Effect of Sludge Loading and Dissolved Oxygen Concentration on Proliferation of *Thiothrix* and Eikelboom Types 1851 and 0041 in Activated Sludge Wastewater Treatment Plants

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Abstract

Filamentous bacteria have been implicated in poor solids separation in activated sludge plants, a problem referred to as sludge bulking. Chemical treatment of this condition remains costly and only offers short-term remedy. In this study, two full-scale wastewater treatment plants were monitored in order to establish relationships between filamentous overgrowth and plant operational conditions with the aim to develop specific strategies for bulking control. Morphological and molecular techniques were applied for identification of dominant filamentous bacteria while plant process parameters were determined using standard methods. Low dissolved oxygen concentration (< 2.0 mg/l) and high sludge age (> 14 days) were confirmed as major factors favoring the proliferation of Thiothrixspp and Eikelboom Types 1851 and 0041 in industrial and municipal plants respectively. Additionally, the results point to a specific limiting substrate effect in the occurrence of filamentous bacteria since similar conditions resulted in the proliferation of different filaments in the various plants. An aerobic selector designed with a short hydraulic residence time and sufficient oxygen supply is a possible strategy to control bulking caused by these filaments.

Key words: Sludge loading, dissolved oxygen concentration, filamentous bacteria proliferation.

1. Introduction

Environmental pollution due to discharge of untreated or insufficiently treated wastewater remains a serious problem especially in developing countries. Destruction of aquatic ecosystems due to cultural eutrophication has been associated with discharge of poor quality effluents (Horan 2003; Mara 2004).

In addition, there is a potential risk of transmission of water-related diseases when such water bodies are dependent upon for domestic and recreational purposes (Bitton 2005). To address this problem, various methods of wastewater treatment have been developed. The activated sludge process is the most widely used method due to its small land requirement, high effluent quality and flexibility with regard to process configuration (Grady et al. 2011). However, a major operational difficulty that has bedeviled this method worldwide for decades is sludge bulking caused by overgrowth of filamentous bacteria (Guo et al. 2012). This condition results in poor sludge settling and compaction, deterioration of effluent quality and loss of active biomass. Non-specific methods involving the application of chemicals have been traditionally applied to control this menace. Unfortunately, the use of these methods is limited due to several reasons. For instance, serious filamentous bulking and worsening sludge settle ability after chemical addition was discontinued have been reported (Juang 2005), while chlorination has been found to be ineffective in treating *Microthrixparvicella* (Guo et al. 2012), and actually worsens *Nocardia* foaming (Jenkins et al. 2004). Also, these methods are costly and only offer temporary solution as they do not remove the causes for the excessive growth of the filamentous bacteria (Tsang et al. 2008).

In an attempt to overcome these limitations, studies have focused on the development of specific methods of bulking control. These preventive strategies involve establishing relationships between the overgrowth of the bulking filaments and plant process parameters to achieve a more permanent solution to the bulking problem (Guo et al. 2010). Based on such studies, certain process parameters have been identified to generally influence bulking (Van Der Waarde et al. 2002; Gaval & Pernelle, 2003; Thompson & Forster, 2003). However, successful control of bulking still remains a major challenge because most of the studies were performed on laboratory and pilot-scale which makes their applicability at full-scale plants to sometimes produce different results. Also, the possible effect of geographic differences on the growth of these filaments has not been fully investigated. The aim of this research was to optimize the activated sludge process by correlating plant operation conditions with overgrowth of specific filamentous bacteria. The findings would contribute to the protection of the environment and human health.

2. Materials and Methods

Two wastewater treatment plants, one treating municipal waste and the other industrial waste, were used for this study. Plants configuration is based on the completely mixed method without nutrient removal. Aeration in both plants is done intermittently using diffuse aerators. The capacity of the industrial plant is 1600 m^3 while that of the municipal plant 1000 m^3. The plants operate at a sludge loading of < 0.1 Kg BOD/KgMLSS. day. Samples were collected over a one-year period from the aeration tanks of each treatment plant for chemical analysis and filamentous bacteria identification. On-site measurements were performed for dissolved oxygen, pH and temperature. Samples for chemical analyses were centrifuged (Megafuge 3.0R) at 5000 rpm for 5 min and the clear supernatants analyzed according to standard methods (APHA 1998). $NO_{3,}$ NH_4, $PO_{4,}$ and total phosphorus (TP) measurements were performed using a double-beam spectrophotometer (UV-2450, Shimadzu). All spectrophotometric measurements were preceded by running a quality control sample (blank) and the readings used to provide an unbiased reference point (auto zero). TN and TOC were measured with DIMA-N connected to a DIMATOC® 2000 analyzer (Dimatec Analysis, GmbH). Samples for molecular analysis were fixed immediately upon arrival in the laboratory. Fixation was done overnight at 4^oC and the final pellet gently suspended in 500 µL 1×PBS and 500 µL pure ethanol and stored at -21 ^oC.

Dominant filamentous bacteria were identified based on the identification keys of Eikelboom (2000) and Jenkins et al. (2004). Fluorescence *in situ* hybridization (FISH), involving the use of 16S rRNA probes, was applied to confirm the results of the morphological identification where necessary. Filament index (FI) was also rated according to the scoring technique outlined by Jenkins et al. (2004).

The following oligonucleotide probes were applied: (i) HHY, complementary to a region of the 16S rRNA of *Haliscomenobacterhydrossis*; (ii) TNI, complementary to a region of the 16S rRNA of *Thiothrixnivea*; (iii) CHL 1851, complementary to a region of the 16S rRNA of Eikelboom Type 1851; and (iv) EUB, complementary to a conserved region of bacterial 16S rRNA molecules. Detail information on these probes can be found in probe Base (Loy et al. 2007). Fixation and hybridization were performed according to the procedure described by Amann et al. (1995), and the protocol of the Chair of Biotechnology for Water Treatment, BTU. Detailed description of the procedure is contained in Adonadaga & Martienssen (2015). Hybridization was then visualized with Nikon Eclipse epifluorescence microscope (LV100), using a Nikon Intensilight C-HGFI as a light source.

3. Results and Discussion

Dissolved oxygen concentration in the aeration tanks was generally below the recommended 2 mg/l required for optimal operation, with an average temperature of 30 ^{o}C recorded in each of the plants. Nutrient levels were generally high in both plants although much higher in the municipal plant (Table 1). A higher nutrient level in the municipal plant is expected considering that such plants are normally nutrient rich. Also, the industrial wastewater is expected to have a certain level of municipal character considering that the factory processes food and beverages. A likely reason for the TP values being higher than the TN values in the industrial plant is the fact that industrial activities as well as synthetic detergents and other cleaning products are major sources of phosphorus in wastewater (Sedlak 1991).

Table 1: Levels of chemical parameters in aeration tank

	Aeration Tank	
Parameter	Industrial Plant	Municipal Plant
NH_4-N (mg/l)	3	31.1
TN (mg/l)	11.0	33.9
PO_4-P (mg/l)	15.8	2.7
TP (mg/l)	41.3	13.8
TOC (mg/l)	124	44
COD (mg/l)	460	45
pH	8.3	7.8
Temp (^{o}C)	28	29.7
DO (mg/l)	1.3	0.7

Thiothrix was confirmed as the dominant filament and responsible for the bulking observed in the industrial plant (Figure 1) while *H. hydrossis* was observed as the co-dominant filament. *H. hydrossis* was, however, not observed as contributing to the bulking despite being co-dominant. Low dissolved oxygen and long residence time of the influent were determined as favoring the overgrowth of *Thiothrix*. These process conditions provided a competitive advantage to *Thiothrix* in terms of substrate utilization. As reported by Larkin & Shinabarger (1983), type species *Thiothrixnivea* is an obligate mixotroph that requires a reduced sulfur compound and an organic compound for growth. Also, Waarde et al. (2002) revealed that sludge bulking in a wastewater treatment plant handling potato-processing waste was as a result of *Thiothrix* proliferation due to the presence of reduced sulfur compounds from the protein content in the wastewater. Considering that the studied industrial plant handles effluent from a food and beverages processing factory, the presence of reduced sulfur compounds is highly likely. The finding that *H. hydrossis* was not contributing to the bulking despite its high numbers is consistent with other reports that this filament is rarely involved in bulking incidences (Kragelund et al. 2008). The absence of these filaments in the municipal plant despite the similar loading rates, however, suggests a likely limiting substrate effect. This conclusion is collaborated by Martins et al. (2003) that whereas appearance of bulking sludge is related to process conditions, the presence of specific filamentous bacteria is likely due to the presence of a specific limiting substrate.

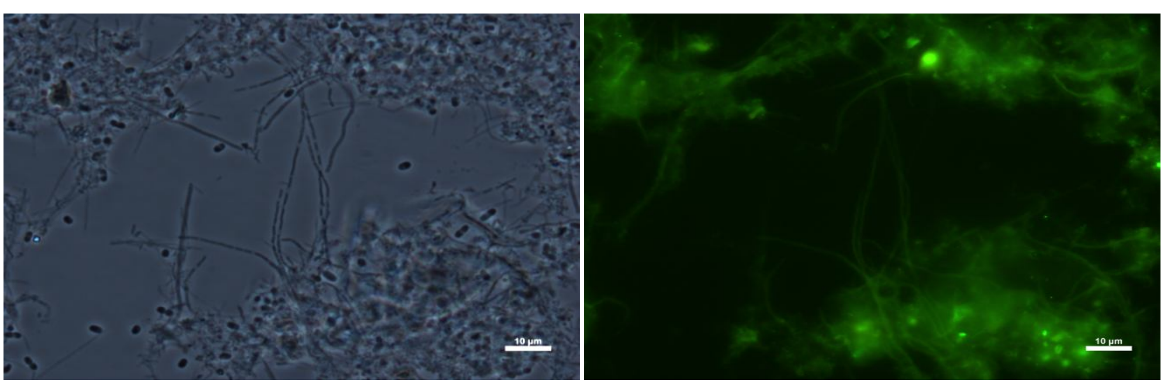

Figure 1: (a) Phase contrast and (b) FISH micrograph of same microscopic field showing bridging caused by overgrowth of Thiothrix

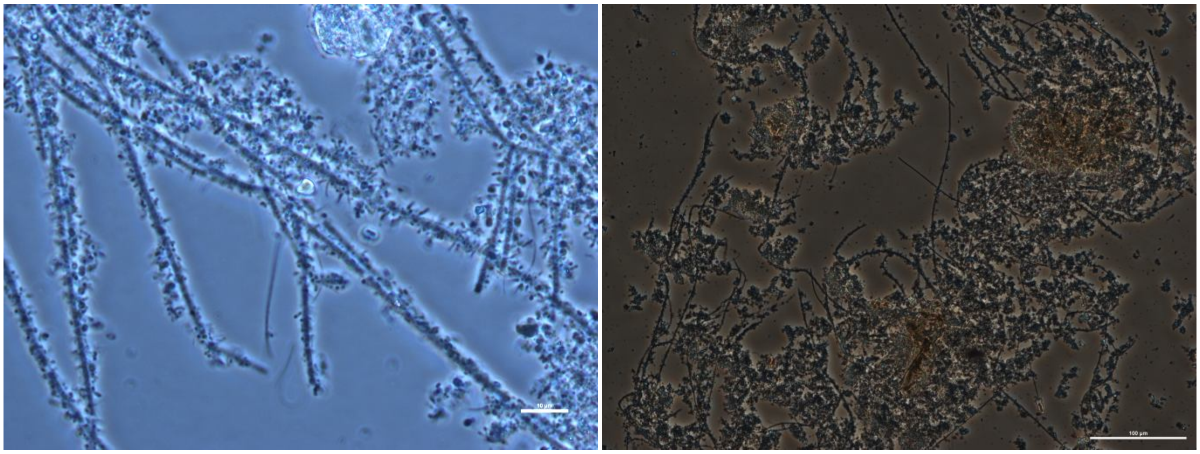

Figure 2: Phase contrast micrographs of EikelboomType 0041 showing open-flocs formation

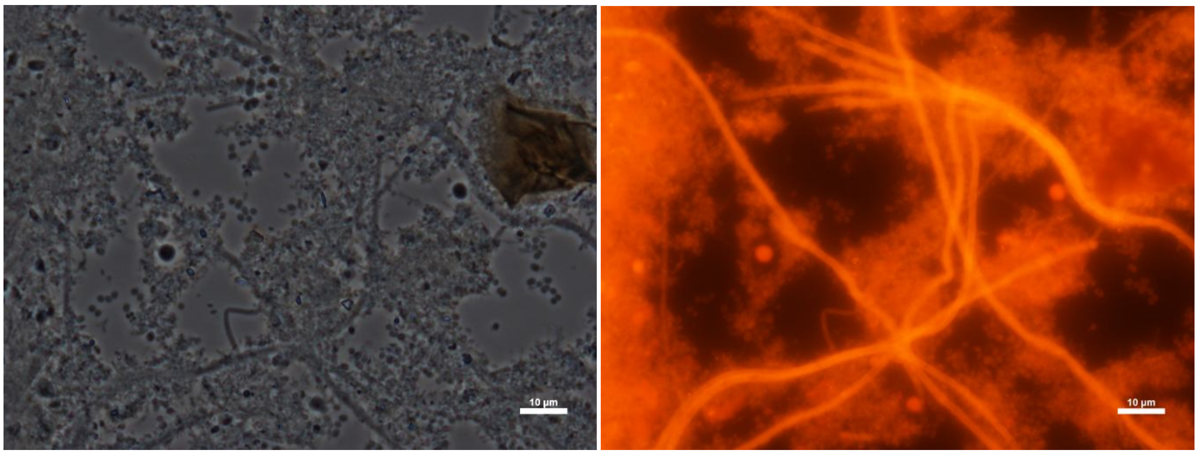

Figure 3: (a) Phase contrast and (b) FISH micrograph of same microscopic field showing interfloc bridging by Eikelboom Type 1851

The two dominant filaments in the municipal plant were identified as Types 0041(Figure 2) and 1851 (Figure 3). The overall filament index in this plant was 4. The involvement of Type 0041 in the bulking agrees with findings by Seviour et al. (1994) and Madoni et al. (2000) who respectively ranked this filament as one of the two dominant filaments responsible for bulking in Australia and Italy. The proliferation of these filaments was directly related to the DO concentration as well as the sludge loading rate of this plant. A low loading rate results in a low food-to-microorganisms (F/M) ratio which favors the growth of these slow growing filaments.

Other studies have reported similar conditions as favoring the occurrence of these filaments. According to Eikelboom (2000), Type 1851 is regularly observed to proliferate in plants operating at a sludge load of < 0.15 kg BOD_5/kg MLVSS.d while Kohno et al. (2002) suggested that the overgrowth of this filament exclusively in long solids retention time (SRT) systems is due to its extremely slow growth rate (0.48–0.93 day^{-1}). Beer et al. (2002) also observed Type 1851 to dominate in only conventional activated sludge plants with long sludge ages (> 20 days). These findings are consistent with those of the present study that the long sludge age as a result of the low loading at which this municipal plant operates accounted for the overgrowth of this filament. Although Kohno et al. (2002) reported that a temperature of 25-30 ^{o}C is optimum for the growth of Type 1851, the results of this study suggest that the role of temperature is secondary. This is because temperature was comparable in both plants yet the filament was observed in only the municipal plant. This observation further alludes to the suggestion that the presence of a particular filament is dependent on a specific limiting factor while operation parameters are responsible for its proliferation.

4. Conclusion

This research aimed at identifying the main process parameters favoring the proliferation of filamentous bacteria in full-scale activated sludge plants. The results provide useful information for the development of strategies to control sludge bulking.

Low DO concentration and high sludge age were identified as the major process parameters influencing the overgrowth of *Thiothrix* and Eikelboom Types 1851 and 0041. However, a limiting substrate effect on the occurrence of specific filaments is strongly suggested based on the fact that similar operational conditions resulted in the proliferation of different filaments in the various plants.

It is recommended that continuous aeration regimes should be practiced, with DO concentrations of not lower than 2 mg/l maintained. An aerobic selector with a low hydraulic residence time and high DO concentration should be incorporated into the existing configuration, preferably before the aeration tank, as a way to control overgrowth of these filamentous bacteria. Further long-term *in situ* research on the combined effects of the process parameters on filamentous bacteria growth is needed for a complete understanding of the ecophysiology of these bulking filaments.

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