Troubleshooting of filamentous bulking using biofilm reactors

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ABSTRACT

The present work aims to evaluate filamentous bulking control in systems combining suspended biomass with biofilm growth. For this study, four sequencing batch reactors (SBR) fed with an easy biodegradable substrate (acetate) were operated without (SBR1) and with support for biofilm growth [5 % (SBR2), 10 % (SBR3) and 20 % (SBR4) of the reactor volume]. The results demonstrated an overabundance of a filamentous fungi-like microorganism in the SBR operating just with suspended biomass. The incorporation of an optimized amount of support for biofilm growth (10 % and 20 %) seemed to suppress the overgrowth of this filamentous microorganism probably due to the combined effect of a decreased biomass loading rate and the physical cut or breakdown of filaments induced by particle-to-particle collisions. Besides the observed differences in terms of the filamentous fungi-like microorganism, the incorporation of a support material for biofilm growth was also found to induce increasing differences in the bacterial community structure as the concentration of support increased in the SBR.

KEYWORDS

Biofilm reactors, Filamentous bulking, Filamentous fungi-like microorganism, Microbial community composition, Support concentration.

INTRODUCTION

Activated sludge processes are among the most common technology used for the biological treatment of wastewaters. The conventional process consists of two stages: (i) the removal of organic material and nutrients from the wastewater by the activated sludge (biochemical stage) and (ii) the activated sludge separation from the effluent through gravity sedimentation (physical stage). The latter often becomes the critical stage of the process due to the frequent problems that severely affect the settling and compaction of the sludge. The most common cause of settling problems is the filamentous bulking - a term used to describe the overgrowth of filamentous microorganisms (bacteria or/and fungi). Filamentous bulking promotes the formation of open aggregates with low settling velocity as well as low compaction which cause serious difficulties in the retention of sludge within the reactor, thus impairing the overall performance and efficiency of the treatment process.

Several approaches have been used to reduce the overgrowth of filamentous microorganisms. For instance, the compartmentalisation of the aeration tank (i.e., plug-flow reactors), or its conversion to a batch process [such as sequencing batch reactors (SBR)] have been used to increase sludge settleability and compaction, being the use of a selector reactor the most widespread engineering tool to control filamentous bulking. Although these technologies

have been successful and have reduced filamentous bulking in many activated sludge systems, there are some reports that point out to their failure (Gabb et al., 1996; Teichgräber et al., 2001; Van den Eynde et al., 1984). For further details and history about filamentous bulking and development of technologies for its control, the readers are invited to read the reviews provided, among others, by Martins et al. (2004) and Jenkins et al. (2004).

An alternative to the existing technologies for filamentous bulking control might be the incorporation of a support material for biofilm growth into suspended growth reactors. Interestingly, no problems with excessive growth of filamentous microorganisms have been reported in the cases where activated sludge processes were combined with biofilm growth. Wanner et al. (1988) found experimental evidences that the presence of a biofilm in the activated sludge significantly improves its settling properties, especially in systems with plastic foam supports. According to the authors, the effect of decreasing the activated sludge loading and the predominance of the fixed form of biomass contributed to suppress the overgrowth of filamentous microorganisms in the mixed liquor; however, this line of research was not continued and further experiments are required to thoroughly elucidate the investigators about the filamentous bulking control mechanisms.

The present work aims to evaluate filamentous bulking control in systems combining suspended biomass with biofilm growth. For this study, sequencing batch reactors (SBR) fed with an easy biodegradable substrate (acetate) operating with or without (control unit) biofilm growth were used. The biofilm grew on small polyethylene supports that were moving freely along with liquid in the reactor. Microscopic and molecular techniques were used to follow the changes in the microbial community developed in each reactor. The use of different support concentrations in the SBR and the characterization of the microbial community involved in the experimented systems allowed the assessment of the filamentous bulking control mechanisms.

MATERIALS AND METHODS

Experimental setup

Four SBR with a working volume of 1.5 L were operated with a constant cycle time of 4 h. The sequence of the operating phases was as follows: 5 min fill, 225 min aerated, 5 min settle and 5 min draw. At the end of each cycle, 0.75 L of effluent was pumped out of the reactors. One reactor was operated just with suspended biomass (SBR1 – control unit) while the others combined suspended biomass with biofilm growth. The biofilm was formed on a polyethylene support developed by the University of Minho, consisting of a hollow, star-shaped support with 17 mm external diameter, a height of 10 mm and a specific surface area of 407 m^2 m^{-3} (Nogueira et al., 2009). The density of the support is about 510 g L^{-1}, so it can be easily suspended by aeration. The support concentration was 5 % (SBR2), 10 % (SBR3) and 20 % (SBR4) of the reactor working volume and the resulting hydraulic retention time (HRT) was 8 h, 7.6 h, 7.2 h and 6.4 h for SBR1, SBR2, SBR3 and SBR4, respectively. During the aerated phase, airflow of 2 L min^{-1} was applied through membrane diffusers, making the reactor contents, including the supports, to circulate. The reactors were operated with synthetic wastewater containing acetate as the only carbon source and the volumetric organic loading rate was 6 g COD L^{-1} day^{-1}, 6.3 g COD L^{-1} day^{-1}, 6.7 g COD L^{-1} day^{-1} and 7.5 g COD L^{-1} day^{-1} for SBR1, SBR2, SBR3 and SBR4, respectively. The slight differences in

the organic loading rates are due to the different HRT resulting from the different liquid volumes of the reactors.

The reactors were inoculated with activated sludge coming from the Serzedelo I Wastewater Treatment Plant (Guimarães, Portugal) at an initial concentration of 1000 mg L^{-1}.

Regular cleaning of feed storage vessels, feed lines and reactors was performed to avoid the proliferation of microorganisms on tubes and walls.

Medium

The synthetic wastewater used as influent contained $NaAC \cdot 3H_2O$ 4270 mg L^{-1} (2000 mg COD L^{-1}), $NaHCO_3$ 2437 mg L^{-1}, NH_4Cl 776 mg L^{-1}, KH_2PO_4 89 mg L^{-1}, $MgCl_2 \cdot 6H_2O$ 45 mg L^{-1}, $FeCl_3 \cdot 6H_2O$ 40 mg L^{-1}, $CaCl_2 \cdot 2H_2O$ 55 mg L^{-1} and 1 mg L^{-1} of the following trace elements: $MnCl_2 \cdot 4H_2O$, $(Ni)_2SO_4 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$ and $ZnSO_4 \cdot 7H_2O$. A refrigerated (4 - 8 ºC) vessel was used to contain the influent.

Analytical methods

Suspended biomass and biofilm concentration and standard sludge volume index (SVI) were determined during the experiments.

SVI of suspended biomass was determined using Standard Methods (APHA, 1998). Considering the volume of the reactors, the SVI was determined in unstirred 25 mL cylinders. Suspended biomass concentration was estimated as total suspended solids (APHA, 1998) using 47 mm fiber glass membrane filters (Whatman, Maidstone, UK) and the biofilm concentration was measured as total solids according to Lazarova et al. (1994).

Microscopic observations

Microscopic observations of the microbial communities of the suspended biomass as well as those of the biofilm were carried out at least once a week in all systems in a phase contrast microscope (Leitz, Laborlux S). Additionally, the presence of filamentous structures were analysed with Calcofluor™ White M2R (American Cyanamid, Eugene, OR, USA) stain. Calcofluor was added to samples to a final concentration of 10 µM and the binding of calcofluor to sample cell walls was practically immediate. After calcofluor staining, the filaments were observed under an epifluorescence microscope (Olympus BX51) using an excitation wavelength of 365 - 370 nm and an emission longpass filter by 421 nm.

Molecular microbial analysis

DNA extraction and amplification

Approximately 2 mL of well homogenized suspended biomass was washed and resuspended in sterile phosphate-buffered saline (PBS) at the time of the sampling and stored at -20 ºC until further use. The biofilm was sampled by removing two supports from the reactors and placing them into a falcon tube containing 40 mL of sterile PBS. The tube was vigorously vortexed for 1 min. Subsequently, the clean supports were removed and the biofilm suspension was stored at -20 ºC.

Total genomic DNA was extracted using a modified protocol of the PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) in which the cells were disrupted by the FastPrep instrument used in the FastDNA® Spin Kit for Soil (MP Biomedicals, LLC, Solon, OH, USA) . The rest of the DNA extractions steps were performed according to the original manufacturer's protocol.

Selected DNA target regions were amplified by polymerase chain reaction (PCR) using a *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA, USA) in a 50 μL reaction mixture containing: 5 μL of 10X PCR buffer [20 mM Tris-HCl (pH 8.4), 500 mM KCl], 3 mM $MgCl_2$, 200 μM of each of the four deoxynucleoside triphosphates (dNTP) (Frilabo, Porto, Portugal), 1.25 U of *Taq* polymerase, 200 nM of each primer and 1 μL of appropriately diluted template DNA. All primers used were synthesized by STAB Vida (Oeiras, Portugal). For DGGE analysis, bacterial 16S rRNA gene fragments were amplified using the primers U968-GC-f (5' – CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAACGCGAAGAACC TTAC - 3') and L 1401-r (5' – CGGTGTGTACAAGACCC - 3'), targeting the V6 to V8 region (Nübel et al., 1996). The thermocycling program used for amplification was: an initial denaturation step of 2 min at 95 ºC; 35 cycles of denaturation at 95 ºC for 30 s, annealing at 56 ºC for 40 s and elongation at 72 ºC for 60 s; and post-elongation at 72 ºC for 5 min. The reactions were subsequently cooled to 4 ºC. Bacterial 16S rRNA gene fragments were also amplified for cloning using the forward primer Bact27-f (5'-AGAGTTTGATCMTGGCTCAG - 3') and the universal primer Uni1492-r (5'-ACGCCTACCTTGTTACGACTT - 3') (Heuer et al., 1997). The program of amplification was similar to the one described above but with an annealing temperature of 52 ºC. The size and yield of the PCR products was estimated using a 100 bp DNA ladder (Frilabo, Porto, Portugal) by electrophoresis in a 1% (w/v) agarose gel stained with ethidium bromide (VWR International, Carnaxide, Portugal).

DGGE analysis

Denaturing gradient gel electrophoresis analysis of the amplicons was carried out using the Dcode system (Bio-Rad, Hercules, CA, USA) in gels containing 8% (w/v) polyacrylamide (37.5:1 acrylamide/bis-acrylamide). A linear denaturant gradient of 35 to 60% was used for all analyses, where a denaturing strength solution of 100% was defined as 7 M urea (Sigma-Aldrich, St. Louis, MO, USA) and 40% formamide (Fluka Chemie, Buchs, Switzerland). Gels were run for 16 h at 85 V in a 0.5X TAE buffer (50X Tris acetate: 242 g Tris, 57.1 mL glacial acetic acid, 100 mL 0.5 M EDTA (pH 8.0) per liter) at 60 ºC. Subsequently, DGGE gels were stained with silver as previously described by Sanguinetty et al. (1994), scanned in an Epson Perfection V750 PRO (Epson, USA), and the DGGE profiles were compared using the BIONUMERICS^{TM} software package (version 5.0; AppliedMaths BVBA, Sint-Martens-Latem, Belgium). Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles using the Pearson product-moment correlation coefficient (Häne et al., 1993).

Cloning and sequencing of PCR amplified products

PCR products obtained with the primers set Bact27f/1492r were purified with the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany) and cloned into *E. coli* JM 109 (Invitrogen, Carlsbad, CA, USA) using the Promega pGEM-T Easy vector system I (Promega, Madison, WI, USA), according to the manufacturer's instructions. 96 white-coloured recombinant colonies were collected and screened by DGGE. PCR was carried out

on the cell lysates using the primer pair above described for DGGE analysis. The DGGE mobility of amplicons was compared to the band-pattern of the biomass samples. The clones whose amplicon's corresponded to bands in the biomass samples community profile were selected for sequencing. Clones with sequences showing identical DGGE mobility were also selected for replicate sequencing. Plasmids of selected clones were purified using the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany) and subjected to DNA sequence analysis. Sequencing reactions were performed at STAB Vida using SP6 (5' - ATTTAGGTGACACTATAG - 3') and T7 (5' – TAATACGACTCACTATAGG - 3') sequencing primers. The sequence information was imported into the BioEdit v7.0.5.3 software package (Hall, 1999) for assembly. Consensus sequences obtained were manually checked and corrected when necessary. They were also checked for potential chimera artefacts by the CHECK_CHIMERA program of the Ribosomal Database Project II (http://35.8.164.52/cgis/chimera.cgi?su=SSU). Similarity searches for the 16S rRNA gene sequences derived from the suspended biomass and biofilm clones were performed using the NCBI Blast search program within the GenBank database (http://www.ncbi.nlm.nih.gov/blast/).

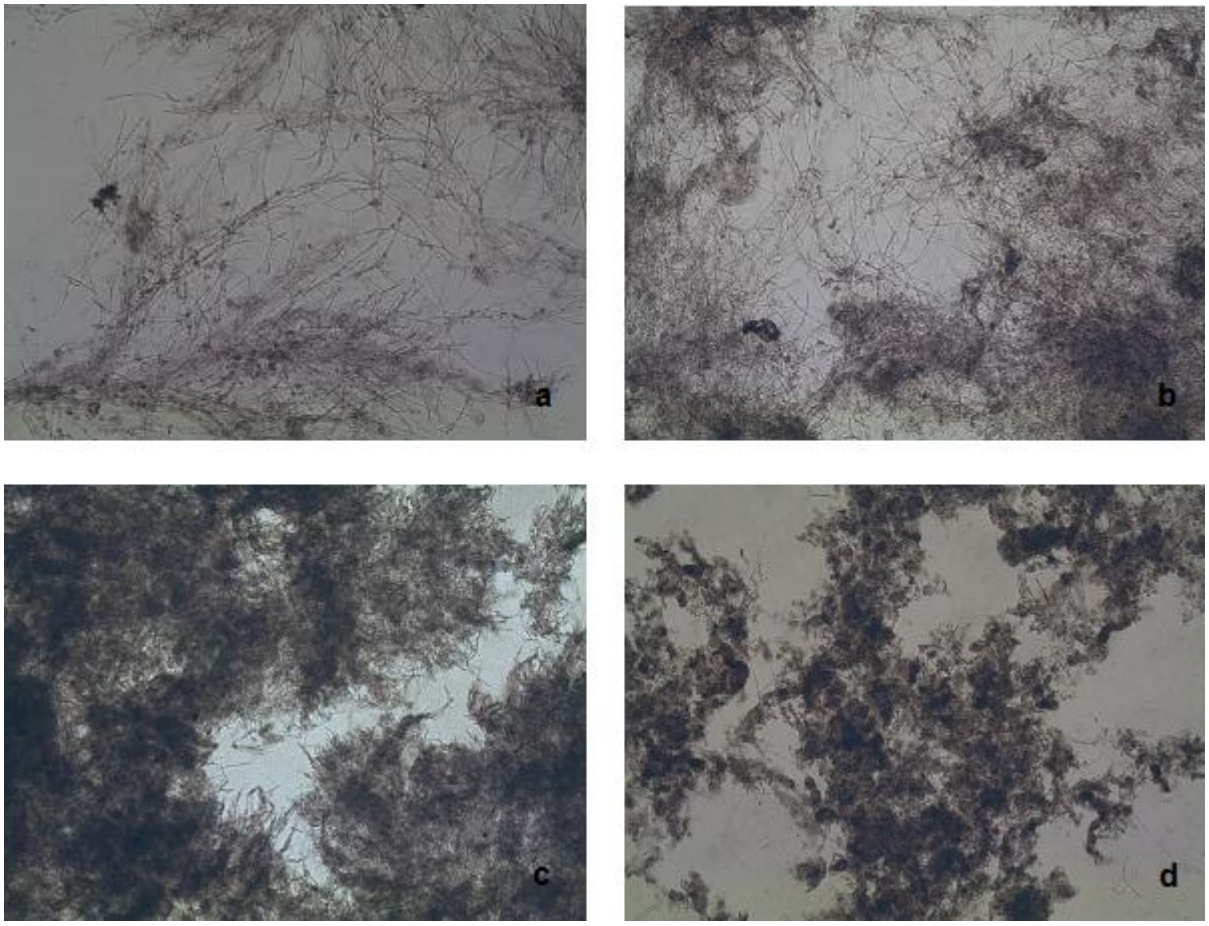
RESULTS AND DISCUSSION

SBR performance

Four reactors (SBR1 – SBR4) were operated with different amounts of support for biofilm growth. Before reactor´s start up, a microscopic analysis of the inocula used in the experiment was firstly done to verify the presence of filamentous microorganisms, being this a crucial step to the subsequent experiments. The seed sludge had small, disperse and not consistent flocs and revealed a large diversity and abundance of filamentous microorganisms (data not shown).

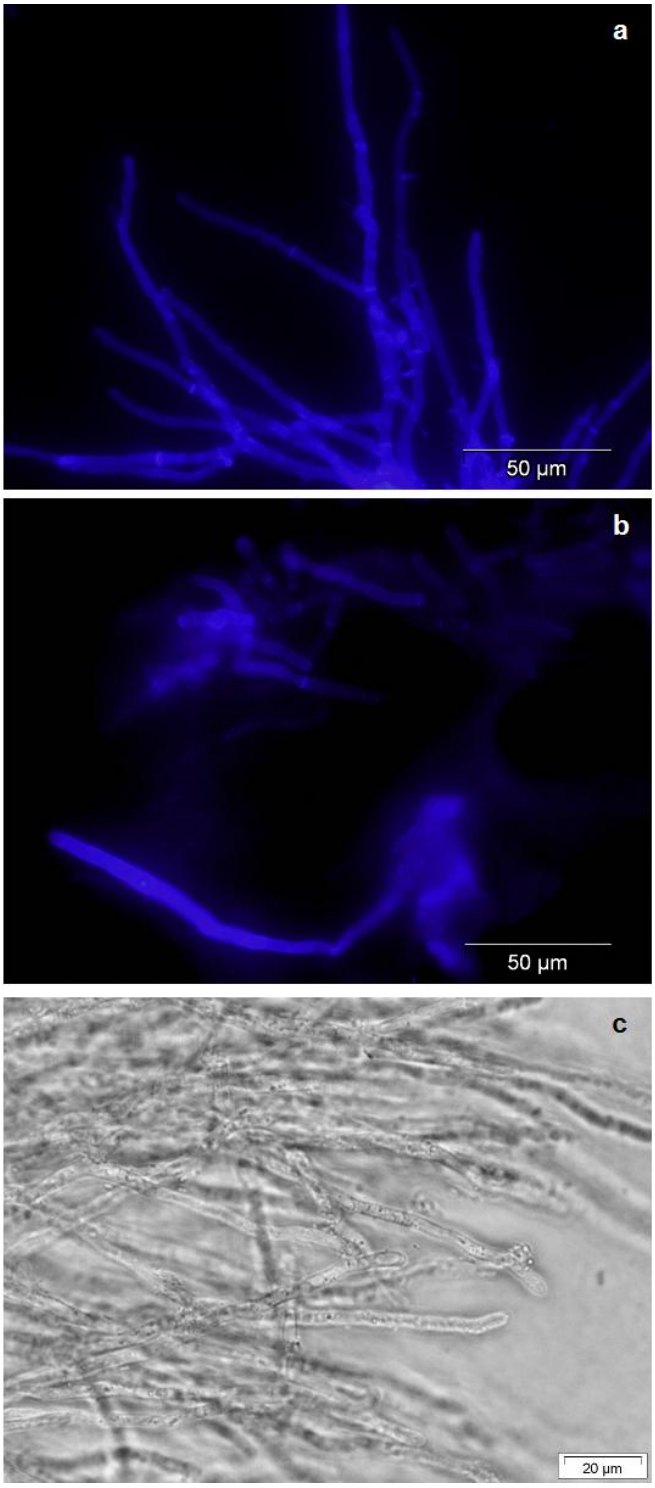
Subsequent microscopic inspections showed that the suspended fraction of biomass in the reactors started to differ very soon after the inoculation. The initially seed sludge gradually developed into large and compact flocs in SBR3, small and structurally weak flocs in SBR4 and flocs dominated by filamentous microorganisms growing in profusion beyond the confines of the flocs into the bulk solution in SBR1 and SBR2 (Figure 1).

Figure 1 - Micrographs of the suspended biomass from SBR1 (a), SBR2 (b), SBR3 (c) and SBR4 (d) on day 120 taken with an Olympus Altra-20 camera in a Leitz phase contrast microscope.



A detailed microscopic inspection revealed that filamentous microorganisms were also presented in the suspended biomass from SBR3 and SBR4; however, these filaments were integrated in the flocs structure (internal filaments) and were considerably shorter when compared to SBR1 and SBR2 filaments (Figure 2a and 2b).

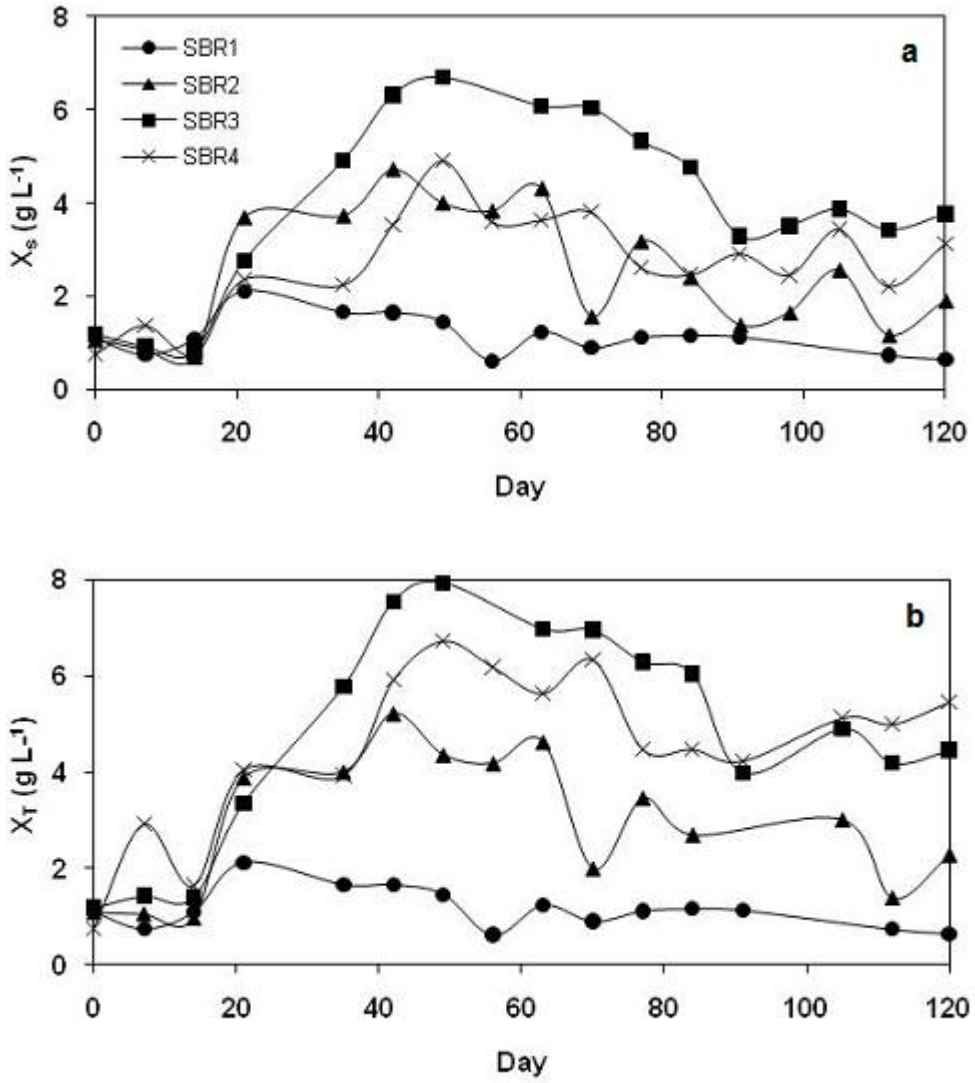
Figure 2 - Micrographs of the suspended biomass from SBR1 (a) and SBR4 (b) on day 120, after calcofluor staining, taken with an Olympus DP71 camera in an epifluorescence Olympus BX51 microscope. Phase contrast micrograph (Leitz, Laborlux S) of the observed filamentous microorganisms is also present (c).



The SVI values were in direct agreement with the microscopic inspection. The SVI in SBR3 did not exceed 150 mL g^{-1} but in SBR1 and SBR2 ranged between 130 mL g^{-1} and 680 mL g^{-1} as a result of the excessive occurrence of filaments (Figure 1a and 1b). SBR4 presented SVI values ranging between 140 mL g^{-1} and 220 mL g^{-1} and no problems with excessive filamentous growth were observed. The formation of large flocs might have been hindered in SBR4 by the high particle-to-particle collision frequency due to the high support concentration. As a result, small and structurally weak flocs developed during the experiments (Figure 1d) negatively affecting the settling and compaction of the suspended

biomass (high SVI values). Due to the different settleability and compaction of the suspended biomass, the four SBR differed considerably in the suspended biomass content (Figure 3a).

Figure 3 - Suspended biomass concentration (a) and total (suspended biomass and biofilm) biomass concentration (b).



The reactor presenting lower SVI values had also higher suspended biomass concentration. A relatively high suspended biomass concentration between 2.8 g L^{-1} and 6.7 g L^{-1} was maintained in SBR3 (low SVI values, Figure 1c). Along the experiments, the suspended biomass concentration decreased up to 0.6 g L^{-1} in SBR1, to 1.2 g L^{-1} in SBR2 and to 2.2 g L^{-1} in SBR4, likely because suspended biomass was constantly washed out of these reactors as a result of the low settling velocity as well as low compaction of their suspended biomass (high SVI values, Figure 1a, 1b and 1d).

The results obtained suggested that increasing the support concentration for biofilm growth in the reactors apparently suppressed the excessive growth of filamentous microorganisms. During the entire period of experiments (120 days), the microscopic observation revealed that filamentous microorganisms in suspended biomass were quite common in SBR1 and SBR2, while in the other reactors (SBR3 and SBR4) their occurrence was negligible. It was

observed that the suppression of the overgrowth of filamentous microorganisms in the suspended biomass of SBR3 and SBR4 might be related to the decrease of the biomass loading rate, i.e. to the increase of the total amount of biomass in the system (Figure 3b). SBR3 and SBR4 had higher total biomass concentration (3.4 – 7.9 g L^{-1} and 3.9 – 6.7 g L^{-1}, respectively) and excessive occurrence of filaments was not observed in these reactors (Figure 1c and 1d). On the other hand, a lower total biomass concentration was maintained in SBR1 and in SBR2 (0.6 – 2.1 g L^{-1} and 1.4 – 5.2 g L^{-1}, respectively) where a relative high proliferation of filamentous microorganisms was observed (Figure 1a and 1b). The results suggest that the filamentous microorganisms causing bulking problems in the reactors operated in this study seems to be high F/M filaments.

Apart from the results mentioned above, microscopic observations suggested another filamentous bulking control mechanism. It was observed that filamentous microorganisms were presented in all the reactors; however, in SBR3 and SBR4 filamentous bulking problems did not occur. Furthermore, filaments length in SBR3 and SBR4 seemed to be considerably shorter than in SBR1 and SBR2. These results suggest that filamentous bulking in SBR3 and SBR4 was suppressed due to physical cut or breakdown of filaments by collisions between supports. SBR3 and SBR4 had a support concentration of 10 % and 20 % which led to a high particle-to-particle collision frequency (Buffière and Moletta, 2000) and accordingly, the supports were induced to physically cut or break down filamentous microorganisms. Consequently, filaments were washed out from the reactors or might have become too short to cause filamentous bulking problems. In SBR2, it seemed that the particle-to-particle collisions established were not enough to control filamentous bulking as this reactor presented lower support concentration (5 %). It has been already reported, in airlift reactors using bare supports, that biofilm damage due to particle-to-particle collisions is proportional to the increased in support concentration (Gjaltema et al., 1997). The results obtained in this study suggested that a similar trend was observed for the suspended fraction of biomass in the reactors.

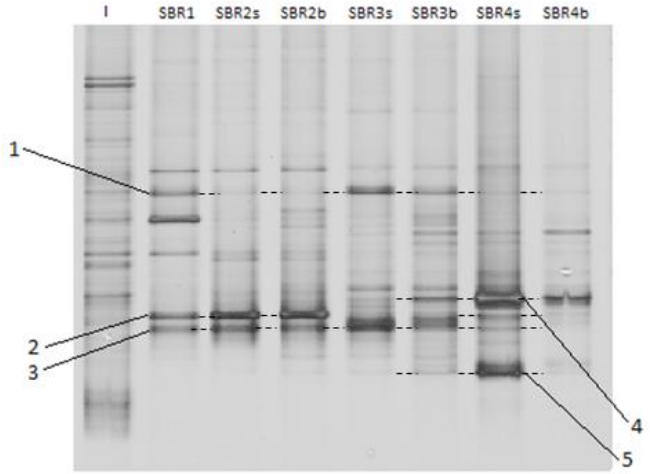
The results obtained showed that the reactor operating without biofilm growth faced filamentous bulking problems. It was observed that the incorporation of an optimized amount of support material for biofilm growth seemed to overcome this problem, apparently due to the particle-to-particle collisions established and to the increase of the overall quantity of biomass and subsequent decrease of the biomass loading rate.

Molecular microbial analysis

To gain insights into the diversity and changes induced in the microbial communities by the increase in the reactor's support concentration, as previously revealed by microscopic inspections, a molecular approach combining PCR-DGGE, cloning and sequencing techniques was further used. Diversity and shifts in bacterial community was estimated based on DGGE patterns of the partial 16S rRNA genes amplified from the inoculum (I) and from suspended biomass (s) and biofilm (b) collected from the SBR (1, 2, 3 and 4, respectively) at the end of the experiments (120 days). Figure 4 depicts the obtained DGGE profiles as well as correspondent similarity indices (SI).

Figure 4 - DGGE patterns of bacterial 16S rRNA gene fragments amplified from suspended biomass (s) and biofilm (b), collected from the SBR (1, 2, 3 and 4, respectively) on day 120, and from the inoculum (I). Corresponding similarity (in %)

matrix is also presented. Numbered DGGE bands (1-5) were further identified by cloning and sequencing.



	SBR4b	SBR4s	I	SBR3b	SBR3s	SBR2b	SBR2s	SBR1
SBR4b	100							
SBR4s	62.4	100						
I	30.7	38.3	100					
SBR3b	55.2	47.4	33.2	100				
SBR3s	23.4	25.5	24.5	84.6	100			
SBR2b	39.4	36.1	30.5	60.5	59.6	100		
SBR2s	32.9	31.7	30.0	63.6	67.3	97.7	100	
SBR1	13.1	11.6	24.2	54.5	55.7	61.7	63.2	100

At the end of the experiments, the bacterial community patterns were significantly different from the initial pattern of the inoculum, as indicated by the comparison of the calculated similarity indices: bacterial similarity between the inoculum and the biomass (suspended and fixed) from the SBR ranged between 24.2 % and 38.3 %. This clear shift in the bacterial structure suggests that a decrease in the diversity resulting from the biomass adaptation to the laboratory conditions occurred. At the end of the experiments, the similarity index between the biomass from SBR1 (control unit) and the suspended biomass from SBR2, SBR3 and SBR4 was 63.2 %, 55.7 % and 11.6 %, respectively, suggesting that higher differences in the bacterial community structure were obtained in the reactor with a higher support concentration. Furthermore, the increase in the concentration of support used in the reactor was found to induce increasing differences between the bacterial community present in the suspended biomass and in the biofilm (SBR2s/SBR2a - 97.7%, SBR3s/SBR3a – 84.8% and SBR4s/SBR4a – 62.4%).

To identify the prominent (more intense) bands in the bacterial community represented in the DGGE patterns, 16S rRNA-genes of two representative suspended biomass samples (SBR1 and SBR4s) were amplified, cloned and sequenced. The DGGE mobility of amplicons obtained from a total of 192 clones were compared to DGGE profiles of both biomass samples to determine to which fragments they corresponded. Table 1 summarizes the sequencing results and Figure 4 depicts their corresponding position in each DGGE profile.

Table 1 - Sequencing results of the selected bacterial clones.

Clone	Sequence length (bp)	Closest relatives (accession number, % sequence similarity)	Phylogenetic division[1]
1	1491	Bacterium rM5 (AB021340.1, 99 %), *Simplicispira psychrophila* strain CA 1 (AB076845.1, 97 %)	Betaproteobacteria
2	1501	*Pseudomonas* sp. BBTR25 (DQ337603.1, 99 %)	Gammaproteobacteria
3	1491	Uncultured beta proteobacterium clone OS1L-16 (AB076869.1, 99 %), *Comamonas* sp. PG6-1 (AB277849.1, 99 %)	Betaproteobacteria
4	1496	Uncultured bacterium clone BE16FW031401IDW-SAK15 (DQ088753.1, 99 %), *Thauera* sp. 27 (AY838760.1, 99 %)	Betaproteobacteria
5	1492	Uncultured bacterium clone 65 (DQ413124.1, 98 %), *Acidovorax ebreus* TPSY (CP001392.1, 97 %)	Betaproteobacteria

[1] According to Wang et al. (2007).

Sequencing and blast searching of the selected bacterial clones resulted mainly in matches with unknown and uncultured microorganisms assigned to the beta subclass of *Proteobacteria* group (clones 1, 3, 4 and 5). In addition, a close relative to *Pseudomonas* sp. (clone 2) belonging to the gamma subclass of *Proteobacteria* was also found. Bacteria from beta and gamma subclasses of *Proteobacteria* represent a collection of microorganisms that are commonly present in conventional activated sludge (Liu et al., 2007; Snaidr et al., 1997). *Pseudomonas*-like organisms (clone 2) are known for their production of extracellular polymeric substances (EPS) and ability to bind cells together (Ivanov et al., 2006; Li et al., 2009). Other members related to *Thauera* genera (clone 4) and *Comamonadaceae* family (clone 1, 3 and 5) are generally present in biological organic oxidation and nitrifying-denitrifying activated sludge (Li et al., 2008; Osaka et al., 2006).

None of the sequenced clones showed affiliation to genera possessing filamentous morphology and therefore could not be related to the filamentous microorganisms observed microscopically. As not all the discrete bands of the DGGE patterns of both biomass samples (SBR1 and SBR4s) matched with the total of the 192 clones collected for comparison, we may have missed this group of microorganisms. Nevertheless, a detailed microscopic inspection revealed that the filamentous microorganisms appeared to be fungi rather than bacteria (Figure 2). Large, truly branched and septate filaments resembling fungi hyphae were observed during the inspection. Intracellular vacuoles, organelles and granules seemed to be also present (Figure 2c). Additionally, microscopic observations after Calcofluor™ M2R staining revealed a positive result with a strong fluorescence signal (Figure 2a and 2b). Calcofluor™ M2R binds with chitin and cellulose and chitin is a constituent of the skeletal of the fungal cell wall (Hickey et al., 2004). Furthermore, filamentous fungi have been recently observed in aerobic granular sludge SBR treating high strength wastewaters (Weber et al., 2009; Weber et al., 2007) like that one used in this study.

The results obtained from the molecular microbial analysis showed clear shifts in the bacterial community induced by the increase in the reactor's support concentration. Phylogenetic analysis of the sequences corresponding to prominent ribotypes in the DGGE profiles suggested that the prevalent groups of bacteria present in the reactors are commonly related to bacteria known to thrive in conventional activated sludge. Moreover, the identified bacterial groups are not generally correlated with filamentous bulking problems, suggesting

that the filamentous bulking observed in this study has not a bacterial origin, but rather a filamentous fungi-like one.

CONCLUSIONS

From this work it can be concluded that:
- Filamentous bulking caused by an overabundance of a filamentous fungi-like microorganism was developed in the SBR operating just with suspended biomass.
- Filamentous bulking problems were successfully overcome through the incorporation of an optimized amount of support for biofilm growth. Two filamentous bulking control mechanisms were found to be of major importance: (i) physical cut or breakdown of filaments induced by particle-to-particle collisions and (ii) decrease of the biomass loading rate as a result of the increase of the overall quantity of biomass.
- Besides the observed differences in terms of the filamentous fungi-like microorganism, the incorporation of a support material for biofilm growth into the SBR was also found to induce differences in the bacterial community structure: as the concentration of support used in the SBR increased, increasing differences in the bacterial community structure were induced.

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