Bulking and Foaming Filamentous in Modified Bardenpho Process during Hot Season

Ahmet Aygun, Sezen Kucukcongar, Zehra Gok, Merve Sogancioglu, Esra Yel, and Murat Kucukhemek

Abstract—The hot season filamentous species in wastewater samples weekly taken from oxic and anoxic zones of the two parallel aeration basins at Konya WWTP were identified and associated with the operational conditions of the plant. Primarily, N. Limicola II, III, Types, 021N, 0092, 0041/0675, 0581, 0803, M. Parvicella and Nocardia found to dominate, which represents the biodegradable industrial discharge, high θc and low F/M. In order to avoid the bulking/foaming problems, specific control measures should be preferred to non-specific ones. Operational conditions were more critical. Type 0092, M. parvicella, N. limicola III were determined as the most dominant species in all basins and zones with their rate above 75% observation frequency. When it is compared with the aeration basins whether added or not added antifoaming and different zones (oxic and anoxic) of basins, it was observed that different species were dominant.

Keywords—Activated sludge, bulking, filamentous bacteria, foaming, temperature.

I. INTRODUCTION

ACTIVATED sludge is a commonly used biological process in the treatment of domestic and industrial wastewaters. Activated sludge represents a complex microbial ecosystem containing a large variety of microorganisms, such as bacteria, protozoa, fungi, metazoan, viruses and algae, as it is a rich source of organic matter and nutrients. Despite the diversity of microorganisms entailing activated sludge, researchers have always been focused on bacteria [1]. The performance of this process relies on a great deal on solid–liquid separation in the final clarifier [2]. Filamentous bulking and foaming are among the most important problems preventing effective solid-liquid separation. Although these problems have been known for more than half a century, no final or comprehensive solutions are available [3].

Filamentous bulking sludge, a term used to describe the excessive growth of filamentous bacteria, is a frequent problem in activated sludge process that has severe effects on operational performance and increase the treatment cost [4]. A better understanding of the phenomena behind the bulking appearance is essential for treatment plant management and to preserve the effluent quality. Some factors responsible for filamentous bulking sludge are oxygen deficiencies, nutrient deficiencies, presence of reduced sulphur compounds, decrease in food/micro-organism ratio (F/M) and wastewater composition [5].

Wastewater composition is one of the significant factors affecting filament formation, as it has been found that slowly degradable organic material and especially lipids favour the growth of both M. parvicella and Nocardia which are the most common foam causing filament types [6,7]. Although, these two are very common, they are not the only types, several other filamentous species are also causing foaming. Within the normal ambient temperature range found in activated sludge aeration basins (8 to 25 °C), in can be generally stated that filamentous organisms grow more rapidly as the temperature increases within this range [8].

Research using classical microbiological techniques, such as microscopic observation and culture-based enumeration, has demonstrated a link between the predominance of filamentous microorganisms and instances of foaming in laboratory, pilot and full-scale wastewater treatment plants [6, 9,10]. The diversity of filaments in foam and identification difficulties may have contributed to the lack of understanding. To help resolve these issues, population shifts of putative foam-causing organisms before, during, and after foaming need to be quantified.

The environmental conditions encountered by the organism in the N and P removal system (advanced biological treatment system) are very different to those in conventional fully aerobic systems. Nevertheless, the N and P systems did not appear to be exempt from filamentous bulking problems. Indeed it appeared that these systems had a propensity to
produce generally poorer settling sludge than their more conventional short sludge age fully aerobic counterparts [11]. Antifoaming agents have been utilized for foam control in activated sludge units. The chemical structure and the antifoaming mechanism of most of the commercial antifoaming agents are not clear. They can either remove foam by destroying the foam causing microorganism, or simply by destroying the foam physically.

The aim of this study was to identify the hot season period filamentous species in activated sludge aeration basin KonyaWWTP, to associate them with the operational conditions of the plant, to compare the species observations for anoxic and oxic zones, and to compare the basins that antifoaming agent was added or not.

II. MATERIAL AND METHOD

A. Material

Description of Konya WWTP

Konya WWTP (Qavg: 0.2 Mm³/d) includes preliminary treatment, modified Bardenpho for organic carbon and partial nitrogen degradation, disinfection, thickener and anaerobic sludge digestion. Bardenpho aeration basins consist of 4 subsequent zones ordered as: anoxic-oxic-oxic-anoxic. Same operating conditions were applied at aeration basins (T1 and T2) that worked as two parallel lines in Konya WWTP. 4 mg/L water-based antifoam was added interminently in 1st aeration basin (T1). Wastewater samples were taken from the first (anoxic zone) and the third (oxic zone) each week between April and July 2012. The working period wastewater influent and effluent water quality data were given in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average value</th>
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<tbody>
<tr>
<td>COD, mg/L</td>
<td>816</td>
</tr>
<tr>
<td>BOD₅, mg/L</td>
<td>415</td>
</tr>
<tr>
<td>SS, mg/L</td>
<td>414</td>
</tr>
<tr>
<td>TP, mg/L</td>
<td>13</td>
</tr>
<tr>
<td>NH₃-N, mg/L</td>
<td>66</td>
</tr>
<tr>
<td>TN, mg/L</td>
<td>96</td>
</tr>
</tbody>
</table>

B. Method

Bacteria in aqueous medium on the slide were hardly seen under the light microscope due to their structural features. These microorganisms were merely made more apparent by staining and increasing their concentration at their location. Gram and Neisser staining were applied to obtain more reliable imaging procedures in wastewater samples weekly taken from oxic and anoxic zones of the two parallel aeration basins at Konya WWTP between April and July 2012.

The Gram Hucker staining protocol was used according to Standard Methods [12]. A smear of activated sludge sample was deposited on a glass slide. The slide was thoroughly air dried and fixed by passing slide through a flame. First, the smear was stained for 1 min with ammonium oxalate-crystal violet solution. The slide was rinsed in tap water and drained of excess; Lugol’s solution was applied for 1 min. The stained slide was rinsed in tap water and decolorized for approximately 15-30 s with acetone alcohol. It was counterstained with safranin for 15 s, rinsed with tap water and dried.

As the second staining method, the Neisser staining protocol was used for the same samples [13]. Another smear of activated sludge sample was deposited on a different glass slide. The slide was thoroughly air dried and fixed by passing slide through a flame. A freshly made mixture of 2 part solution A (methylene blue, glacial acetic acid and ethanol solution) and 1 part solution B (crystal violet and ethanol solution) was placed onto the slide for a contact period of 10-15 s. Afterwards the excess dye was rinsed with tap water and the slide was stained with solution C (chrysoidin Y solution) for a contact period of 45 s. The slide was rinsed with tap water and dried.

Gram- and Neisser-stained glass slides were examined under oil immersion at 100X magnification with direct illumination with Olympus BX51T-32P01 microscope equipped with a color camera. Filamentous bacteria were characterized to genus or to a numbered “type” using the dichotomous key suggested by Jenkins et al. [8]. At assessment period, available filamentous species’ percentage distribution and filamentous bacteria’s frequency of observation were determined. The rank of filaments was recorded by using a scale ranging from 0 (filaments absent) to 3 (dominant). Determining the dominant species, the operating conditions that caused foaming and bulking were investigated.

III. RESULTS AND DISCUSSION

After Gram and Neisser staining procedures, sample photomicrographs are indicated as in Fig 1(a) and 2(b) for wastewater samples, respectively. While purple-blue species in Gram staining are gram positive, pink or reddish species are gram negative. Similarly, blue-colored species were neisser negative while neisser positive species are yellow-brown in Neisser staining (Fig. 1(a) and 2(b)). During the period between April and July 2012, observation frequency percentages of observed filamentous bacteria in all basins and zones are given in Figure 1.

As shown in Figure 1, Type 0092, M. parvicella, N. limicola III were determined as the most dominant species in all basins and zones with their rate above 75% observation frequency, while Spirochaetae, Type 411, Spirillum, Beggiatoa and Streptococcus were determined the least with their rate below 25% observation frequency. The first and second basin (T1 and T2) were operated under the same conditions, the only difference was water-based antifoaming agent which was added in T1. In addition to application of antifoaming, in anoxic zone, Type 1863, Type 0411, Type 0581, Type 1851, Type 021N, S. natans and N. limicola II...
were lesser observed in samples. Besides this, after antifoaming addition, Type 0803 and Streptococcus was never observed. Depending on antifoaming addition in oxic zone, Type 0803, Type 1701, Type 0581, H. hydrosis, Type 1851, Type 021N and Thiotrix II species were lesser observed. Similar to that of in anoxic zone, Type 0411, Fungi, Beggiatoa was never observed after antifoaming addition.

The dissolved oxygen deficiency is one of the most typical factors that cause most filamentous bacteria in activated sludge process. Increasing dissolved oxygen concentrations approximately 2 mg/L or even more is the effective method to avoid filamentous bulking [4].

Independent from application of antifoaming species were observed alterations between both of two basins’ oxic and anoxic zones of the two basins.

While Type 0803, Type 1701, H. Hydrosis, Type 0914, Type 0041, Type 21N, Spirochaetae, Fungi, S. natans, Thiothrix II and N. limicola I species, taken from the samples in oxic zones, were observed higher; they were observed rare in anoxic zones. Concordantly with this, with increasing dissolved oxygen, Type 1863, Type 0411, Type 0581, Zooglea, Streptococcus and Thiothrix I were observed lesser.

Filamentous bacteria are normal components of activated sludge but they may compete successfully with the flocc-forming bacteria under specific conditions. Percentage ratios of filamentous bacteria in the overall observations were determined and dominant species generated by operating conditions were presented in Figure 2. N. Limicola III (9%); M. parvicella and Type 0092 (7%); Type 0041 and 021N and N. Limicola II (6%); Type 0581, 0914 and 0675 and Nocardia (5%); Type 0803 and 1851, S. natans, Thiothrix I and Thiothrix II (4%) were the dominant filamentous microorganisms in the 81% of the samples taken at time of hot season period. The F/M and θc were 0.07-0.20 and 11.2 days,
Types 0092, 0675/0041, *M. parvicella* proliferate under low F/M ratios (long sludge age), and/or nutrient deficiency conditions [8]. Type 0803 dominates in case of industrial influent and anaerobic supernatant recycle [13], which was the case during the study period. *S. natans*, Types 1701, 021N, 1851, *Thiothrix* spp., *H. hydrosis*, *N. limicola* II, III increases with biodegradable industrial effluents. Konya sewerage system receives wastewaters from a number of small and medium scale industries distributed to the several regions of the city. Most of these are working on food processing and automotive spare part production/maintenance. The ambient temperature varied between 14.8-22.2 °C (avg 19.3 °C). The seasonal variation of the filamentous population is primarily attributed to the temperature effect on the population size of *M. parvicella* and Type 0092 [14,15]. Operational conditions and influent variations affect the dominating filaments. The complementarities of the dominance of these filamentous microorganisms are strong evidence that they compete for the same substrate. It was hypothesized that increasing temperatures are responsible for foam formation as growth rate increases with increasing temperature in the growth range [14]. *M. parvicella* is dominant at the temperatures below 15 °C and Type 0092 is dominant at temperature above 15 °C [8]. *Nocardia* appears to be favored at higher aeration basin temperatures and *M. parvicella* at lower aeration basin temperatures. This phenomena not observed in this study. Filamentous growth is not only temperature-dependent. Therefore, the operational conditions should be strictly followed and controlled to prevent filamentous growth.

![Fig. 2 Observed filamentous species percentage relative to each other in Konya WWTP modified Bardenpho unit during 2012 hot season.](image)

Species and percentage distribution of dominant filamentous species observed in each zone, were compared in Table 2. When T1 (antifoaming agent was added) and T2, (antifoaming agent was not added) were compared in terms of distribution of dominant filamentous bacteria, it was observed that different species were dominant. For example, in anoxic zone of basin, while Type 0092 and *N. limicola* III were more dominant, whereas, in anoxic zone of basin 2, *N. limicola* III was dominant specie. While the most dominant *N. limicola* III with the rate 11% and *M. parvicella*, Type 0041 and Type 0092 became dominant also. In anoxic zone of basin 2, *N. limicola* III was the most dominant specie, but in oxic zone, Type 0092 and Type 021N became dominant also. Recently antifoaming agents have been produced and utilized at aeration basins as temporary solution to foaming problems. This study was performed hot season, when excessive amount of *Nocardia* had been observed.

### Table II

<table>
<thead>
<tr>
<th>T1-Zox</th>
<th>T2-Zox</th>
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<tbody>
<tr>
<td>8% <em>N. limicola</em> III Type 0092</td>
<td>7% <em>Nocardia</em>+&lt;br&gt;7% <em>M. parvicella</em> Type 021N*&lt;br&gt;7% Type 0675*&lt;br&gt;8% Type 0041</td>
</tr>
<tr>
<td>7% <em>N. limicola</em> III Type 0092</td>
<td>6% <em>N. limicola</em> III Type 0092</td>
</tr>
<tr>
<td>11% <em>M. parvicella</em> Type 0092</td>
<td>7% Type 0092 Type 0803*&lt;br&gt;6% Type 0092 Type 0581*&lt;br&gt;6% Type 0803*&lt;br&gt;6% Type 0092 Type 0041</td>
</tr>
</tbody>
</table>

*Difference between T1 and T2 basins (antifoaming agent added or not) +Difference between Anoxic and Oxic zone

As seen in Table 2, as the most abundant filament type, *N. limicola* III was observed at all zones. This indicated that the utilized antifoaming agent was either only destroy *Nocardia* or can destroy the foam physically without any adverse effect to the other filamentous microorganisms. The number and types of filamentous species differ slightly in anoxic and oxic zones. In antifoaming agent added basin (T1) the number of filamentous species was higher, whereas, number of observed types was lesser in the oxic zone of T2.

### IV. Conclusion

*N. Limicola* II, III, Types, 021N, 0092, 0041/0675, 0581, 0803, *M. Parvicella* and *Nocardia* dominated in Konya WWTP in hot season. Operational conditions and biodegradable influent characteristics had higher influence. Specific control measures, i.e. creating environmental conditions in the plant to inhibit or suppress the growth of the filamentous organisms are suggested in this study. Due to the variety of types of species, non-specific measures (e.g. the use of chemicals which selectively control the excessive growth) are not suggested. Presence of more than one condition, forming bulking and foaming in Konya WWTP, resulted in lower percentage distribution of different filamentous bacteria. To resolve present problems in this plant, it is suggested that controlling of the industrial wastewater discharge, F/M rate and regulation of sludge age, setting desired value of dissolved oxygen and removal of nutrient deficiency.
ACKNOWLEDGMENT

This study was technically supported by Konya Metropolitan Municipality, Konya Water and Sewerage Administration (KOSKI).

REFERENCES


