

BIOFILM REACTOR TECHNOLOGY AS AN ALTERNATIVE TO CONTROL FUNGAL FILAMENTOUS BULKING CAUSED BY *GALACTOMYCES GEOTRICHUM*

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Abstract

The present work aims to evaluate a strategy for solving fungal filamentous bulking caused by *Galactomyces geotrichum*. 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 *G. geotrichum* in the SBR operating just with suspended biomass. The incorporation of an optimized amount of support for biofilm growth (10 %) seemed to suppress the overgrowth of the *G. geotrichum* filaments probably due to the combined effect of a decreased biomass loading and an increased shear force.

Keywords

Biofilm reactors, filamentous bulking control, *Galactomyces geotrichum*, support concentration, wastewater treatment

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. 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 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 biomass 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.

Filamentous bulking studies have been focused only on bacteria being that fungi have not been considered. Fungi were not considered important members of the community being indicated as the dominant or secondary bulking filament in approximately 1 % and 2 % of wastewater treatment plants; however, it is generally agreed that they are probably more common than reported because researchers are not looking for fungi and fungi have atypical forms in wastewaters. In this context, the present work aims to evaluate fungal filamentous bulking caused by *Galactomyces geotrichum* (a fungus commonly isolated from WWTP) in systems combining suspended and biofilm growth.

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⁻¹ 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) of the working volume (V_T). During the aerated phase, airflow of 2 L min⁻¹ 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⁻¹ day⁻¹. The experimental conditions of the control unit were known to promote fungal filamentous bulking caused by *G. geotrichum* (Matos et al., in preparation). The reactors were inoculated with activated sludge coming from the Serzedelo 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 fungal 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: SBR1 – 0 % V_T , SBR2 – 5 % V_T , SBR3 – 10 % V_T and SBR4 – 20 % V_T . Figure 1 shows the micrographs of the suspended biomass on day 120.

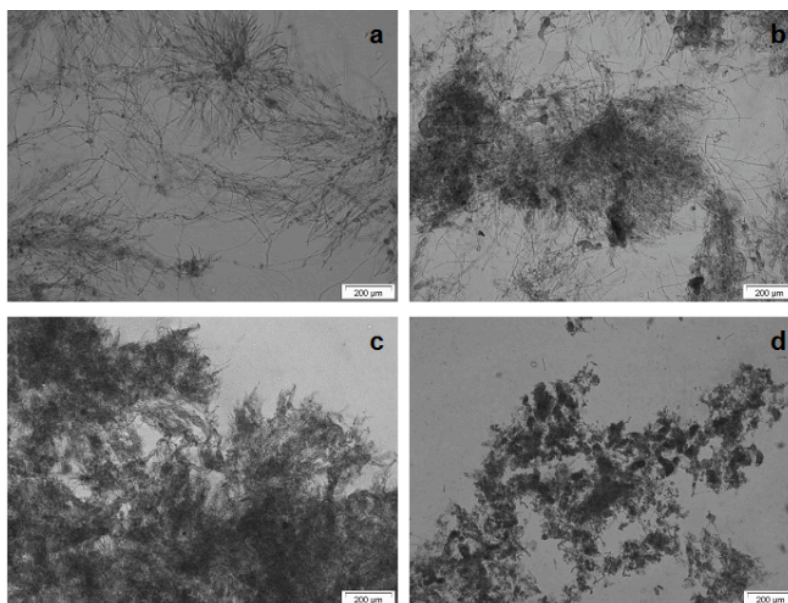


Figure 1. Micrographs of the suspended biomass from SBR1 (a), SBR2 (b), SBR3 (c) and SBR4 (d) on day 120 taken with an Olympus Altra-20 camera in a Leitz phase contrast microscope.

The microscopic observations revealed that *G. geotrichum* filaments 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 *G. geotrichum* filaments.

It was observed that filaments length in SBR3 and SBR4 seemed to be considerably shorter than in SBR1 and SBR2 (Figure 2). These results suggested that filamentous bulking in SBR3 and SBR4 was suppressed due to the shear force established by collisions between supports. In fact, additional tests with pure *G. geotrichum* culture (data not showed) demonstrated that a higher shear force may induce the growth of *G. geotrichum* with a different morphology. SBR3 and SBR4 had a support concentration of 10 % and 20 % which led to a high support-to-support collision frequency and accordingly, to a higher shear force. 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 %).

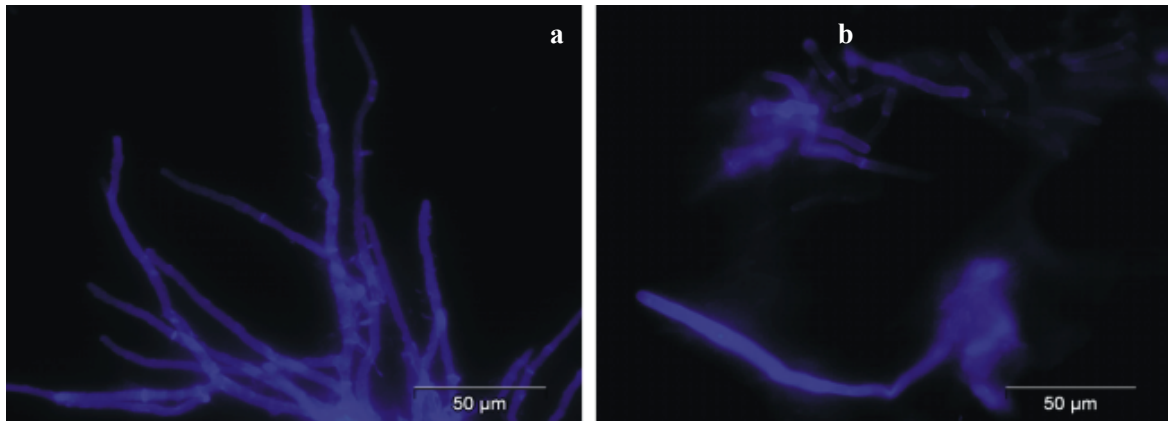


Figure 2. Micrographs of the suspended biomass from SBR1 (a) and SBR4 (b) on day 120, after calcofluor staining, taken with an Olympus DP71 camera in an epifluorescence Olympus BX51 microscope.

It was also observed that the suppression of the overgrowth of *G. geotrichum* filaments 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 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 *G. geotrichum* filaments was observed (Figure 1a and 1b).

Conclusions

From this work it can be concluded that fungal filamentous bulking problems caused by *G. geotrichum* 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) increase of the shear force 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.

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