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APPLICATION OF SELECTED CHEMICAL COMPOUNDS TO LIMIT THE GROWTH OF FILAMENTOUS BACTERIA IN ACTIVATED SLUDGE

Excessive growth of filamentous bacteria in activated sludge is one of the most common reasons for sludge bulking. This phenomenon is responsible for disturbances in sewage treatment plant operation and adds up to the deterioration of chemical characteristics of treated sewage. This study presents research results on the application of selected chemical agents to limit the growth of filamentous bacteria. The research was conducted such that it was possible to identify an agent that effectively limited bacterial growth. At the same time, the agent was to be non-toxic to the microfauna in the sludge and was not to reduce the effectiveness of the sewage treatment process. Sodium hypochlorite, aluminum sulfate, and hydrogen dioxide were found to be the most effective agents for controlling filamentous bacteria.

1. INTRODUCTION

Activated sludge bulking is the most frequent and serious biological disturbance of the sewage treatment process. Bulking sludge can be defined as sludge with insufficient sedimentation properties and poor thickening capacity caused by excessive growth of filamentous or zoogleal organisms [3], [9]. However, the most common problems are associated with filamentous bacteria, whose mass growth causes sludge bulking. Excessive growth of these bacteria disrupts the contact of flocs with sewage substances, which inhibits biochemical processes. [11]. Flocs overgrown with filamentous bacteria settle poorly in secondary sedimentation tanks, which prevents maintenance of proper sewage flow in facilities and causes outflow of sediments from a plant.

Currently, there are ca. 30 identified types of bacteria that occur with variable frequency in the activated sludge [3]. Different filamentous organisms are often

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present simultaneously in a given activated sludge. However, only one, or at most two, strains of bacteria occur in greater numbers than the remaining strains. For that reason, dominant and subdominant filamentous bacteria are distinguished. In a properly functioning sewage treatment plant, these bacteria are constantly present in the activated sludge, but do not have a significantly negative effect on the treatment process as long as their numbers do not exceed normal levels. However, their mass growth leads to sludge bulking and causes disturbances in sewage treatment plant operation.

The purpose of the study presented here is to control the growth of filamentous bacteria. The study included isolation and identification of filamentous microorganisms that occur in activated sludge in the Municipal Sewage Treatment Plant in Toruń and determination of the methods and means of limiting their growth.

2. MATERIALS AND METHODS

2.1. OBJECT OF THE SURVEY

Activated sludge samples were collected from the Municipal Sewage Treatment Plant in Toruń. This plant was opened in 1998 for the purpose of mechanical and biological treatment of domestic and industrial sewage from the area around the city of Toruń. The sewage retention time in the plant is approximately 24 hours, and the plant neutralizes ca. 58 000 m^3 per day.

2.2. ISOLATION AND IDENTIFICATION OF FILAMENTOUS BACTERIA

Activated sewage for laboratory analyses was collected directly from an aeration tank. The collected sludge was shaken in a 10% solution of potassium sulfate in order to partially dilute biopolymers. After appropriate solutions had been prepared, the bacteria were inoculated onto spread plates with IAM culture medium [6]. After several days of incubation at 22 °C, bacterial cultures were examined under a 1×400 microscope. The filamentous cultures were inoculated into liquid medium IAM, and after several days of incubation were smeared on a glass slide that was stained with alkaline fuchsine. After a pure strain of filamentous bacteria has been obtained, the culture was inoculated into slant tubes containing the IAM culture medium.

Identification of the filamentous microorganisms was carried out based on Eikelboom and Buijsen’s Microscopic Sludge Investigation Manual [3].

2.3. CONTROLLING GROWTH OF FILAMENTOUS BACTERIA

The purpose of the analyses was to determine the impact of selected chemical compounds, including potassium permanganate, sodium hypochlorite, aluminum sulfate, aluminum chloride, iron (II) chloride, and hydrogen dioxide, on the development of filamentous bacteria occurring in the activated sludge.

The experiment was conducted in a system containing 9 flasks, including one control. All 9 flasks were filled at time t_0 with 100 cm^3 of activated sludge from the above-mentioned plant. A specific chemical compound was added to all 8 flasks such that the final concentrations equalled 25, 50, 75, 100, 125, 150, 175, 200 mg/dm^3. The testing of each chemical compound was carried out for 7 days at 20 °C.

10 cm^3 subsamples were collected every 24 hours from all 9 flasks, and were subjected to macro- and microscopic analyses. The remaining volume was set aside for 30 minutes in order to separate the activated sludge from the sludge liquor. After the sludge liquor had been removed, the mixture was topped up to a final volume of 100 cm^3 with sterile raw sewage containing one of the substances surveyed with an appropriate concentration (0–200 mg/dm^3).

The macro- and microscopic examination included the following analyses: activated sludge index, floc morphology, number of filamentous organisms (based on Eikelboom and Buijsen's approximation method [3]), abundance, and taxonomic affiliation of the protozoa.

3. RESULTS AND DISCUSSION

Activated sludge collected in the Municipal Sewage Treatment Plant in Toruń was found to contain high abundances of filamentous organisms. They were clearly visible in all flocs. The number of filamentous bacteria, according to the approximate scale of Eikelboom and Buijsen [3], was classified in category 3 (abundant filamentous microorganisms) or 4 (highly abundant filamentous microorganisms).

During the study, seven strains of filamentous bacteria were isolated from the activated sludge from the aforementioned plant. *Microthrix parvicella* was found to be the dominant strain and it occurred in all samples. The remaining strains (*Haliscomenobacter hydrossis*, *Nostocoida limicola*, *Streptococcus*, Type 021N, Type 0961, Type 0803, Type 1701) were subdominant ones and occurred with varying frequency (table 1).

A great variety of microfaunal species were observed in the sludge surveyed. *Protozoa* were represented by *Ciliata* (*Carchesium*, *Epistylis*, *Opercularia*, *Vorticella*, *Aspidisca*, *Euplotes*, *Linotus*, *Paramaecium*), *Flagellata* (*Bodo* sp., *Hexamitus* sp.) and *Rhizopoda* (Amebas). *Rotatoria* and *Nematodes* were also observed.

Table 1

Morphological and physiological characteristics
of isolated filamentous bacterial strains

Characteristics		Filamentous microorganisms present in sludge							
		Strain No. 1	Strain No. 2	Strain No. 3	Strain No. 4	Strain No. 5	Strain No. 6	Strain No. 7	Strain No. 8
Predominant		+							
Submitted			+	+	+	+	+	+	+
Branching	No branching	+	+	+	+	+	+	+	+
	False branching								
	Real branching								
Motility	Non motile	+	+	+	+	+	+	+	+
Sulphur deposits	Present								
Crosswalls isibility	Visible		+	+	+		+	+	
	Invisible	+				+			+
Filament shape	Direct					+		+	
	Curved	+	+	+	+		+		+
	Bent								
Gram staining	Positive	+					+		+
	Negative		+	+	+	+		+	
Cell diameter	<1.0 μm	+				+		+	
	1.0–2.2 μm		+	+	+		+		+
	2.5 μm								
Attached growth	Other bacteria cells attached to filaments				+				
	No/poor attached growth	+	+	+		+	+	+	+
Constrictions	Visible		+	+	+		+		+
Sheath	Visible				+	+			
Cell shape	Discus shaped		+				+		
	Coccus								+
	Rod				+				
	Square		+					+	
	Rectangular			+				+	
	Cell shape almost invisible	+				+			

The activated sludge index in the majority of control samples exceeded 100 cm^3/g (average of 124 cm^3/g), while the volume of activated sludge in 100 cm^3 of the sample being analyzed fluctuated around an average of 70 cm^3. The surface area of the sludge equalled on average ca. 3000 μm^2.

Table 2

Abundance of filamentous bacteria in active sludge after application of selected chemical compounds according to the approximate scale of Eikelboom and Buijsen [3]

Substances	Concentration [mg/dm^3]	Abundance of filamentous bacteria				
		Category 0	Category 1	Category 2	Category 3	Category 4
Sodium hypochlorite	25					+
	50					+
	75					+
	100				+	
	125			+		
	150			+		
	175		+			
	200		+			
Hydrogen dioxide	25					+
	50				+	
	75				+	
	100				+	
	125				+	
	150			+		
	175			+		
	200			+		
Aluminum(III) chloride	25				+	
	50				+	
	75			+		
	100			+		
	125			+		
	150			+		
	175			+		
	200			+		
Potassium permanganate	25					+
	50				+	
	75				+	
	100				+	
	125			+		
	150			+		
	175			+		
	200			+		
Iron(II) chloride	25				+	
	50				+	
	75				+	
	100				+	
	125				+	
	150			+		
	175			+		
	200			+		
Aluminum sulfate	25					+
	50					+
	75					+
	100				+	
	125				+	
	150			+		
	175			+		
	200		+			

3.1. IMPACT OF CHEMICAL SUBSTANCES ON THE DEVELOPMENT OF FILAMENTOUS BACTERIA

A reduction in filamentous bacterial abundance was noted following additions of all the chemical substances tested (table 1). However, sodium hypochlorite and aluminum sulfate had the most limiting impact on the development of filamentous bacteria. These substances, when applied in concentrations of 200 mg/dm^3, reduced the number of filamentous bacteria from category 4 to 1. However, the impact of these two substances was notable only above a concentration of 100 mg/dm^3; at lower concentrations, no impact on the growth of filamentous bacteria was observed, confirming earlier studies [1]. MADONI et. al [10] found that sodium hypochlorite yields favourable results, particularly in the case of *Microthrix parvicella*, which was the prevailing strain. The susceptibility of *M. parvicella* to aluminum salts has been described many a time [2], [9]. An impact of this compound on other filamentous bacteria was also observed. In the sewage treatment plants described by EIKELBOOM and ANDREASEN [2], simultaneous precipitation with aluminum salts always resulted in a reduction in filamentous bacterial numbers and effective sedimentation. This observation was confirmed by research conducted by KALISZ [5] and by the present study (figure 1).

The remaining chemical compounds reduced the content of filamentous bacteria from category 4 to 2 (H_2O_2 and $KMnO_4$) and from 3 to 2 ($AlCl_3$ and $FeCl_2$). In this group of substances, $KMnO_4$ and H_2O_2 were given special attention due to the fact that these compounds reduced the content of filamentous bacteria beginning at the lowest concentration applied (25 mg/dm^3).

The activated sludge index decreased significantly as a result of the addition of three compounds tested: sodium hypochlorite, hydrogen dioxide, and aluminum chloride. At 100 mg/dm^3, these compounds reduced the value of the activated sludge index by over 50% in comparison to the control (figure 2). KOŚCIELNIAK and BARBUSIŃSKI [8] obtained similar results during a week-long analysis of activated sludge under addition of hydrogen dioxide; a decrease in the sludge index occurred at 100 mg/dm^3. The authors of this study obtained similar results. Other substances had a lesser impact (figure 2).

Furthermore, the effectiveness of $FeCl_2$ varied significantly depending on the concentration applied. This compound reduced the value of the sludge index by ca. 20% at a concentration of 100 mg/dm^3, with over 50% reduction occurring at a concentration of 200 mg/dm^3.

Results describing the impact of the substances being tested on protozoan survivorship are presented in figure 3. $FeCl_2$ was noted to have the least toxic impact on protozoa. This compound, at a concentration of 100 mg/dm^3, had no negative impact on ciliates and dinoflagellates. However, at a concentration of 200 mg/dm^3 a weak negative impact on dinoflagellates was observed. The effect of other chemicals tested

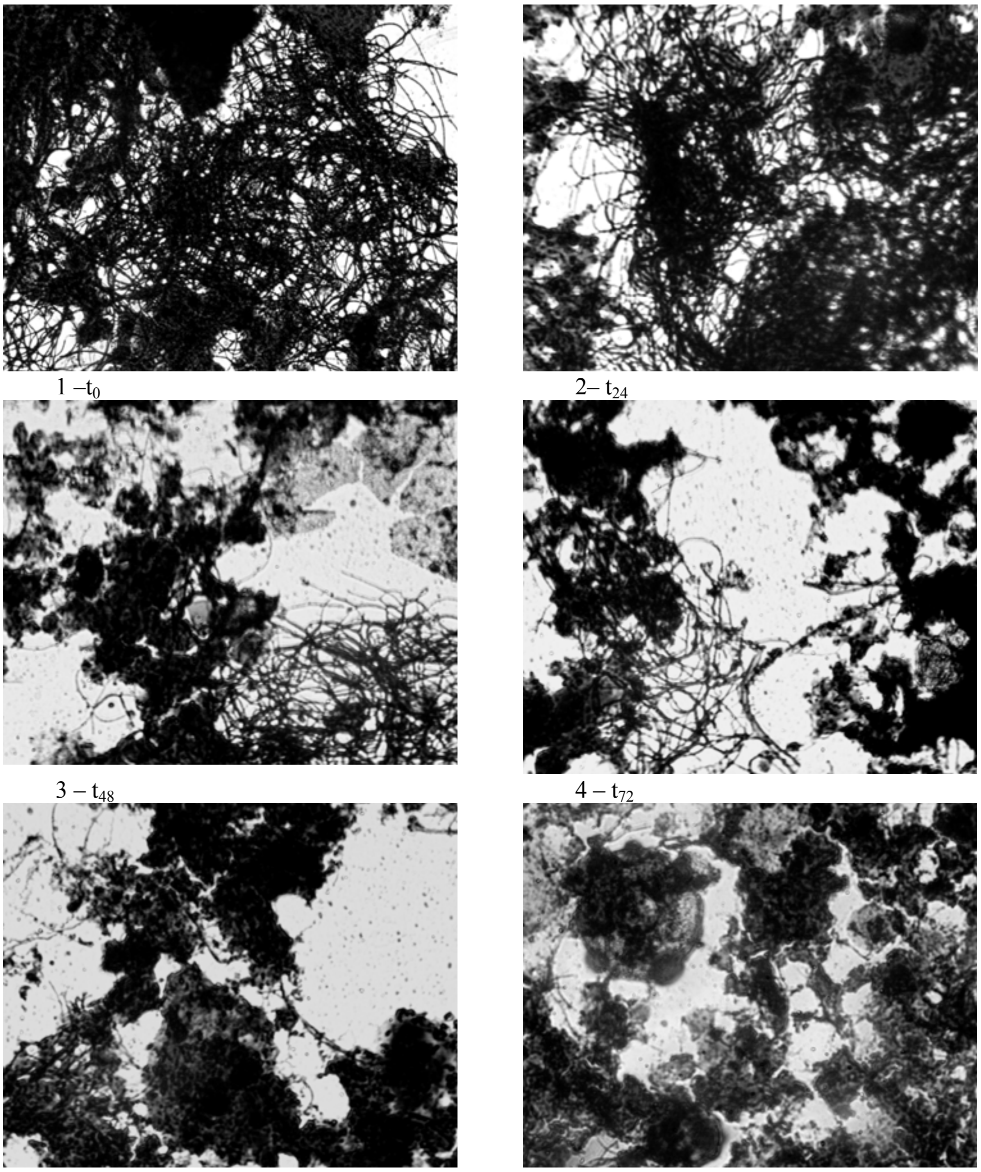

Fig. 1. Abundance reduction of filamentous bacteria in activated sludge after application of aluminum sulfate

was highly dependent on concentration. Such compounds as sodium hypochlorite, H_2O_2 and $Al_2(SO_4)_3$ proved to be highly toxic for protozoa at a concentration of 100 mg/dm^3, but their impact was relatively small: survivorship generally exceeded 60%

in comparison to the control. However, when the concentration of these compounds reached 200 mg/dm^3, their toxicity was very high: survivorship did not exceed 40%. KALISZ [5] obtained slightly different results for sodium hypochlorite. Her research confirms that this compound reduces the number of filamentous microorganisms and the activated sludge index, but repudiates the idea that this substance inhibits the growth of microfauna.

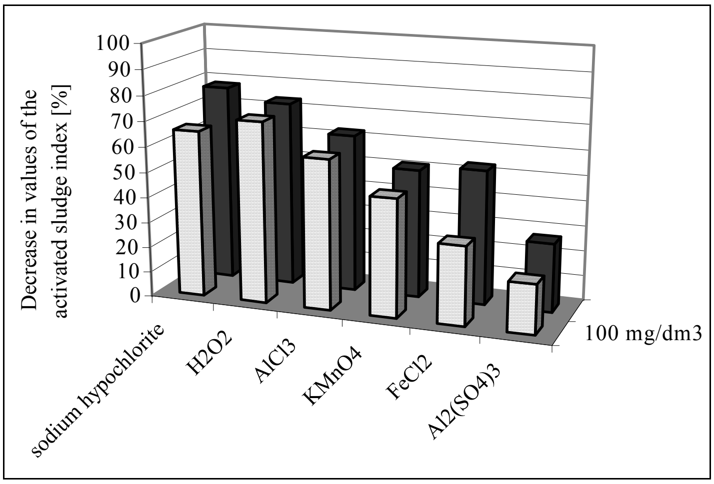

Fig. 2. Changes of values in the activated sludge index after treatment with chemical substances during a 7-day experiment

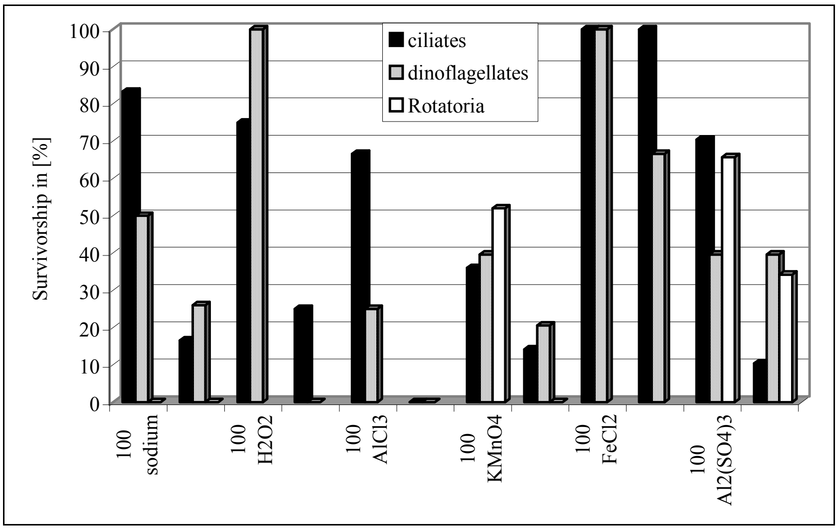

Fig. 3. Impact of substances being tested on protozoan survivorship in the activated sludge

According to GIERŻATOWICZ [14], a 200 mg/dm^3 concentration of hydrogen dioxide is well tolerated by activated sludge; this result was not confirmed by the present study. Furthermore, according to KOŚCIELNIAK and BARBASIŃSKI [8], 100 and 200 mg/dm^3 concentrations of hydrogen dioxide are toxic for microfauna of sludge, a result partially confirmed by this study.

In contrast, aluminum chloride was highly toxic at a concentration of 100 mg/dm^3 and, in higher concentrations, caused complete mortality of all protozoa, which does not confirm results presented by KALISZ [5].

As mentioned previously, non-specific methods of control of excessive growth of filamentous bacteria include immediate neutralization of organisms by addition of chemical substances. Of these, strong oxidants, such as chlorine and hydrogen peroxide, are the most effective. Unfortunately, these substances usually have an impact on other microorganisms inhabiting the sludge [7], which was also confirmed by our experiments.

4. CONCLUSIONS

1. The following substances can be used for neutralizing filamentous microorganisms in activated sludge: sodium hypochlorite, aluminum sulfate, and hydrogen dioxide.
2. Aluminum sulfate and sodium hypochlorite demonstrate the strongest properties for growth limitation of filamentous bacteria in comparison to other substances. These substances are not effective until a concentration of 100 mg/dm^3 has been reached.
3. Sodium hypochlorite was found to be hazardous for sludge microfauna, especially in concentrations exceeding 100 mg/dm^3, and aluminum sulfate reduced the active sludge index insignificantly.
4. Hydrogen dioxide is also effective in reducing numbers of filamentous bacteria in activated sludge. Furthermore, it is effective at low concentrations: below 100 mg/dm^3.
5. Hydrogen dioxide was also observed to significantly reduce the active sludge index, and in concentrations below 100 mg/dm^3, displayed no toxic impact on sludge microfauna.
6. Other tested compounds were not found to be useful in neutralizing filamentous bacteria due to a weak impact on these bacteria or high toxicity for protozoa.

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ZASTOSOWANIE WYBRANYCH ZWIĄZKÓW CHEMICZNYCH W CELU OGRANICZENIA ROZWOJU BAKTERII NITKOWATYCH OSADU CZYNNEGO

Nadmierny rozwój bakterii nitkowatych w osadzie czynnym jest jednym z najczęstszych powodów puchnięcia (pęcznienia) osadu. Zjawisko to prowadzi do zaburzeń w funkcjonowaniu oczyszczalni i przyczynia się do pogorszenia parametrów chemicznych ścieku oczyszczonego. W prezentowanej pracy prowadzono badania nad zastosowaniem wybranych środków chemicznych do ograniczenia rozwoju bakterii nitkowatych. Badania prowadzono w taki sposób, aby na podstawie otrzymanych wyników można było wskazać środek skutecznie ograniczający rozwój tych bakterii, a jednocześnie nietoksyczny dla mikrofauny osadu i nieobniżający skuteczności procesu oczyszczania ścieków. W wyniku przeprowadzonych badań stwierdzono, że do zwalczania bakterii nitkowatych występujących w osadzie badanej oczyszczalni najlepiej nadawały się: podchloryn sodu, siarczan glinu i nadtlenek wodoru.