



# Influence of feeding pattern and hydraulic selection pressure to control filamentous bulking in biological treatment of dairy wastewaters



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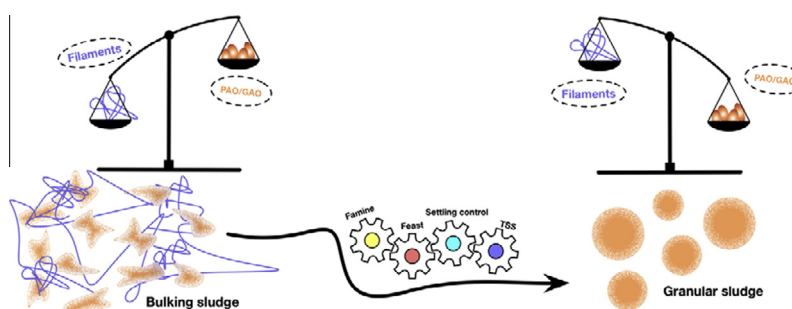
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## HIGHLIGHTS

- AGS technology as a tool to control bulking sludge.
- Low hydraulic selective pressure is needed to limit filamentous bulking.
- High hydraulic washout of particles is unnecessary to get well-settling granular sludge.
- Process efficiency, in terms of hydraulic and organic loads, is enhanced.

## GRAPHICAL ABSTRACT



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## ABSTRACT

In sequencing batch reactors (SBRs) treating dairy wastewaters, the overgrowth of filamentous bacteria is a frequent cause of operational problems. The present study aimed at understanding to what extent the operating conditions of a SBR can be optimized to convert a bulking activated sludge into a well-settling biomass at low aeration velocity. The abundance of filament morphotypes and floc-formers able to store biopolymers were analysed by PCR-DGGE and 16S amplicon sequencing. The results indicated that a combination of an anaerobic-microaerated feeding pattern with a low selective pressure was beneficial to suppress filamentous overgrowth and to form aerobic granules, while increasing the efficiency of suspended solid removal. Average removal efficiencies for total chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) were  $94 \pm 2\%$ ,  $95 \pm 1\%$  and  $83 \pm 13\%$ , respectively.

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## 1. Introduction

Biological treatments, especially activated sludge (AS) processes, are common techniques to eliminate biodegradable matter from urban and industrial wastewaters. Despite hundred years of operation and progress, serious operating issues still occur with AS technology. One major problem is the bulking of the sludge

characterized by an excessive growth of filamentous bacteria. This problem causes biomass washout, environmental pollution and high discharge costs.

Despite the recent progress in microbial ecology, little is known about the physiology and kinetics of specific types of filamentous bacteria. The limitation or deficiency of dissolved oxygen (DO) is generally considered as an operational factor leading to the proliferation of filamentous bacteria (Martins et al., 2003). The increase of aeration rate is a common operational method to mitigate sludge bulking but it results in a waste of aeration energy

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consumption. Other factors leading to the development of loose filamentous structures are low concentrations of readily biodegradable substrates (rbCOD), diffusion-limited substrate uptake rate by microorganisms and poor storage capacity of the biomass (Martins et al., 2004a).

Until now, preventive actions against bulking are based on the construction of bio-selectors (including aerobic, anoxic and anaerobic selectors) that enable the rbCOD to be consumed at high concentrations into a first selector tank. In the case of sequencing batch reactor (SBR) technologies, selector-like systems can be obtained by pulse-feeding patterns. Even though the incorporation of periods with (feast) and without (famine) rbCOD can give competitive advantages to non-filamentous bacteria, this is not necessarily always the case, especially for enhanced biological phosphorus removal (EBPR) systems (Vaiopoulou et al., 2007). In fact, the worsening of sludge settling characteristics is often attributed to the presence of microaerophilic conditions in the anaerobic step, which are generated during the mixing and pumping steps of wastewater (Martins et al., 2004b). Consequently, the wastewater treatment plant (WWTP) suffering recurrent bulking sludge phenomena often need to be partially redesigned which generates additional costs.

Another promising alternative to improve the settleability of biomass is based on the selective removal of filamentous bacteria with the purge of the effluent. This strategy was previously developed in SBR systems to generate dense microbial aggregates, viz. aerobic granular sludge (AGS). They were obtained by applying a short settling time that acts as a hydraulic selection pressure to eliminate undesirable particles displaying a low settling velocity (e.g. filaments or loose sludge flocs) and to simultaneously promote the formation of denser granular architectures within the reactor (de Kreuk and van Loosdrecht, 2006; Rocktäschel et al., 2015). However, aerobic granules are also susceptible to be colonized by filamentous morphotypes (Wagner et al., 2015; Pronk et al., 2015) especially in the cases of treatment of wastewaters from industrial origins (Schwarzenbeck et al., 2004; Figueroa et al., 2011).

The control of filamentous bulking is therefore lacking a deeper knowledge on fundamentals, including the role of the sludge inoculum, the impact of hydraulic selective pressure on the decantation process and effluent turbidity, as well as the long-term stability of the process. In particular, it is still unknown if a hydraulic selection pressure would worsen or improve the properties of a bulking sludge.

The present study aimed at evaluating how the combined hydraulic selection pressure with an anaerobic-microaerated feeding pattern can promote the formation of a well-settling biomass and selectively reduce the abundance of filament morphotypes. Managed with a low aeration velocity, the performances of a lab-scale SBR treating nutrient rich industrial wastewater were monitored over 300 days. The findings allow tailoring an optimal strategy to eliminate the filaments in conventional activated sludge SBR.

## 2. Material and methods

### 2.1. Lab-scale system set-up

The experiment was conducted in a sequencing batch reactor with an internal diameter of 12 cm, a height of 40 cm and a working volume of 4.5 L.

The reactor was operated over 300 days at room temperature ( $20 \pm 3$  °C). The pH was initially adjusted between 7.3 and 7.6 with sulfuric acid and sodium hydroxide during the 160 first days of experiments. Then the pH value remained between 7.0 and 8.0

without any correction. The oxygen concentration was set between 3.0 and 8.0 mg L<sup>-1</sup> by varying the air to nitrogen ratio. The mixed gas was introduced through a fine bubble aerator at the bottom of the reactor with a variable flow rate ( $<4$  L min<sup>-1</sup>,  $<0.006$  m s<sup>-1</sup>). The inoculated bulking sludge was collected at the full-scale dairy WWTP between 03.02.2014 and 14.02.2014. Wastewater was collected from a local dairy industry (different from the full-scale plant) once a week and stored at +3 °C. The feed was warmed up to  $20 \pm 3$  °C prior to feeding into the reactor. The feeding mode was either an aerated fill or an anaerobic-microaerated fill. In the latter case, the anaerobic feeding was combined with short aeration pulses of 10 s (at a flow rate of 3 L min<sup>-1</sup>) every 20 min in order to provide a sequential mixing process while keeping oxygen concentration below 0.1 mg L<sup>-1</sup>. The average composition of the wastewater used throughout the experiment was similar to the pre-treated influent of the full-scale plant described in Table 2. The experimental approach was divided into five periods (Table 1). During the first period (Initiation), the SBR was initially operated in the same conditions as the full-scale plant to confirm the reproducibility of the bulking event at the lab scale. The second and third periods (Storage metabolisms) were devoted to the selection of floc-forming microorganisms able to store polymers such as phosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) under high or low hydraulic selective pressures. The fourth period (Intensification) was dedicated to evaluate the robustness of the process by decreasing the length of the cycle. In these conditions, the average organic loading rate (OLR) was set at 2.5 kg of COD m<sup>-3</sup> d<sup>-1</sup>. Finally, the fifth period (Maturation) focused on the improvement of effluent quality by optimizing the settling time.

### 2.2. Description of the full-scale plant

The industrial WWTP treats on average 860 m<sup>3</sup> d<sup>-1</sup> of dairy wastewater characterized by a high COD concentration. The influent was first collected in an equalisation buffer tank (900 m<sup>3</sup>). About half of the COD content was removed with a dissolved air flotation unit. The stream was then treated with two conventional SBRs operated in parallel ( $2 \times 1600$  m<sup>3</sup>). In order to reduce the aeration time required in the reaction step, the filling phase was performed under aerated conditions. The dissolved oxygen concentration was controlled at 3 mg L<sup>-1</sup>. The reaction step involved the addition of a metal salt for the precipitation of phosphorus. The settling was achieved with a floating decanter. The whole cycle varied between 8 and 12 h. Wastewater characteristics are given in Table 2. The designed sludge loading rate was 0.2 kg COD kg<sup>-1</sup> total suspended solids (TSS) d<sup>-1</sup>.

### 2.3. Physical and morphological characteristics of the inoculum

The inoculum contained both filamentous biomass and spherical particles. Regarding the operating conditions of the plant, the particles were assumed to be formed through a physico-chemical mechanism (i.e. coacervation process). In order to support this hypothesis, the composition of this particular type of granules was examined by field emission scanning electron microscopy (FE-SEM) on a JEOL JSM-7500F electron microscope with sample sputter-coated with gold. Briefly, samples were rinsed with ethanol and supercritically dried with liquid carbon dioxide to preserve the porous network by avoiding the collapse of pores through capillary action. The chemical composition of the samples was characterized by an energy-dispersion X-rays (EDX) analysis system using an acceleration potential of 15 kV and a working distance of 8 mm.

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