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ALICJA MACHNICKA*

FILAMENTOUS MICROORGANISMS IN PHOSPHORUS REMOVAL FROM WASTEWATER

Phosphorus removal from wastewater effectively prevents water in closed systems from eutrophication. Traditionally phosphates have been removed from wastewater due to chemical precipitation. An alternative approach to this way of phosphate removal is its biological removal. Many microorganisms have the ability to accumulate phosphorus as polyphosphates in volutin granules. The aim of the research was to compare the uptake of phosphorus carried out by filamentous microorganisms present in the foam with that by activated sludge microorganisms. The results obtained have indicated that filamentous microorganisms abundantly occurring in the scum have the ability to uptake and accumulate phosphorus at the rate at least similar to that of activated sludge microorganisms.

1. INTRODUCTION

Phosphates are present in high concentrations in several municipal and industrial wastewaters. If present in excess amounts they can lead to many water quality problems, including, e.g., an increase in treatment cost, worse recreational values, a decrease in the size of populations of plants and animals as well as possible sub-lethal effects caused by algal blooming [1]. Phosphate (and nitrogen compounds) removal from wastewater effectively prevents lakes and other natural aquatic systems from eutrophication.

Chemical precipitation has been considered to be a conventional method of phosphate removal from wastewater. Although this method is relatively easy, it seems relatively expensive and primarily results in production of large quantities of sludge. The quantity of sludge to be disposed can increase in volume up to 30%. Moreover the sludge can be contaminated with heavy metals.

An alternative approach to this method of phosphate removal is its biological removal. Microorganisms (e.g., *Acinetobacter, Pseudomonas, Aerobacter, Moraxella, E. coli, Mycobacterium, Corynebacterium*) assimilate phosphorus which is

* Institute of Environmental Protection and Engineering, University of Bielsko-Biała, Willowa 2, 43-309 Bielsko-Biała, Poland.

indispensable component of several macromolecules in the cell [2], [3]. Some microorganisms have the ability to accumulate polyphosphates in special granules (volutin granules). Polyphosphates are linear polymers whose residues linked together by high-energy phosphoanhydride bonds may account for up to 10–20% of the cellular dry weight [4], [5]. Polyphosphate can be observed under bright-field or phase-contrast microscope. Neisser's stain is used to observe these granules under bright-field microscope. Nuclear magnetic resonance (NMR) has been also recently used to detect polyphosphate granules in wastewater microorganisms [6], [7]. Phosphorus accumulation by flock-forming bacteria is at present well-known and confirmed.

Up to the present, the contribution of filamentous microorganisms present in wastewater to phosphorous removal was not evaluated. The filamentous microorganisms are responsible for foam formation and activated sludge swelling [8]. Foaming usually takes place in many wastewater treatment plants world wide [9]–[13], especially in those designed for carbon and nutrient removal. The foam formed can cover the entire surface or at least the surface where anaerobic dephosphatation and anoxic denitrification take place. Also settling tanks can be partially or totally covered with the foam. It is difficult to remove the foam, therefore it is considered to be the burden. And finally it affects adversely the process of anaerobic sludge digestion. Consequently, the process of foam formation absorb attention of many investigators and treatment-plant operators. However, filamentous microorganisms are the usual components of the activated sludge microflora. The importance of filamentous organisms and/or their contribution to phosphorus uptake were not studied so far. There are only some controversial findings and no distinction was made between the role of filamentous organisms present in the foam and those associated with the activated sludge flocks.

On the basis of our own investigations it was discovered that in the liquid comprising the microorganisms present in the foam we can found as much as 200 mg PO_4^{3-}/dm^3. Release of phosphates to the effluent in secondary settling tanks during the wastewater treatment can affect adversely their quality and makes it impossible to reach their required level of, e.g., 1.0 mg P_{total}/dm^3. It seems therefore important to explain the mechanisms of phosphorus accumulation and release by filamentous bacteria. The investigations carried out should have some practical implications for an overall biological process of phosphorus removal.

2. MATERIALS AND METHODS

The aim of our investigations was to compare the rate of phosphorus uptake by filamentous bacteria present in the foam with that typical of the activated sludge microorganisms.

The samples of foam floating on the surface of bioreactors were taken from a large municipal wastewater treatment plant. The plant was designed for a flow of 60 000 m^3/d. At present the volume of the wastewater treated is about 40 000 m^3/d. Hydraulic retention time (HRT) approaches 6 hrs. The wastewater is partially diluted with infiltration water which results in a modest organic load in the order of 0.12 g BOD/g·d. In order to remove the nitrogen compounds, anaerobic, anoxic and aerobic zones have been designated.

The investigations presented were performed in 4 stages. The samples were always taken from the surface of foam in the nitrification (aerobic) zone. The time that elapsed from foam sampling to laboratory analyses was about 20 minutes. The foam samples were analysed chemically and microscopically. Samples were analysed for pH, temperature, redox (oxidation reduction potential – ORP), phosphates, total phosphorus, various nitrogen forms, i.e. nitrates, nitrites and ammonium ($N–NO_3$, $N–NO_2$ and $N–NH_4$), COD, BOD and dissolved oxygen. Determinations were carried out according to the Standard Methods for the Examination of Water and Wastewater (the 17th edition) [11] using a spectrophotometer HACH DR 400. The concentrations of potassium, magnesium and calcium were determined with an atomic absorption analysis instrument – AAnalyst 100 Perkin Elmer. For microscope morphological analyses a bright-field microscope and a contrast-phase microscope coupled with a camera for observations were used. The microscope used – Nikon Alphaphot – 2 YS coupled with camera Panasonic GP–KR 222 allowed also size measurements by a programme *Lucia* – ScMeans Version 4.51. Samples for microscopic examinations were stained according to the Neisser method. Bacteria present in the foam were analysed on the basis of biochemical test (API ZYM, API 20E, API Staph) and using selective agar and selectively differentiated agars.

The clusters containing volutin granules were examined by means of a scanning electron microscope equipped with the attachment for an energy dispersive analysis of X-ray (EDX). The examinations aimed at confirmation of the cluster composition.

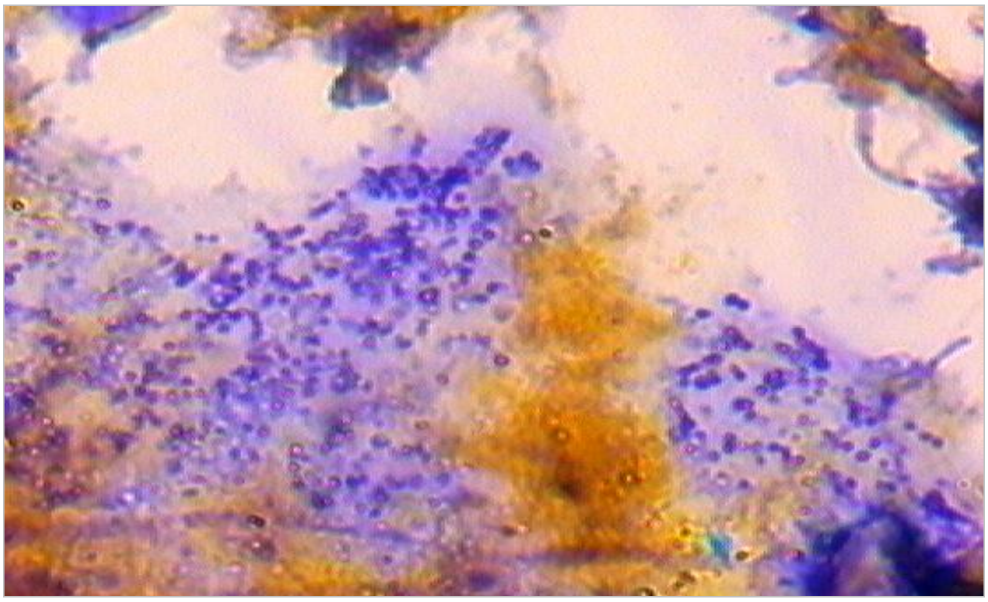
3. RESULTS AND DISCUSSION

An example of raw wastewater quality was given in the table. The results present the phosphorous uptake by the microorganisms of activated sludge and filamentous microorganisms that are prevailing in the scum floating in bioreactors. It was clearly shown that the filamentous microorganisms are capable of uptaking and accumulating phosphorous at a rate similar to that of activated sludge biomass. The removal of phosphorous from sewage was supported by the results of microscopic examinations of bacteria stained according to the Neisser tests (Photos 1, 2 and 3). Blue volutin granules which are visible in the cells of filamentous bacteria present in foam corroborate accumulation of phosphorus by these microorganisms.

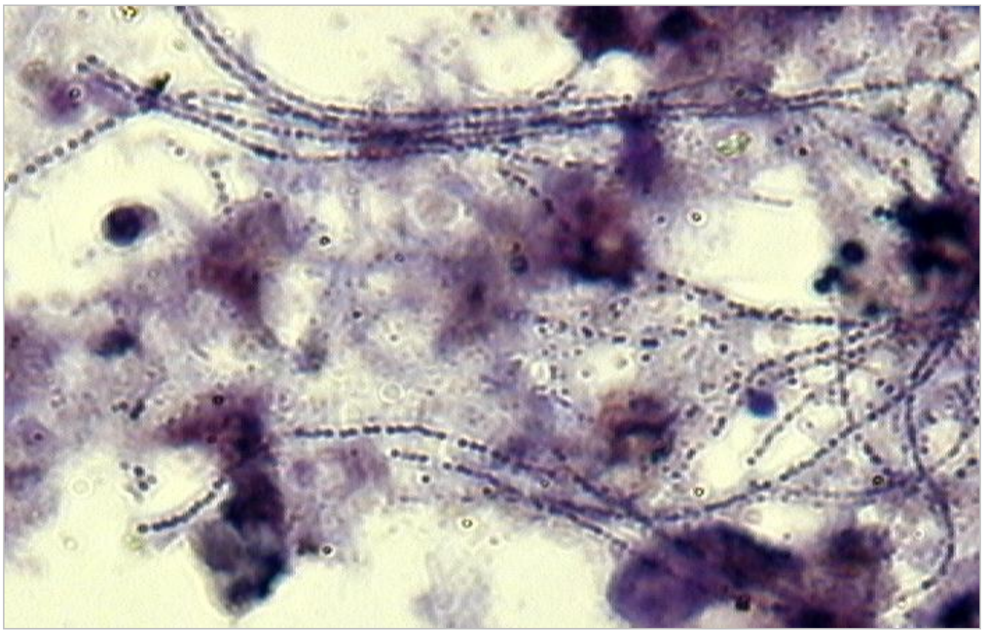
Table

The quality of raw wastewater

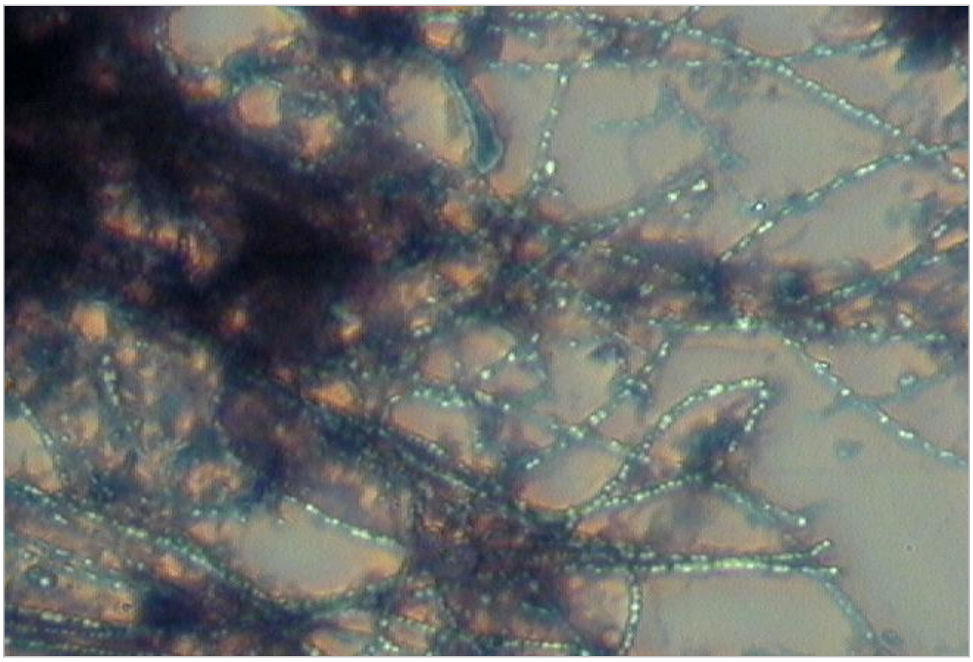
Parameter	Units	Values
pH		6.34
ORP	[mV]	58.2
Dissolved oxygen	[mg O_2/dm^3]	1.73
Ammonium nitrogen	[mg N_{NH_4}/dm^3]	12.0
Nitrite nitrogen	[mg N_{NO_2}/dm^3]	0.06
Nitrate nitrogen	[mg N_{NO_3}/dm^3]	0.50
Phosphates	[mg PO_4^{3-}/ dm^3]	10.76
Total phosphorus	[g P_t /kg d.s.]	3.7
COD	[mg O_2/dm^3]	285
BOD	[mg O_2/dm^3]	144
Total suspended solid	[mg/dm^3]	80–90
Calcium	[mg Ca^{2+}/dm^3]	47.6
Magnesium	[mg Mg^{2+}/dm^3]	5.106
Potassium	[mg K^+/dm^3]	10.335



Phot. 1. Polyphosphate granules in the activated sludge biomass – bright-field microscope



Phot. 2. Polyphosphate granules in the filamentous microorganisms – bright-field microscope



Phot. 3. Polyphosphate granules in the filamentous microorganisms – contrast-phase microscope

Based on chemical determinations it can be concluded that the uptake of phosphorus by filamentous bacteria is higher than that required for the biomass synthesis, and its substantial part is accumulated within cells as high polymers (polyphosphates). Up to now that ability was mainly attributed to the bacteria present in the flocks of activated sludge (Phot. 1). The results from the Neisser tests were confirmed by the analysis carried out with electron microscope equipped with the EDX attachment which will be the subject of the next publication. Under aerobic conditions an increase in the phosphorous accumulated by microorganisms was measured in parallel with the phosphates decrease in the liquor (figures 1 and 2).

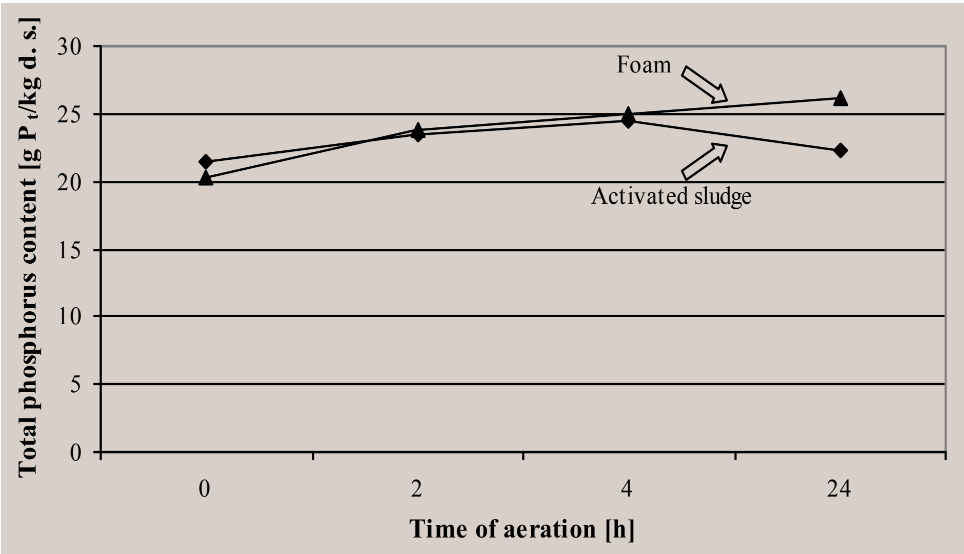


Fig. 1. Concentration of total phosphorus in activated sludge biomass
and filamentous microorganisms versus aeration time

The maximum concentration of phosphorous (24.6 g P_{tot}/kg $_{dry\ solids}$ as an average of 4 series of experiments) in the biomass of activated sludge was obtained after 4 hours of sludge aeration (figure 1). Simultaneously after 4 hours of sludge aeration the lowest concentration of phosphates was measured in the liquor. The concentration of phosphates in the liquor decreased on an average from 17.6 mg PO_4^{3-}/dm^3 at the beginning to 3.8 mg PO_4^{3-}/dm^3 at the end of the aforementioned period of aeration (figure 2). Surprisingly, further aeration to some extent resulted in the release of phosphates from the activated sludge flocks.

In contrast, the filamentous microorganisms proved to be able to uptake phosphates for longer, within the entire period of 24 hours of the experiments, and to accumulate them in the form of polyphosphates. An average concentration of total phosphorous of 20.4 g P_{tot}/kg $_{dry\ solids}$ measured in the filamentous microorganisms at the beginning of the experiments, after 24 hours of aerobic conditions, increased to 26.2 g P_{tot}/kg $_{dry\ solids}$ (figure 1).

Obviously, the inclusion of polyphosphates in the microorganisms cells resulted in a decrease of phosphates concentration in the liquor from 54.9 (on an average) to 8.0 mg PO_4^{3-}/dm^3 (figure 2).

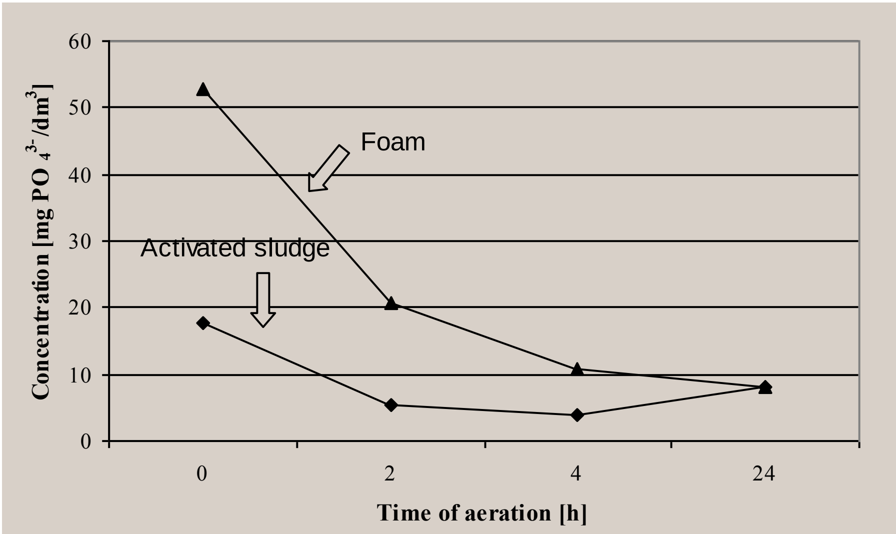


Fig. 2. Concentration of phosphates in the liquid associated with activated sludge and foam versus time of aeration

The rate of phosphates uptake was certainly related to its initial concentration both in the liquid associated with the activated sludge flocks and in the foam. A higher initial PO_4^{3-} concentration in the foam liquid was probably due to depletion of dissolved oxygen (no direct oxygen supply to the floating scum) approaching anaerobic conditions.

4. CONCLUSIONS

1. Filamentous microorganisms that predominate in the floating scum in bioreactors have the capability to uptake and accumulate phosphorus at the rates similar to these of microorganisms of activated sludge.

2. The removal of phosphorus from wastewater by filamentous microorganisms was corroborated by microscopic examination of polyphosphate granules in bacterial cells.

3. Filamentous microorganisms are characterized by the capability to uptake phosphorous for longer during 24 hours of aeration, while the microorganisms forming activated sludge flocks are able to uptake phosphates only for the first 4 hours of aeration. A further uptake of phosphates by filamentous bacteria was confirmed by microscopic examination of polyphosphate granules whose number and volume grow.

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ZNACZENIE MIKROORGANIZMÓW NITKOWATYCH W USUWANIU FOSFORU ZE ŚCIEKÓW

Efektywnym sposobem zapobiegania eutrofizacji zamkniętych systemów wodnych jest m.in. usuwanie fosforu ze ścieków. Tradycyjnie fosfor eliminuje się, dokonując jego chemicznej precypitacji. Alternatywnym rozwiązaniem problemu jest biologiczne usuwanie fosforu. Wiele gatunków mikroorganizmów ma zdolność gromadzenia tego pierwiastka w postaci ziaren wolutyny.

Celem badań było porównanie możliwości pobierania fosforu ze ścieków przez mikroorganizmy nitkowate występujące w pianie i mikroorganizmy osadu czynnego. Uzyskane wyniki wskazują, że mikroorganizmy nitkowate dominujące w pianie wykazują zdolność pobierania i gromadzą fosfor w stopniu co najmniej podobnym do mikroorganizmów osadu czynnego.