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GROWTH OF *GALACTOMYCES GEOTRICHUM* IN SEQUENCING BATCH REACTORS UNDER DIFFERENT ORGANIC LOADING CONDITIONS

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KEYWORDS
Fungal filamentous bulking, *Galactomyces geotrichum*, organic loading rate, sequencing batch reactors

ABSTRACT

The present work aims to study the effect of the organic loading rate on the growth of filamentous fungi. For this purpose, three sequencing batch reactors (SBR), fed with an easy biodegradable substrate (acetate), were operated at different organic loading rates: 4.3 g COD L^{-1} day^{-1} (SBR1), 1.0 g COD L^{-1} day^{-1} (SBR2) and 0.5 g COD L^{-1} day^{-1} (SBR3). High amounts of fungal filaments were observed in the SBR operating at higher organic loading rate, as ascertained by direct microscopic inspection, while, at lower organic loading rates, overabundance of fungal filaments was not observed. Sequence retrieved from the isolated fungal filaments presented high similarity (99 %) to *Galactomyces geotrichum.*

INTRODUCTION

Recent studies reporting the useful implementation of fungi in wastewater treatment plants triggered the need to improve fungi based systems. Therefore, it is crucial to investigate the conditions that promote their selection.

The literature on causes and control of filamentous bulking provides information on the conditions that promote fungal growth, given that most fungi grow as hyphae. According to the existing literature, low pH has been the unique characteristic associated with fungal filamentous bulking; however, recent studies observed fungal filamentous bulking in reactors operating in neutral pH ranges meaning that other factors such as wastewater type and organic loading rate might provide selective pressure. In this context, the present work aims to evaluate the effect of the organic loading rate on the filamentous bulking caused by fungi.

MATERIALS AND METHODS

Three sequencing batch reactors (SBR) with a working volume of 1.5 L were operated with a constant cycle time of 4 h, a volume exchange ratio of 0.5 L L^{-1} and a resulting hydraulic retention time of 8 h. The duration of the individual operating phases was: 5 min fill, 225 min aerated, 5 min settle and 5 min draw. During the aerated phase, airflow of 2 L min^{-1} was applied through membrane diffusers and the dissolved oxygen (DO) concentration in the reactors was above 2 mg L^{-1}. The mean pH values in the SBR were between 7.5 and 8.5. The reactors were operated with synthetic wastewater containing acetate as carbon source and other nutrients. The three reactors were operated in the same conditions except for the applied organic loading rate, i.e. for the feeding acetate concentration. SBR1 was operated at an organic loading rate known to promote fungal filamentous bulking (4.3 g COD L^{-1} day^{-1}) (Matos et al. 2010). The organic loading rate was changed in the other SBR (1.0 g COD L^{-1} day^{-1} and 0.5 g COD L^{-1} day^{-1} for SBR2 and SBR3, respectively) and the presence of fungal filaments was assessed by microscopic observations (Matos et al. 2011). Conventional plating, isolation and microscopic techniques together with molecular methods were used to identify the fungal filaments (Matos et al. 2011).

The reactors were inoculated with activated sludge coming from the Serzedelo I Wastewater Treatment Plant (Guimarães, Portugal).

Acetate, biomass concentration and standard sludge volume index (SVI) were monitored weekly according to the *Standard Methods*.

RESULTS AND DISCUSSION

Influence of the Organic Loading Rate on the Growth of Fungal Filaments

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Three reactors (SBR1 – SBR3) were operated at different organic loading rates (4.3 g COD L^{-1} day^{-1}, 1.0 g COD L^{-1} day^{-1} and 0.5 g COD L^{-1} day^{-1} for SBR1, SBR2 and SBR3, respectively) in order to study its effect on the growth of fungal filaments.

Figure 1 depicts the quality of the sludge in all experimented systems on day 60. Filaments were abundant in SBR1 (with SVI values of about 680 mg L^{-1}), while in SBR2 and SBR3 their occurrence was occasional (with SVI values of 109 mg L^{-1} and 129 mg L^{-1}, respectively).

The observed filaments were identified as fungi (data not shown). The filaments were Gram positive and Neisser negative. They were large, truly branched and septate resembling fungi hyphae. Intracellular vacuoles, organelles and granules were also observed. Additionally, microscopic observations after Calcofluor™ M2R staining revealed a positive result with a strong fluorescence signal. Calcofluor™ M2R binds with chitin and cellulose and chitin is a constituent of the skeletal of the fungal cell wall.

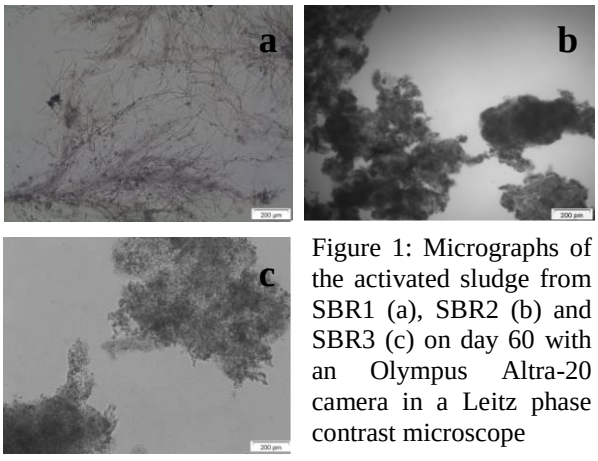

Figure 1: Micrographs of the activated sludge from SBR1 (a), SBR2 (b) and SBR3 (c) on day 60 with an Olympus Altra-20 camera in a Leitz phase contrast microscope

Fungal Filaments Identification

In order to identify the fungal filaments, culturing and isolation techniques were used followed by DNA extraction, amplification and sequencing of PCR amplified products.

Diluted SBR1 sludge samples (grab samples taken on day 60) were spread plated onto NGRBA medium (Matos et al. 2011). The cultures revealed filamentous colonies and a few counts of non-filamentous colonies. The non-filamentous colonies were considered to be yeasts. The filamentous colonies were transferred to MEA for isolation. Only one fungus type was isolated. The isolated fungus produced rapidly growing white powdery colonies (Figure 2).

Sequencing and blast searching of the isolated fungus ITS region resulted in match with *Galactomyces geotrichum* (99 % sequence similarity). *G. geotrichum* is a teleomorph of *Geotrichum candidum*. The fungus was already isolated from wastewater sludge by others and it is known for its ability to decolorize synthetic dyes and olive mill wastewater.

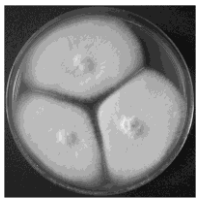

Figure 2: Photograph of the isolated filamentous fungus on MEA

CONCLUSIONS

Fungal filamentous bulking caused by an overabundance of *G. geotrichum* filaments developed in a SBR operating at a high organic loading rate of 4.3 g COD L^{-1} day^{-1}. At lower organic loading rates of 1.0 g COD L^{-1} day^{-1} and 0.5 g COD L^{-1} day^{-1}, the fungal filaments were not observed.

ACKNOWLEDGEMENTS

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REFERENCES

Matos, M.; Pereira, A.P; Nicolau, A.; Rodrigues, A.L.; Brito, A.G; and Nogueira, R. 2010. "Troubleshooting of filamentous bulking using biofilm reactors". In Proceedings of the 2010 *WEF/IWA Biofilm Reactor Technology Conference* (Portland, OR, USA, Aug. 15-18). 162-175.

Matos, M.; Pereira, A.P; Nicolau, A.; Rodrigues, A.L.; Brito, A.G; and Nogueira, R. 2011. "Influence of the organic loading rate on the growth of *Galactomyces geotrichum* in activated sludge". Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substance & Environmental Engineering (in press).