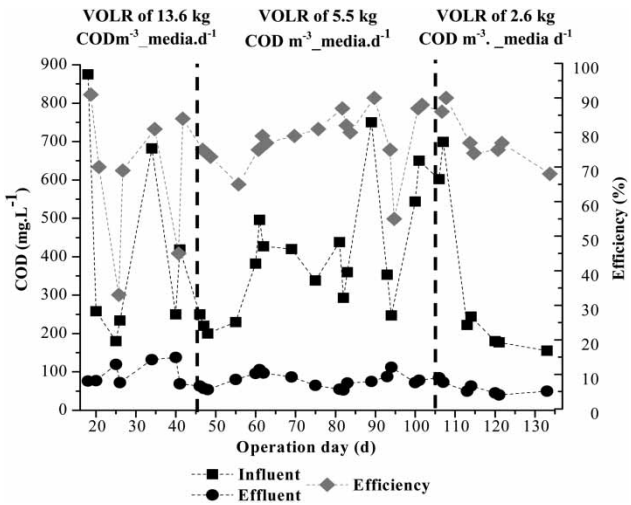

Figure 2 | Concentrations of COD and removal efficiencies of organic matter expressed as COD for different VOLR.

The concentration of recycle sludge in terms of TSS was (5,800± 3,608) mg L^{-1} (phase 1), (2,300± 2,669) mg L^{-1} (phase 2) and (296 ± 127) mg L^{-1} (phase 3) and for volatile suspended solids was (3,680 ± 2,278) mg L^{-1} (phase 1), (1,360 ± 1,562) mg L^{-1} (phase 2) and (193 ± 75) mg L^{-1} (phase 3).

The concentration of TSS in the reactor was calculated using the data from Table 2 and the concentration of recycle sludge which resulted in (1,213 ± 829) mg L^{-1} (phase 1), (1,025 ± 1,093) mg L^{-1} (phase 2) and (206 ± 93) mg L^{-1} (phase 3).

The limit for effluent is for BOD instead of COD (BOD <120 mg L^{-1}). However, since the average for COD effluent was below this limit, the SAF will satisfy the limit for organic matter removal.

With the introduction of phase 3 (VOLR = 2.6 kg COD m^{-3}_media d^{-1}) an improvement in COD effluent

concentrations was observed (49 ± 8 mg L^{-1}), which corresponds to a removal of 74 ± 4%. Similar results were found by Gonçalves *et al.* (1998) who studied a submerged aerated biofilter as post-treatment of UASB. The reactor was filled with polystyrene beads and the authors obtained a COD concentration in the effluent equal to 49 mg L^{-1} resulting in an efficiency of 56% under the application of a VOLR = 2.3 kg COD m^{-3}_media d^{-1}.

The SAF showed good conversion of NH_4^+–N when subjected to VOLR lower than 5.5 kg COD m^{-3}_media d^{-1} adopted from phase 2 (Figure 3). During phase 1 (VOLR = 13.6 kg COD m^{-3}_media d^{-1}) the effluent NH_4^+-N was 35 ± 5 mg L^{-1}, which represents a conversion efficiency of 15 ± 12%. With the introduction of phase 2 (VOLR = 5.5 kg COD m^{-3}_media d^{-1}), the NH_4^+-N concentration in the effluent was 16 ± 7 mg L^{-1} which represented a conversion efficiency of 63 ± 17%. In phase 3 (VOLR = 2.6 kg COD m^{-3}_media d^{-1}), the effluent NH_4^+–N showed values of 11 ± 7 mg L^{-1} and a conversion efficiency of 78 ± 15%.

One limiting parameter for controlling nitrification is the organic matter, owing to the sensitivity of autotrophic nitrifying microorganisms to this substrate. The removal of organic matter and ammonia conversion can be performed in a single unit, as reported by Pujol *et al.* (1992), Fdz-Polanco *et al.* (2000), and Ling & Chen (2005), and this behavior was also observed in this study during the experimental phases. Nitrification in conjunction with the biodegradation of organic matter was observed at VOLR below 5.5 kg COD m^{-3}_media d^{-1} (Figure 4) which resulted in a higher production of nitrite and nitrate at these loads. At VOLR of 13.6 kg COD m^{-3}_media d^{-1} (Figure 4), which

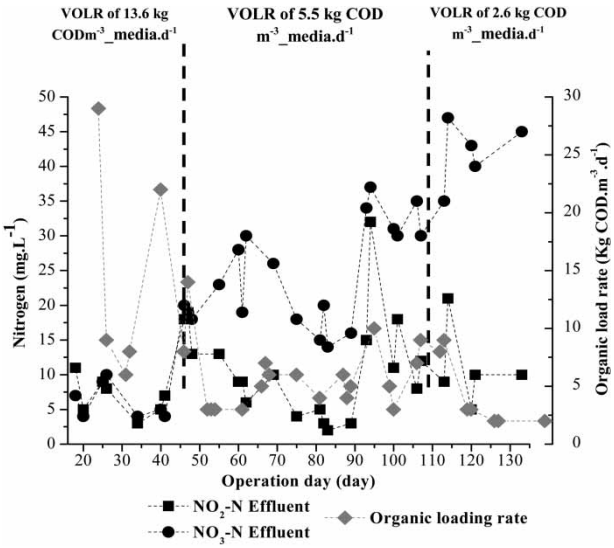

Figure 4 | Concentrations of NO_2- N and NO_3-N at different VOLR.

can be considered a high organic loading rate, the heterotrophs will outcompete the nitrifiers because the growth rate of heterotrophs is much higher than for autotrophs. At low organic loading rates, not all oxygen is consumed by heterotrophs so that the nitrifiers can co-exist with the heterotrophs in the biofilm. Morgan-Sagastume & Noyola (2008) operating a BAF using volcanic rocks as support medium, observed inhibition of nitrification from organic loads exceeding 9.4 kg COD m^{-3}_media d^{-1}. Loading rates and removal of COD and NH_4^+-N are shown in Figure 5. The NH_4^+-N removals (approximately 78%) for VOLR of 2.6 kg COD m^{-3}_media d^{-1} showed similar results for a

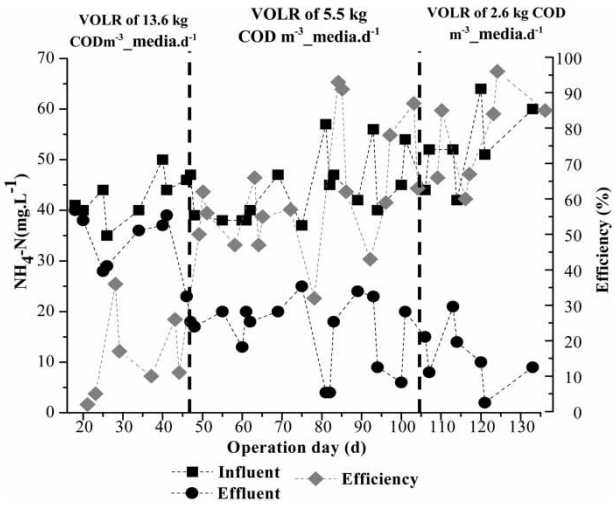

Figure 3 | NH_4-N concentrations and conversion efficiencies in SAF at different VOLR.

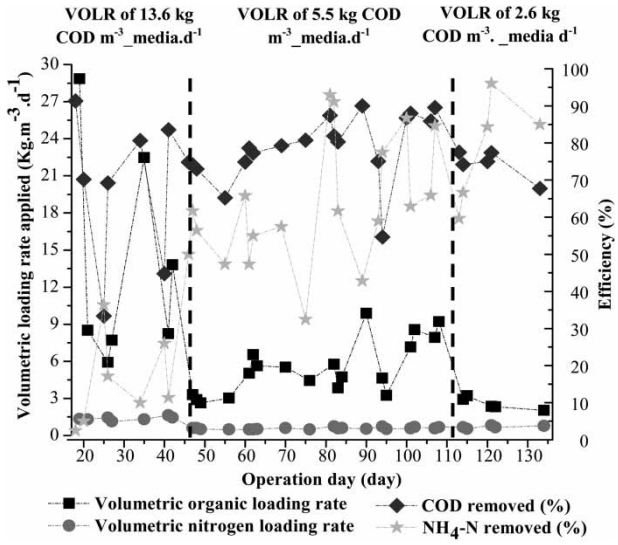

Figure 5 | Loading rates and removal of COD and NH_4^+-N.

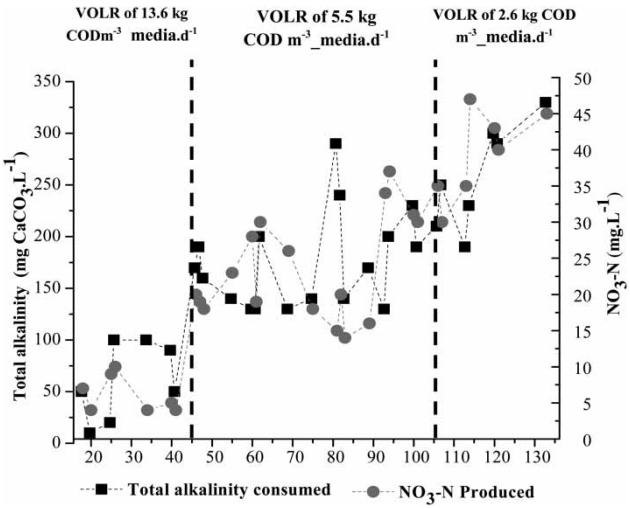

Figure 6 | Total alkalinity consumption and production of NO_3-N in VOLR.

trickling filter post-UASB operating with organic loading rates (OLRs) varying from 0.45 to 0.55 kg COD m^{-3} d^{-1} (Almeida *et al.* 2013).

The alkalinity during the three phases is shown in Figure 6. For phase 1, there was a consumption of total alkalinity of 60 ± 37 mg L^{-1} $CaCO_3$ and an effluent nitrate concentration of 6 ± 2 mg L^{-1}. Phase 2 allowed an increased rate of nitrification, which showed consumption in total alkalinity of 181 ± 47 mg L^{-1} and an effluent nitrate concentration of 24 ± 7 mg L^{-1}. With the introduction of phase 3, there was a higher consumption of total alkalinity of 268 ± 57 mg L^{-1} and a nitrate concentration of 42 ± 5 mg L^{-1}. It is well known that there is alkalinity consumption during the conversion of ammonia to nitrate. The results presented in Figure 6 confirm those data presented in Figure 4. A relationship between average nitrate

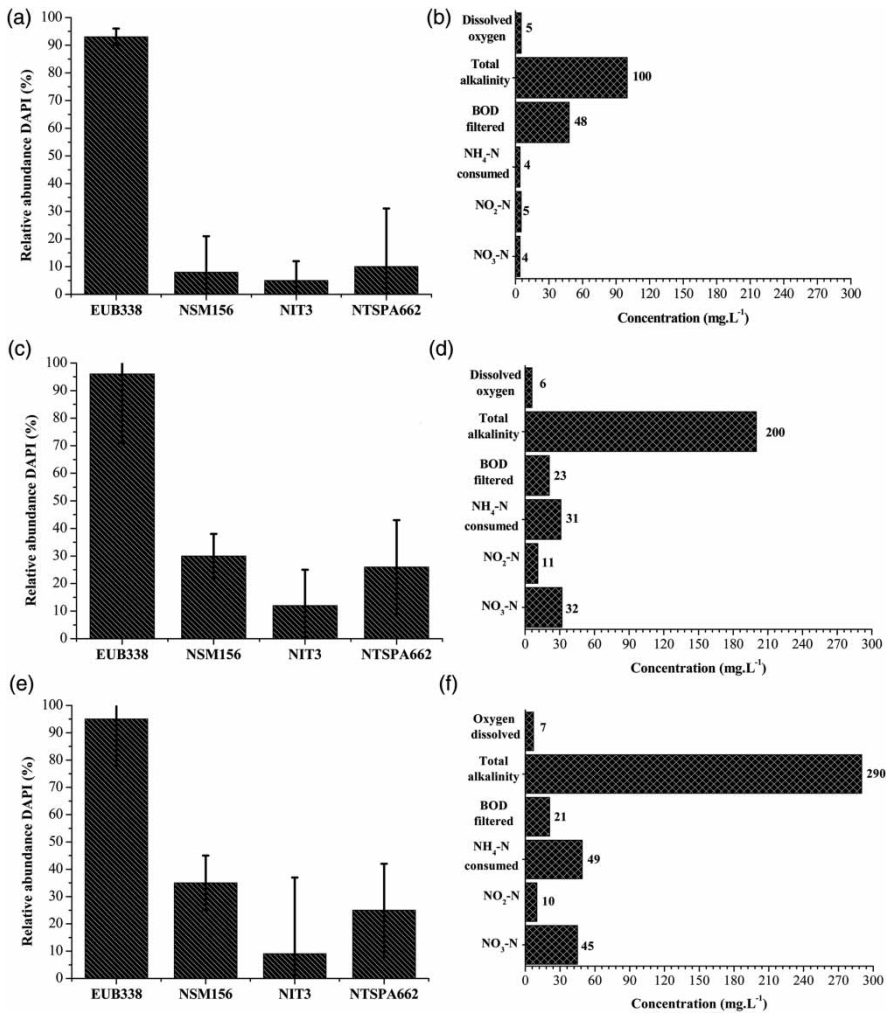

Figure 7 | Distribution of bacteria (EUB338), AOB (NSM156) and NOB (NIT3 and NTSPA662) on top of the SAF. Each graph shows the relative amount of each probe relative to DAPI (y axis) during three phases (Phase 1: 1.29 kg BOD m^{-3}_media d^{-1}; Phase 2: 0.40 kg BOD m^{-3}_media d^{-1}; Phase 3: 0.19 kg BOD m^{-3}_media d^{-1}). Right-hand side: graphs of dissolved oxygen, total alkalinity, filtered BOD, NH_4-N, NO_2-N, NO_3-N. Phase 1 ((a) and (b)) Phase 2 ((c) and (d)) and Phase 3 ((e) and (f)).

concentration and alkalinity consumed for the three phases would be 1:10 (Phase 1), 1:7.5 (Phase 2) and 1:5.7 (Phase 3).

Two groups of microorganisms are involved in the process of nitrification: ammonia-oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Studies indicate *Nitrospira* as the dominant NOB genus in wastewater treatment (Juretschko *et al.* 1998; Okabe *et al.* 1999; Daims *et al.* 2000). *Nitrospira* bacteria, adapted to low concentrations of nitrite and dissolved oxygen, are known as K-strategist, while the genus *Nitrobacter* is considered an r-strategist as it is adapted to environments with high concentrations of nitrite and dissolved oxygen (Schramm *et al.* 1999). Thus, the genus *Nitrospira* together with *Nitrobacter* were chosen as representatives of NOB, and *Nitrosomonas* to represent the AOB. Accordingly, oligonucleotide probes were selected to quantify these communities of bacteria.

Fluorescent *in situ* hybridization (FISH) specific groups cell count compared with the DAPI-stained cell results, dissolved oxygen, total alkalinity, filtered BOD, NH_4^+-N, nitrite and nitrate concentrations for the three phases are shown in Figure 7. The relative abundance of AOB compared with total DAPI-stained cells were 8, 30 and 35%, and for *Nitrobacter* NOB were 5, 12 and 9%, while the main genus *Nitrospira* NOB showed 10, 26 and 25% for the VOLR of 1.29, 0.4 and 0.19 kg BOD m^{-3}_media d^{-1}, respectively (Figure 7).

The increase in the nitrifying community from phase 1 when compared with phase 2 and phase 3 due to a decrease in VOLR corroborates results obtained by Aoi *et al.* (2000), Kindaichi *et al.* (2004), Elenter *et al.* (2007) and Fu *et al.* (2010). The fact can be explained by the reduction of the organic loading over the experimental phases that resulted in a lower supply of carbon, and consequently, the population of heterotrophic bacteria decreased in relation to the nitrifying bacteria. This observation, coupled with a higher conversion of NH_4-N, total alkalinity and production of NO_3-N after the second step, shows that the nitrification process started with the reduction of the organic load. Therefore, the reduction of the organic load resulted in higher relative abundance of nitrifying bacteria, which was verified for organic loads of 0.40 kg BOD m^{-3}_media d^{-1}. In addition, due to competition for space and dissolved oxygen between nitrifying and heterotrophic bacteria in biofilms, heterotrophic bacteria that have rapid growth are located on the exterior, while the nitrifying bacteria with slower growth settle in inner regions (Fdz-Polanco *et al.* 2000).

CONCLUSIONS

The results showed that the SAF was efficient in terms of COD removal and conversion of NH_4-N. The pilot plant has achieved an average removal of COD equal to 78% with the application of an organic loading rate below 2.6 kg COD m^{-3}_media d^{-1}. It has also been found that the nitrification process took place simultaneously with biodegradation of organic material subjected to loads less than 5.5 kg organic COD.m^{-3}_media d^{-1} and, in spite of an VOLR of 2.6 kg-COD.m^{-3}_media d^{-1}, the SAF showed a removal efficiency of NH_4-N greater than 76%. The microbial analysis using FISH technique confirmed the dominance of AOB and NOB when organic load rates decreased. *Nitrospira* bacteria population was greater than *Nitrobacter* in all experimental phases. Bottle caps used as support medium in SAF were shown to be a decent alternative material; thus reusing the caps can save energy, raw material used to produce a new support medium. Since they are constantly discarded in the environment, it can also reduce the waste produced by humans.

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