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Original Research Article

Identification of Filamentous Microorganisms Causing Filamentous Bulking and Factors Affecting Their Growth in a Petrochemical Wastewater Treatment Plant

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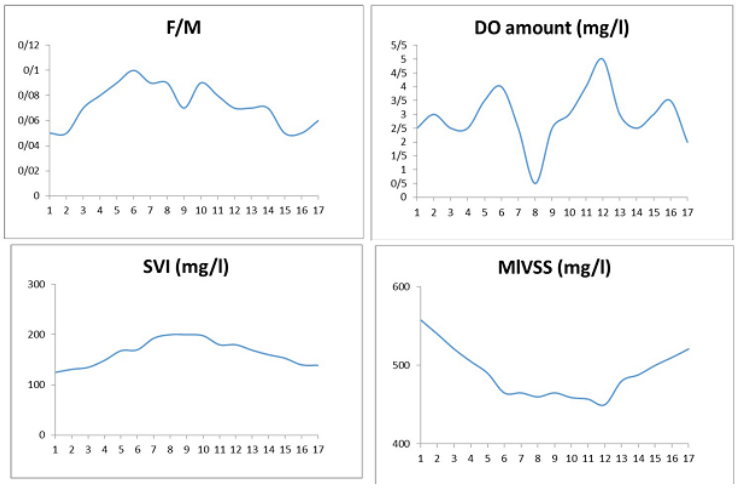
ABSTRACT

Bulking (sludge bulking) is caused by the excessive growth of filamentous or non-filamentous bacteria in the process of wastewater treatment, which subsequently results in a reduction in the quality of the output of wastewater treatment plant. By identifying the main cause of bulking bacteria, we can recognize its formation and find solutions to specific bulking control. The proliferation of Nocardiaforms and Beggiatoa are due to the presence of excess oil compounds and sulfur compounds in the input wastewater, respectively. In this study, the identification of bulking bacteria was carried out in a petrochemical wastewater treatment plant using microscopic methods. Identification of filamentous bacteria was realized by microscopic studying of slides, based on morphological characteristics and their reaction to different staining; therefore, Beggiatoa and Thiothrix identified as predominant filamentous bacteria. According to results characteristics of these bacteria and effective growth factors, the abundance of sulfur compounds, the sulfur content and subsequently the growth of these bacteria are increasing. Thus they are prevented for the specific bulking control in a Petrochemical wastewater treatment plant by removing sulfuric acid in the pH adjustment of the balancing pond and replacement of hydrochloric acid.

GRAPHICAL ABSTRACT



INTRODUCTION

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The active sludge process, which acts in a biological way, has the most efficiency in large-scale wastewater treatment systems, and the aerobic method is common in urban and industrial wastewater treatment [1].

Industrial wastewater treatment is usually in three stages of primary, secondary and final. The primary stage is mainly physical, and suspended solids in the wastewater are eliminated through screening, filtration and flocculation. The secondary stage is biological. In the active sludge method, the organic matter inside the aerator ponds is decomposed by bacteria as a biological reactor and converted to CO_2, H_2O, NH_4, and new live-cell mass. The final stage also includes clarification, disinfection and filtration for the reuse of the treated wastewater [2-4]. Most of biological treatment plants by activated sludge method face with operational problems, such as occlusion filters or lack of sludge settling caused by the bulking phenomenon, leading to a reduction in the efficiency of wastewater treatment and the quality of sludge settling [5-8].

It can be conceived that Filamentous bacteria act as the main skeleton and Zoogloeal bacteria stick to this skeleton and form the flocculation building. The purpose of this study was to identify the bacteria producing filamentous bulking and factors affecting their growth in the active sludge system of Abadan petrochemical wastewater treatment plant in order to adopt more suitable methods for preventing, controlling and inhibiting the bulking phenomenon in active sludge system of Abadan petrochemical wastewater treatment plant.

Since the use of continuous flow reactors, sludge rising, bulking or sludge bulking has been one of the major problems affecting the biological treatment of waste materials. Most of the process problems result from the overcoming of FILAMENTOUS bacteria, creating a condition with the name of filamentous sludge bulking [9-13].

Activated sludge bulking is one of the most common problems in the wastewater treatment plants. This can lead to inappropriate quality of the output wastewater, environmental damages, inappropriate quality of produced sludge for sending to the sludge-processing unit. In critical cases it can lead to activated sludge overflow from secondary settling. As a result, controlling this phenomenon is an essential requirement in all activated sludge wastewater treatment plants [14].

This is the most common and most important problem in the wastewater treatment plants by activated sludge method. Approximately 50% of urban wastewater treatment plants intermittently or permanently are affected by this problem. [15-18]. It should be noted that the filamentous microorganisms are one of the essential parts of the flocculation population in the activated sludge process and their presence can even improve the quality of some of the sludge properties. Serious problems arise when we witness their critical growth and reproduction, and the filamentous bulking occurs due to the critical growth and reproduction of these filamentous organisms [19].

At the intended treatment plant unit, the clarifiers are embedded in two basins of A and B. The creation of bulking in cause's occupation of large amounts by the sludge has settled hardly according to the reduction of sludge compaction. The purification process was carried out with the problem [20-25]. As in this petrochemical industry, the identification of these bacteria creating bulking has not been performed yet, the microscopic examination of the major bacteria creating filamentous bulking was carried out through this study.

Material and methods

In this study, according to the inspection and test procedures of petrochemical company, sampling was done from a biological reactor (aerator pond) for seventeen weeks (one sample per week) and the parameters affecting bulking phenomenon including DO rate, temperature, pH rate , SVI rate, MLSS rate, and MLVSS were measured and calculated [26-29].

Sampling and investigating the diversity of filamentous bacteria

In order to investigate the diversity of the filamentous bacteria, sampling was done weekly for a period of 17 weeks in different parts of the treatment plant. The samples were then inserted separately into 1-liter glass bottles from aerator ponds and clarifiers and transferred to the laboratory. During transference and the whole study

period, samples were stored in a refrigerator at 4 °C in the open air [30-34].

At first, the mobility of the filaments was studied in the fresh samples collected using the wet slide method. This was meant to study the morphology of filamentous bacteria such as length, diameter, shape, and the existence of real and false branches along these filaments. Also having a transverse wall, we examined the presence of pods around bacteria, presence of specific morphological structures such as buds. The presence of stored granules, such as the metachromatic grains in the filaments, as well as the investigation of the reaction of these bacteria to different staining, gram staining and neisser staining on the dried smears were investigated in samples [35-39].

It is worth noting that in the production of smear from filamentous bacteria, the bacteria stabilization on the slide is not by heat according to the structural alteration, the slides stained dry and without stabilization [40-43].

Presence of Sulfur Granules in Filamentous Bacteria

Investigating the presence of sulfur granules in the filamentous bacteria was performed by sulfur oxidation test (S test) and wet slide using aqueous sodium sulfide solution at a concentration of 1 g/l. As a result, light yellow granules were observed [44-46].

Presence of Iron Granules in Filamentous Bacteria

The study of the presence of iron oxide deposits in these bacteria was also performed on dried smears from samples. To investigate ferro-iron oxide deposits, the combination of 2% potassium ferricyanide aqueous solutions and 5% acetic acid was used hotly. Then, 2% safranin aqueous solution was applied for the background staining. Ferric iron oxide deposits were also studied in the same manner, but with the difference that 2% potassium ferricyanide aqueous solutions was used instead of potassium ferricyanide. [47-49].

Result and discussions

Primary examination and results of microscopic images

The primary examination of the samples collected by gram-staining indicated the overcoming of gram-negative bacteria (833000 per liter) over gram-positive ones (333000 per liter). The results were obtained from the observation of wet slide and gram and neisser staining as well as the study of sulfur granules and ferrous and ferric iron oxide deposits in the filamentous bacteria and their specific morphological characteristics. A comparison was made between Table 1 and these studies with the data in other Tables and identification keys of filamentous bacteria. The comparison showed the dominance of Beggiatoa bacteria (553,000 per liter) and Thiothrix (277000 per liter) in the active sludge sample of this petrochemical treatment unit.

In addition, the species of 0041 (50,000 per liter), 1701 (26000 per liter) and Haliscomenobacter hydrosis (33000 per liter) were observed with lower amounts in the sample of this treatment unit. In addition, nocardioform bacteria (83,000 per liter) and Nostocodia limicola (93,000 per liter) were more frequent than species of 1701 and Helicobacter hydroses.

Table 1: Characteristics of Isolated bacteria from Sludge bulking in Abadan Petrochemical Wastewater

Bacteria type	Gram test	Nicer test	Moving	Sulfur supply	Iron supply	String length	String diameter	Shape
Nocardia	+	-	-	-	-	10-20	1	Irregular
helicobacter hydroses	-	-	-	-	-	20-100	0.5	Rod
0041	+	-	-	-	-	100-500	1.4-1.6	Irregular
1701	-	-	-	-	-	20-80	0.6-0.8	Rod

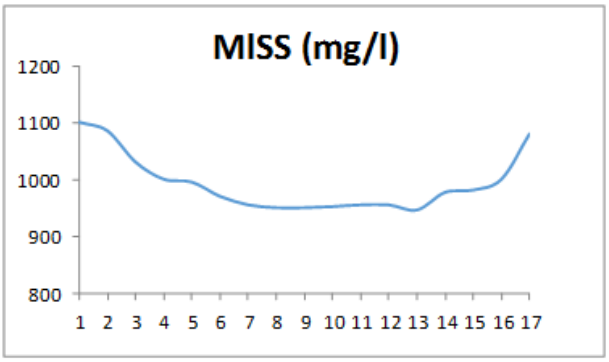

Figure 1: MLSS (mg/l) results in 17 weeks

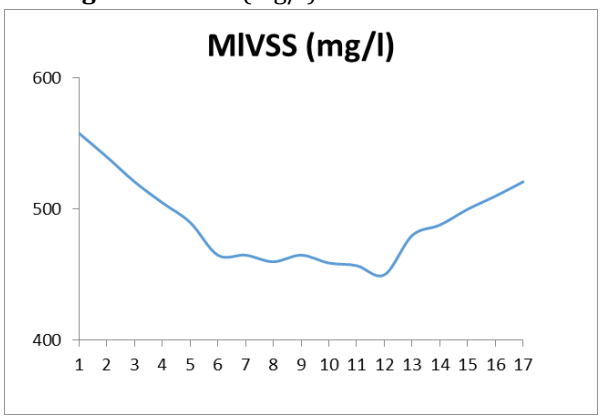

Figure 2: MLVSS (mg/l) results in 17 weeks

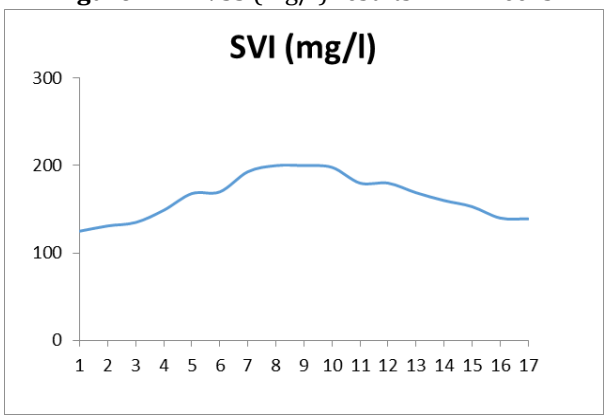

Figure 3: SVI (mg/l) results in 17 weeks

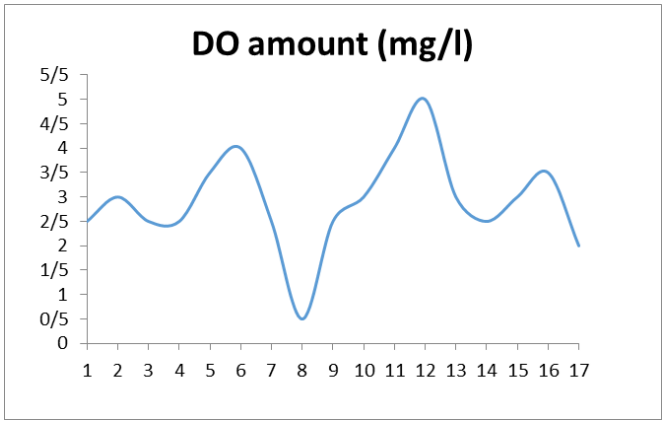

Figure 4: DO (mg/l) results in 17 weeks

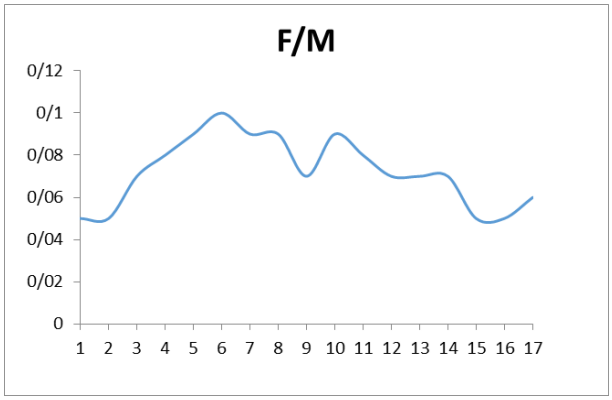

Figure 5: Comparison of F/M results

Trend of filamentous bacteria dominance in different time sequences

The observation indicates fresh samples by gram staining every 7 days from the upward trend of the SVI (Fig. 3) of the activated sludge basin, which sometimes calculated between 170 and 200. In addition to confirmation of the presence of filamentous bacteria, overcoming of certain types of these bacteria in each stage of the investigation and the presence of a variety of filamentous bacteria had not been observed previously in the active sludge liquid, and neither had the existence of unique morphological structures related to some of these bacteria.

After creating the uptrend of SVI rate (Fig. 3) and significant downtrend of MLSS (Figure. 1) in the second week after the sampling began, we observed dominance of Thiothrix bacteria (277,000 per liter) in the form of relatively long filaments. Also, Beggiatoa (553,000 per liter) in the form of short filaments at a relatively high rate and nocardioform (83,000 per liter) and Haliscomenobacter Hydrossis to a lesser extent (33,000 per liter) were observed.

In the eighth week of this study, the frequency of Nostocoida limicola (133,000 per liter) was high in the specimens due to the excessive reduction of MLSS (Fig. 1). There was a significant increase for sludge (190 cc), SVI (Fig. 3). In addition, a very high amount of Haliscomenobacter hydrosis (500,000 per liter) was observed in the form of hair coil due to mechanical problem in one of the aerator surface of activated sludge basin and a

significant decrease in the amount of dissolved oxygen (Fig. 4).

In addition, we observed very long filaments of Thiothrix (110,000 per liter) and Microthrix parvicella (50,000 per liter) not found in the sample significantly until now. However, in the 16th week of the study, filamentous bacteria were found only as small aggregates, in the small sludge amount (13,000 per liter), dispersed, and in microscopic observations.

The uniformity of the diversity of the filamentous bacteria in the different parts of the treatment plant was predictable regarding the operation of wastewater treatment plant in this petrochemical wastewater treatment plant. The continuous rotation of wastewater among different parts of the treatment plant, especially returning a part of sludge settled in clarifiers to the aerator pond was 11%.

Environmental factors and indicators are very effective in dominance of the filamentous bacteria mentioned in the active sludge and the occurrence of bulking phenomenon by them. These factors have been studied in many research studies [22, 23]. High concentrations of oil and grease compounds and the low ratio of F/M (Food/Microorganism), or low levels of organic loading in the wastewater often lead to the dominance of a bunch of filamentous bacteria with the general name of nocardioform bacteria studies [22, 23]. Therefore, the observation of the frequency of Nocardia virus bacteria (83,000 liters per liter) is significant due to the presence of large amounts of oil, lubricants and grease compounds (30 cubic meters per day) in wastewater collected from different parts of this petrochemical company and that it can be a sign of low ratio of food to bacteria in the wastewater.

In some other studies, Gordonia bacteria has been introduced as one of the components of Nocardioform actinomyces [23, 24].

also considered Gordonia as one of the predominant filamentous bacteria in active sludge and bulking of oil wastewater [23, 24]. Jenkins et al. (1993) have introduced nostocoida limicola in the wastewaters with high concentrations of various types of sulfides and organic acids as the main causes of creating bulking. Therefore, due to the abundance of sulfur compounds in oil wastewater, the abundance of filamentous bacteria is inevitable in the active sludge of the petrochemical plant [24].

Conclusion

An important reason for overcoming of Nostocodia limicola is the lack of nitrogen and phosphorus content, as the main food, in all industrial wastewater, Since the oil wastewater as one kind of Industrial wastewater often has encountered with nutrient deficiency, the dominance of this bacteria is justifiable in the desired petrochemical waste, due to deficiency in nitrogen and phosphorus compounds in this wastewater.

In the bulking of this wastewater, we also observed relatively significant amounts of 0041 species (50,000 per liter) due to the low ratio of food to bacteria; in other words, the low rate of organic loading in the wastewater was observed according to the results.

Hence, the presence of 0041 species at relatively high levels in the bulking of the aforementioned petrochemical wastewater indicates the abundance of nocardioforms (83,000 per liter) on the low ratio of F / M in this wastewater. The abundance of Haliscomenobacter hydrosis bacteria and 1701 indicates that there is deficiency of soluble oxygen (DO) in the wastewater. With regard to the relatively lower values of these two types of bacteria in the petrochemical aerator pond, the aeration system of the petrochemical plant probably has not any problem and there is no deficiency of soluble oxygen in the aerator pond. In another part of this research, by studying the active sludge, bulking of the petrochemical wastewater treatment plant, carried out during 17 weeks, in different environmental, process conditions by

using the microscopic method are investigated. The data obtained on other filamentous bacteria are present in this wastewater with lower amounts and dominance in more difficult environmental conditions such as reduction in the dissolved oxygen and an increase in concentrations of oil and sulfur compounds.

On the other hand, the ability and the solution of the resistance of filamentous bacteria species was compared with each other against the hard conditions for survival in these situations. A high abundance of Thiothrix (277,000 per liter) throughout the study indicates high resistance of these bacteria against difficult environmental conditions, especially at high concentrations of sulfur compounds and organic acids. In addition, the emergence of a unique rosette form in early studies can be also considered as a mechanism to overcome these difficult situations. The emergence of relatively high levels of Beggiatoa (553,000 per liter) and Thiothrix (277000 per liter), which were observed during the study in direct microscopic examination of the sample, showed high survival potential especially in the abundance of sulfur compounds. Williams and Unz (1985) also highlighted their dominance in the high levels of sulfur compounds in the wastewater.

A significant increase in Haliscomenobacter Hydrossis (500,000 per liter) at a very low concentration of dissolved oxygen (Figure. 4) indicates the dominance of these bacteria in these difficult conditions. Microthrix parvicella was hardly found in direct examination of the wastewater sample. However, it was abundantly found in high concentrations of oily compounds, as well as in the low ratio of food to bacteria and in active sludge with long sludge age, indicating the resistance of these bacteria in such a difficult situation. Repeating these experiments and observing similar results showed that increasing retention time and the hydraulic retention in this wastewater treatment plant could lead to the dominance of aforementioned species. Considering that Beggiatoa and Thiothrix bacteria have been known as the cause of creation of bulking in the treatment plant, as well as the active role of sulfur compounds in increasing the growth of these bacteria, the reduction or removal of sulfur in the input wastewater is one of the bulking control methods. Therefore, the removal of H_2SO_4 acid injection as a pH regulator in the balancing pond and replacing HCl acid was put on the agenda.

Conflict of Interest

We have no conflicts of interest to disclose.

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