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Filamentous and non-filamentous bulking of activated sludge encountered under nutrients limitation or deficiency conditions

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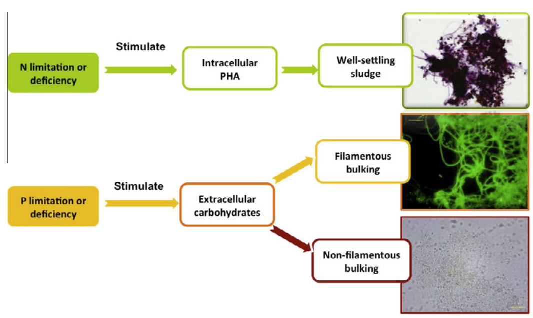
HIGHLIGHTS

- Effects of N/P deficiency on SVI, EPS, PHA and microbial community structure were examined.
- Bulking was not encountered in reactors with nitrogen limitation or deficiency.
- Bulking was encountered in those reactors fed with wastewater deficient in phosphorus.
- N limitation/deficiency stimulates formation of intracellular storage products (PHA).
- P limitation stimulates formation of carbohydrates.

GRAPHICAL ABSTRACT



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ABSTRACT

Although the limitation or deficiency of nutrients, such as nitrogen (N) and phosphorus (P), has been one of the frequently reported factors causing filamentous or non-filamentous bulking of activated sludge, the mechanisms are still unclear. In this work, the long-term effects of N and P limitation or deficiency on sludge settleability and bioflocculation characteristics were investigated in six sequencing batch reactors (SBRs) fed with wastewater with different nutrient availability. The sludge volume index (SVI), microbial community structures, intracellular poly-β-hydroxyalkanoates (PHAs) and extracellular polymeric substances (EPS) were characterised over time. Bulking was not observed in SBRs with N limitation or deficiency, in which SVI remained below 150 mL/g. In contrast, bulking was encountered in those reactors with P deficiency. The occurrence of non-filamentous bulking was associated with a higher carbohydrates fraction and a lower proteins fraction in EPS. In the case of filamentous bulking, SVI correlated negatively with the amount of PHAs. Our experimental data support the hypothesis that the occurrence and/or the type of bulking in activated sludge could be affected by the combination of kinetic selection, microbial storage, as well as the EPS composition.

1. Introduction

The performance of an activated sludge system for biological wastewater treatment is often deteriorated due to sludge separation problems caused by sludge bulking. Bulking consists of filamentous bulking due to excess proliferation of filamentous bacteria [1] and non-filamentous bulking (also known as *Zoogloeal* bulking or viscous bulking) [2], resulting from certain microbes that produce large amounts of biopolymers on their surface [3].

The causes for inducing filamentous bulking are complicated [4] and include factors such as low dissolved oxygen (DO) concentrations [5–7], low organic loading rates [8], low substrate

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concentration gradients [9], low pH [10], and low temperatures [11]. Nutrient limitation [12] has also been identified as a factor for the proliferation of filamentous bacteria in activated sludge. In order to obtain well-settling sludge, the ratio of biological oxygen demand (BOD) to nitrogen (N) to phosphorus (P) in influent should generally satisfy 100:5:1 [1]. Peng et al. [13] showed that filamentous bulking was stimulated by the lack of either N or P in the feed. However, the simultaneous absence of both N and P did not induce filamentous bulking [13]. Low nutrient supplies have also been suggested to cause non-filamentous bulking. It was reported that activated sludge treatment of nutrient-deficient wastewater such as some types of industrial wastewaters led to severe slime formation and consequently biomass separation difficulties due to non-filamentous bulking [2,14,15]. Non-filamentous bulking at a full-scale wastewater treatment plant (WWTP), which was hypothetically due to low concentrations of soluble phosphate (0.2 mg/L), was solved by supplying additional soluble phosphate [16].

However, the mechanisms involved in both filamentous and non-filamentous bulking induced by nutrient limitation/deficiency are not fully understood at present. There is still controversy about which bulking type would be caused under nutrient limitation. On one hand, it is hypothesised that nutrient deficiency has an effect on the competition between floc-forming and filamentous bacteria, causing filamentous bulking when filamentous bacteria proliferate due to their enhanced ability to uptake substrates under stress conditions [13,17]. On the other hand, nutrient limitation has also been hypothesised to induce the production of extracellular polymeric substances (EPS) on the surface of microorganisms [18]. The EPS are important for the physicochemical properties of activated sludge flocs and have been implicated to affect sludge settling properties [19], inducing non-filamentous bulking. In addition, when sludge is subject to nutrients limitation or deficiency, more carbon substrate can be used for accumulation of poly-hydroxyalkanoates (PHA) and glycogen [20], which would affect the competition between filaments and floc-formers, as well as sludge settleability.

The objective of this study was to shed light on the mechanism of filamentous and non-filamentous bulking of activated sludge induced by nutrients limitation or deficiency, through a comprehensive experimental study. Six lab-scale sequencing batch reactors (SBR) were operated for 130–230 days with various nutrients-supplying conditions. The sludge volume index (SVI), PHA storage and EPS composition, Gram and Neisser staining, fluorescent *in situ* hybridization (FISH) and microscopic observations were used to monitor sludge properties and to track the changes of microbial morphology and community structure. These experimental data led to the connections between bulking type and the associated sludge properties, including sludge settleability, microbial structure, intracellular storage and extracellular polymeric substrates under the stress condition of nutrients limitation or deficiency.

2. Materials and methods

2.1. Lab-scale SBR reactors

The experiments were performed in six identical SBRs each with a 12-L working volume. Each reactor was equipped with an air compressor for aeration and a stirrer for mixing. Operation of the SBRs was based on 6 h cycles consisting of a feed phase (10 min) in which 6 L fresh medium was supplied giving rise to a hydraulic retention time of 12 h, an anoxic phase (110 min), an aerobic phase (180 min), a settling phase (50 min) and an effluent withdrawal phase (10 min) in which 5.85 L of reactor supernatant were withdrawn. The bulk liquid DO concentration in aerobic periods was controlled at 2.0 ± 0.2 mg/L under aerobic periods. Temperatures in all reactors were controlled at 25 ± 2 °C. pH was recorded but not controlled, and fluctuated between 7.0 and 7.5. The biomass concentrations in all reactors were kept in the range of 1800–2400 mg/L with sludge wasting that ensured an operation at a sludge age of 20 days of each reactor. The surfaces of tube, pumps and reactors were cleaned manually weekly in order to prevent biomass attachment.

The feed conditions for the SBRs are summarised in Table 1. SBRs 1–4 were operated for 233 days, to investigate the effects of nutrient deficiency on sludge settleability and microbial community structure. With COD/N/P ratios being 300:30:10 in the feed, SBR1 was operated as a control. SBR2 (COD/N/P set at 300:0:10), SBR3 (COD/N/P set at 300:30:0) and SBR4 (COD/N/P set at 300:0:0) were operated to investigate the effects of N, P and simultaneous N&P deficiency respectively. In order to investigate the combined effects of sludge cultivation history and influent nutrient ratios, which may affect the types of bulking and the dominant filaments, two additional reactors (SBRs 5–6) were operated for 130 days in three phases. Phase I (days 1–24) was used to collect base line data with normal feed (COD/N/P set at 300:30:15). The effects of N limitation (COD/N/P set at 300:5:15) and P limitation (COD/N/P set at 300:30:1) on sludge proprieties were investigated during Phase II (days 25–66) in SBR5 and SBR6, respectively. The effects of N deficiency and P deficiency on sludge proprieties were further investigated during Phase III (days 67–130).

2.2. Synthetic wastewater and seeding sludge

The medium for the SBRs consisted of a carbon source, a nutrient solution and a trace element solution. The normal synthetic wastewater contained CH_3COONa of 4.69 mM (300 mg COD/L), NH_4Cl of 2.14 mM (30 mg N/L), KH_2PO_4 of 0.32 mM (10 mg P/L in SBRs 1–4) or 0.48 mM (15 mg P/L in SBRs 5–6), $MgSO_4{\cdot}7H_2O$ of 0.37 mM, KCl of 0.48 mM, $CaCl_2{\cdot}2H_2O$ of 0.10 mM and 1 mL/L of the following trace element solution: EDTA 10 g/L, $ZnSO_4{\cdot}7H_2O$ 0.12 g/L, $Na_2MoO_4{\cdot}2H_2O$ 0.06 g/L, $MnCl_2{\cdot}4H_2O$ 0.12 g/L, KI 0.18 g/L, $CuSO_4{\cdot}5H_2O$ 0.03 g/L, H_3BO_3 0.15 g/L, $FeCl_3{\cdot}6H_2O$ 1.5 g/L. Under the conditions of nutrients limitation and deficiency, N and/or P concentrations in the synthetic wastewater were modified according to the specific values describe in Table 1.

Each SBR was inoculated with 2 L seed sludge from the secondary clarifier of the GaoBeiDian WWTP (Beijing, China). The seed sludge had a good settling property (SVI < 100 mL/g), in which only limited filamentous bacteria (Type 0092 as the dominant filament) were present as a floc backbone.

2.3. Analytical methods

The temperature, pH and DO were monitored on line using WTW pH/DO meters (WTW Multi 340i, Germany). Supernatant

Table 1
Summary of feed conditions in six reactors with different influent nutrient ratios.

Reactor	Phase	Time (days)	C/N/P (mgCOD:mgN:mgP)	Remarks
1	–	1–233	300/30/10	Control
2	–	1–233	300/0/10	N deficiency
3	–	1–233	300/30/0	P deficiency
4	–	1–233	300/0/0	N&P deficiency
5	I	1–24	300/30/15	Control
	II	25–66	300/5/15	N limitation
	III	67–130	300/0/15	N deficiency
6	I	1–24	300/30/15	Control
	II	25–66	300/30/1	P limitation
	III	67–130	300/30/0	P deficiency