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## TROUBLESHOOTING OF FILAMENTOUS BULKING USING HYBRID SYSTEMS

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### KEYWORDS

Filamentous bulking control, hybrid systems, wastewater treatment

### ABSTRACT

The present work aims to evaluate filamentous bulking control in hybrid systems. For this study, four sequencing batch reactors (SBR) fed with 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 support-to-support collisions.

### INTRODUCTION

WWTP frequently face filamentous bulking – a term used to describe sedimentation problems caused by the filamentous microorganisms (bacteria and/or fungi). Several technologies have been used to reduce this problem (e.g. selector reactors). Although these technologies have been successful and have reduced filamentous bulking in many activated sludge systems, there are some reports that point out their failure (Martins 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), but this line of research wasn't continued. An interesting and important question is then how systems combining suspended and biofilm growth (hybrid systems) control filamentous bulking.

### MATERIALS AND METHODS

Four SBR with a working volume of 1.5 L were operated with a constant cycle time of 4 h (5 min fill, 225 min aerated, 5 min settle and 5 min draw), a volume exchange ratio of 0.5 L L<sup>-1</sup> and a resulting hydraulic retention time of 8 h. 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 (Nogueira et al. 2009). The support concentration was 5 % (SBR2), 10 % (SBR3) and 20 % (SBR4). During the aerated phase, airflow of 2 L min<sup>-1</sup> was applied through membrane diffusers, making the reactors' content, 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<sup>-1</sup> day<sup>-1</sup>. The reactors were inoculated with activated sludge coming from the Serzedelo I Wastewater Treatment Plant (Guimarães, Portugal).

Microscopic observations of the microbial communities were carried out 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 in an epifluorescence microscope (Olympus BX51) using an excitation wavelength of 365 - 370 nm and an emission longpass filter by 421 nm. Suspended biomass and biofilm concentration were determined according to the *Standard Methods*.

### RESULTS AND DISCUSSION

Four reactors (SBR1–SBR4) were operated with different amounts of support for biofilm growth. Fig 1 shows the micrographs of the suspended biomass on day 120. The microscopic observations revealed that filamentous microorganisms were quite common in SBR1 and SBR2, while in the other reactors (SBR3 and SBR4) their occurrence was negligible. The results obtained suggested that increasing the support

concentration for biofilm growth suppressed the excessive growth of filamentous microorganisms. It was observed that filaments length in SBR3 and SBR4 seemed to be considerably shorter than in SBR1 and SBR2 (Fig 2). These results suggested 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 support-to-support collision frequency 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 support-to-support collisions established were not enough to control filamentous bulking as this reactor presented lower support concentration (5 %).

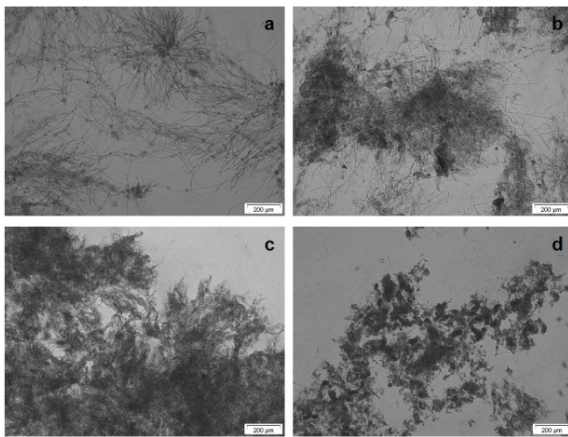


Figure 1: Micrographs of the suspended biomass from SBR1 (a), SBR2 (b), SBR3 (c) and SBR4 (d) on day 120 taken with an Olympus Altra-20 camera in a Leitz phase contrast microscope

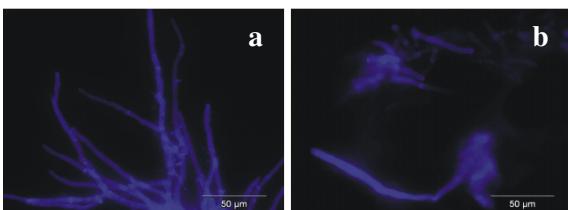


Figure 2: Micrographs of the suspended biomass from SBR1 (a) and SBR4 (b) on day 120, after calcofluor staining, taken with an Olympus DP71 camera in an epifluorescence Olympus BX51 microscope

It was also observed that the suppression of the overgrowth of filamentous microorganisms in 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. SBR3 and SBR4 had higher total biomass concentration ( $3.4 - 7.9 \text{ g L}^{-1}$  and  $3.9 - 6.7 \text{ g L}^{-1}$ , 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

( $0.6 - 2.1 \text{ g L}^{-1}$  and  $1.4 - 5.2 \text{ g L}^{-1}$ , respectively) where a relative high proliferation of filamentous microorganisms was observed (Fig 1a and 1b).

The microscopic inspection revealed that the filamentous microorganisms appeared to be fungi. Large, truly branched and septate filaments resembling fungi hyphae were observed during the inspection. Intracellular vacuoles, organelles and granules seemed to be also present (Fig 3). Additionally, microscopic observations after Calcofluor™ M2R staining revealed a positive result with a strong fluorescence signal (Fig 2). Calcofluor™ M2R binds with chitin and cellulose and chitin is a constituent of the skeletal of the fungal cell wall.

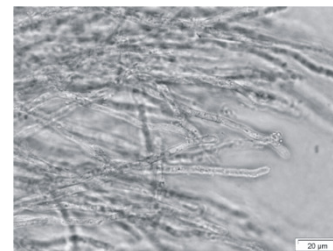


Figure 3: Phase contrast micrograph (Leitz, Laborlux S) of the observed filamentous fungi-like microorganism

## 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 support-to-support collisions and (ii) decrease of the biomass loading rate as a result of the increase of the overall quantity of biomass.

## ACKNOWLEDGEMENTS

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