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EFFICIENCY OF BIOLOGICAL PHOSPHORUS REMOVAL BY FILAMENTOUS BACTERIA

EFEKTYWNOŚĆ BIOLOGICZNEGO USUWANIA FOSFORU PRZEZ BAKTERIE NITKOWATE

Abstract: Phosphorus removal in wastewater treatment plant is carried out by chemical precipitation, advanced biological treatment or a combination of both. One of the biggest problems with high concentration of phosphorus in water environment is eutrophication. Activated sludge flocs have a heterogeneous structure, which consist of a variety of microorganisms. Filamentous bacteria are normally present in the activated sludge and have ability to assimilation of phosphorus. In this study phosphorus accumulation by isolated filamentous bacteria from activated sludge foam was present.

Keywords: filamentous microorganisms, wastewater, activated sludge foam, phosphorus

Introduction

Phosphates are present in high concentrations in wastewaters and can be removed through chemical precipitation, advanced biological treatment or a combination of both. High concentration of phosphorus can lead to many water quality problems, including *e.g.*: increase of the treatment cost, lowering the recreational values, loss of livestock and possible sublethal effects of algal [1]. Conventional activated sludge microorganisms (mixed liquor suspended solids, or MLSS) contain 1.5 to 2.0% phosphorus (dry weight measure). Conventional activated sludge processes achieve removal efficiency of approximately 20% [2].

In the biological phosphorus removal natural capacity of phosphorus accumulation by activated sludge microorganisms has been used. Activated sludge flocs have a heterogeneous structure, which consist of a variety of microorganisms as well as organic and inorganic particles and dead cells surrounded by extracellular polymeric substances [3, 4]. Filamentous bacteria are normally present in the activated sludge and have ability to assimilation of phosphorus, which enters into the composition of several macromolecules in the cell, but same microorganisms (*e.g. Acinetobacter*, *Pseudomonas*, *Aerobacter*,

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Moraxella, *E. coli, Mycobacterium*, *Corynebacterium*) have the ability to store phosphorus as polyphosphates in volutin granules.

Since Fuhs and Chen [5] first described *Acinetobacter* spp. as a microorganism playing an important role in enhanced biological phosphorous removal processes, most subsequent studies have focused on biological reactions and other organisms participating in biological phosphorous removal. Bacterial biomass in conventional activated sludge systems treating municipal sewage usually contains 2-3% of phosphorous. Designed for nutrients removal activated sludge can accumulate as much as 8% of phosphorous. Finally phosphorous is removed from the treated sewage with the surplus activated sludge.

Many wastewater treatment plants have problems in the stage of biological treatment, which are related to the copious growth of filamentous microorganisms. Under specific conditions they proliferate to such an extent that they markedly affect the treatment plant performance causing sludge bulking or foaming. Besides the excessive growth of these bacteria can cause potential problems with the sludge settling that reduce the efficiency of the wastewater treatment plants.

In order to control the growth of these problematic bacteria, their identification has been required. More than 30 different filamentous bacterial morphotypes have been described based on their morphology [6, 7].

Activated sludge systems designed for enhanced nutrients removal are based on the principle of altering anaerobic and aerobic conditions for growth of microorganisms with a high capacity of phosphorous accumulation. Sometimes, to avoid the return of large parts of accumulated phosphorous in the processes of sludge conditioning chemical precipitation is often applied.

Materials and methods

Wastewater and foam samples were taken from a large municipal wastewater treatment plant in Bielsko-Biala. The amount of treated wastewater is approximately 90,000 m^3/d. Solid retention time (SRT) is about 14 days and the concentration of mixed liquor suspended solids (MLSS) fluctuates between 4.3-4.7 g/dm^3.

Table 1

Characterization of sterile wastewater

Parameter	**Unit**	**Wastewater**
pH	-	7.14
T	[ºC]	20.1
ORP	[mV]	209
Oxygen dissolved	[mg O_2/dm^3]	3.4
Ammonia nitrogen	[mg N_{NH4} /dm^3]	29.6
Nitrites	[mg N_{NO2} /dm^3]	0.18
Nitrates	[mg N_{NO3} /dm^3]	2.5
Phosphates	[mg PO_4^{3-}/dm^3]	59.8
Phosphorus	[mg P/dm^3]	19.8
COD	[mg O_2/dm^3]	449
Calcium	[mg Ca^{2+}/dm^3]	60.1
Magnesium	[mg Mg^{2+}/dm^3]	19.0
Potassium	[mg K^{+}/dm^3]	35.6

The wastewater and foam samples were taken from the nitrification chamber. Samples were put in the plastic containers by scoop and then they were immediately (about 20 min) taken to the laboratory and sterilized. Sterile wastewater used in the study was characterized by the parameters presented in Table 1. Prepared wastewater allowed for good growth of filamentous poly-P bacteria.

Samples were analyses for: pH, temperature, redox (oxidation - reduction potential - ORP), phosphates, phosphorus, nitrogen ($N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$), chemical oxygen demand (COD), oxygen dissolved (DO), metal ions: calcium, magnesium and potassium and density of bacteria. The wastewater was determined in accordance to the Standards Methods [8] procedures, using a spectrophotometer HACH DR 4000. Concentration of potassium, magnesium and calcium were determined on an atomic absorption analysis instrument - AAnalyst 100 Perkin Elmer.

The foam samples were also analyses in microbiological way and filamentous microorganisms were isolated according procedure given by Machnicka [9]. In morphological analyses were used a bright field and contrast phase microscope coupled with a camera was used for observations. The microscope used - Nikon Alphaphot - 2 YS coupled with camera Panasonic GP-KR 222 allowed also for size measurements by a programme *Lucia* - ScMeas Version 4.51. Samples for microscopic investigations were stained according to the Gram and Neisser methods.

After identifications of filamentous poly-P bacteria, five reactors, of 500 cm^3 volume each, were filled with wastewater. Reactors including sewages have been sterilized for 30 minutes in an autoclave at a temperature of 121ºC, and a pressure of 150 kPa.

Filamentous poly-P bacteria were analyzed on the basis of biochemical API ZYM test (bioMérieux Polska Sp. z o.o.), which are semi quantitative micro-methods, for the assessment of enzymatic activity and bacteriological selective agar and selective were used. Species choice of activated sludge and identification will be aim of different article.

Results and discussion

Filamentous microorganisms are dominantly present in the scum floating over bioreactors surface (Fig. 1). Confirmation of phosphorus accumulation by microorganisms are visible glowing volutin granules which are noticeable in the cells of filamentous bacteria of foam (Fig. 1).

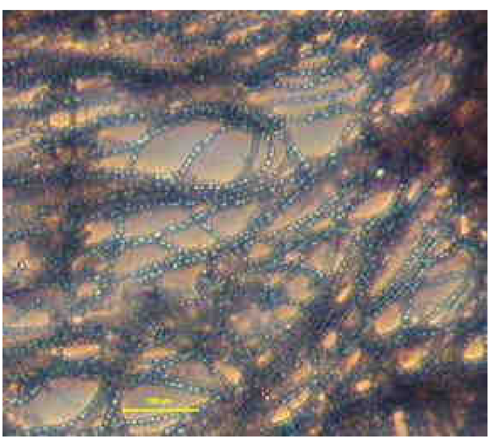

Fig. 1. Filamentous poly-P bacteria in the foam of activated sludge - Neisser method (contrast phase) [authors]

The isolated species have had a similar morphological characteristic. In all of the examined foam samples, among the filamentous microorganisms there was a domination of not branched forms, while the branched - „tree-like" and dichotomic forms were less frequently present (Figs. 2-6 - Gram-method, bright field). Among the isolated filamentous poly-P bacteria, basing on the cultivated, morphological and biochemical tests, the following species have been distinguished: *Microthrix parvicella* (Fig. 2), *Nocardia amarae* (Fig. 3), *Nocardia* sp. (J-27), (Fig. 4), *Nocardia pinensis* (Fig. 5), *Rhodococcus chubuensis* (Fig. 6).

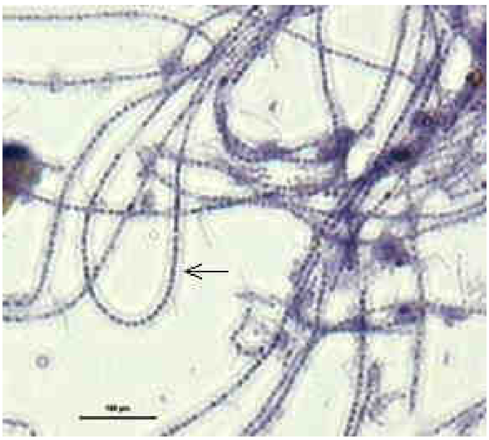

Fig. 2. *Microthrix parvicella* [authors]

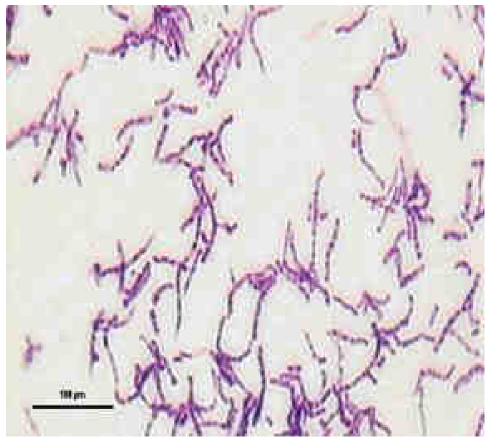

Fig. 3. *Nocardia amarae* [authors]

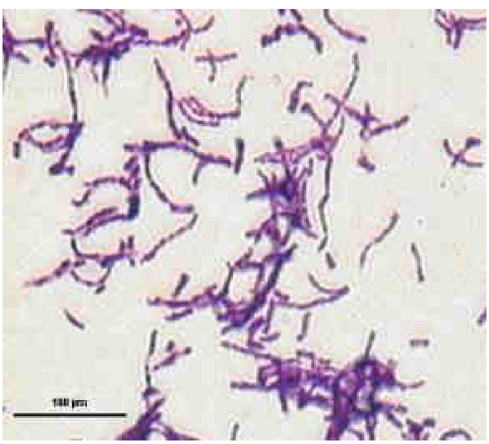

Fig. 4. *Nocardia* sp. (J - 27) [authors]

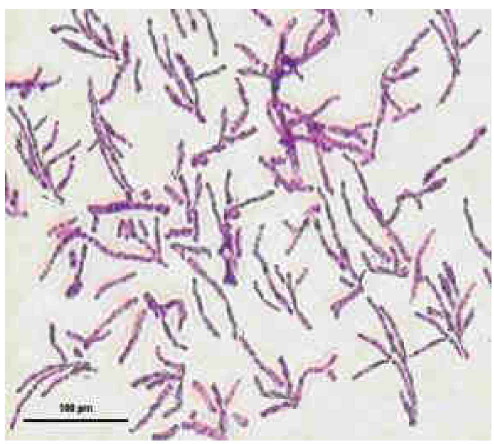

Fig. 5. *Nocardia pinensis* [authors]

The results presented highlight phosphorus uptake by filamentous microorganisms dominantly present in the scum, floating over bioreactors surface. On the basis of chemical determinations it was concluded that the phosphorous uptake was in surplus to that required for biomass synthesis (Fig. 7). Under aerobic conditions increase of stored phosphorous by filamentous microorganisms was measured in parallel to the phosphates decrease in the liquid. The determined removal of phosphorous from sewage was supported by the results of microscopic observations of bacteria stained according to Neisser tests (Fig. 1). Volutin granules which are noticeable in the cells of filamentous bacteria of foam are confirming accumulation of phosphorous by the filamentous microorganisms.

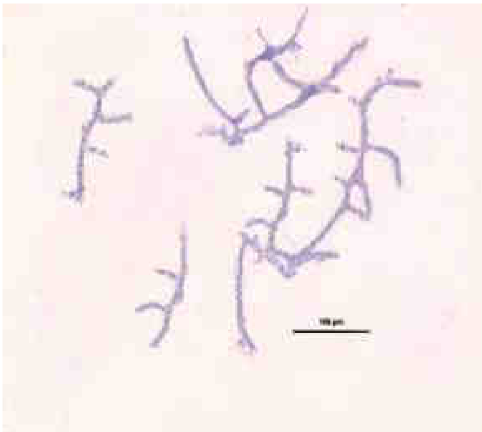

Fig. 6. *Rhodococcus chubuensis* [authors]

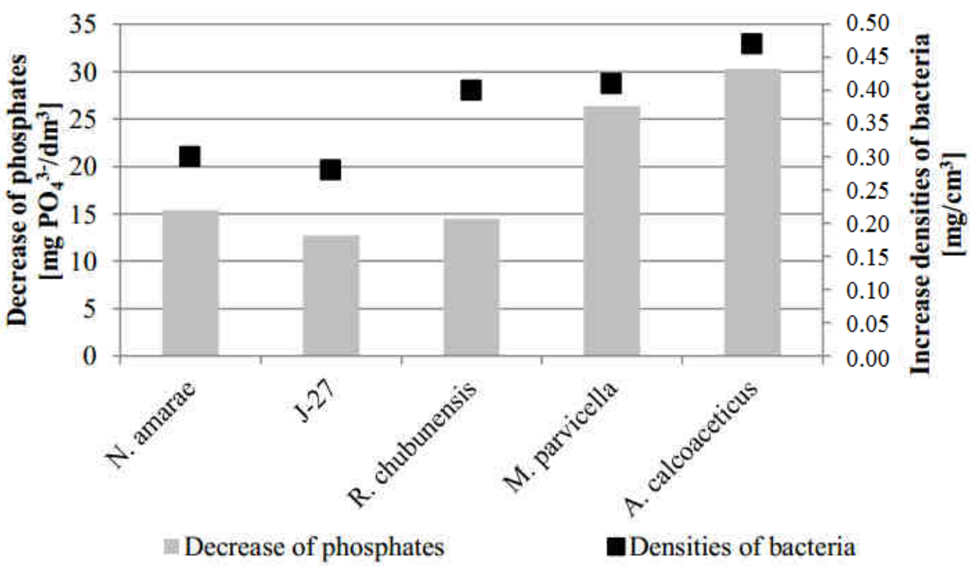

Fig. 7. Changes of phosphates concentration and densities of bacteria

Microthrix parvicella was particularly active in phosphorous accumulation (Fig. 7). *Microthrix parvicella* dominantly present in the floating scum over the bioreactors surface, has had a capacity of phosphorous uptake and accumulation similar to that of *Acinetobacter calcoaceticus* (Fig. 7).

In the stage of logarithmic growth *Microthrix parvicella* and *Nocardia amarae* have shown a distinctly higher ability of phosphorous accumulation in comparison to the ability of volutin granules storage by *Acinetobacter calcoaceticus* (Fig. 7).

According to various authors [10, 11] microorganism's cell comprises 70-86% of water and the hydration depends mainly on: the quantity of materials up, the composition of the culture medium, the growth phase and characteristics of species. Supposing that the cell organisms tested contained only 75% of the water, and not taking into account weight of the inoculum surrounding the cells, after the calculation can be demonstrated that the phosphorous accumulated comprise from 5.0 to 8.7% by dry weight of cells (Table 2). Additionally, converting the weight of accumulated phosphorous per biomass unit of bacteria (1 mg), could be found that these microorganisms assimilated from 0.017 to 0.022 mg P/mg biomass (Table 2).

Table 2.

Content of phosphorous in biomass

Bacteria species	**Accumulated phosporous [mg P/mg biomass]**	**Percentage of phosphorous in biomass [%]**
Nocardia amarae	0.017	6.7
Nocardia J - 27	0.014	5.7
Rhodococcus chubuensis	0.012	5.0
Microthrix parvicella	0.022	8.7
Acinetobacter calcoaceticus	0.021	8.5

Conclusions

Filamentous bacteria have ability to phosphorous accumulation in form of volutin granules. Among analyzed microorganisms highest storage properties characterized by *Microthrix parvicella* (filamentous bacteria) and *Acinetobacter calcoaceticus* (not filamentous bacteria). Percentage of phosphorous in biomass was 8.7 and 8.5%, respectively. Though technological troubles caused by filamentous microorganisms, they could be used for the of biological phosphorous removal processes with good efficiency.

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EFEKTYWNOŚĆ BIOLOGICZNEGO USUWANIA FOSFORU PRZEZ BAKTERIE NITKOWATE

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Abstrakt: Usuwanie fosforu w oczyszczalniach ścieków przebiega w procesach: chemicznej precypitacji, biologicznego oczyszczania oraz jednoczesnym strącaniu i akumulacji. Jednym z głównych problemów wysokich stężeń fosforu w środowisku wodnym jest eutrofizacja. Kłaczki osadu czynnego mają heterogenną strukturę, w skład której wchodzą różnorodne mikroorganizmy. Normalnym składnikiem osadu czynnego są bakterie nitkowate mające zdolność asymilacji fosforu. W przedstawionych badaniach starano się określić zdolność wyizolowanych z piany osadu czynnego bakterii nitkowatych do kumulacji fosforu.

Słowa kluczowe: organizmy nitkowate, ścieki, piana osadu czynnego, fosfor