THE IMPORTANCE OF POTASSIUM AND MAGNESIUM IONS IN BIOLOGICAL PHOSPHORUS REMOVAL FROM WASTEWATER.

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ABSTRACT
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. Traditionally phosphate removal from wastewater has been achieved mainly through chemical precipitation. An alternative approach to phosphate-removal is that of biological phosphate removal. The aim of the research was to compare the phosphorus uptake by filamentous microorganisms present in the foam and that by activated sludge bacteria as well the importance of potassium and magnesium ions in biological phosphorus removal from wastewater.

KEYWORDS
filamentous microorganisms, phosphates and phosphorus removal, potassium and magnesium ions.

INTRODUCTION
Phosphorus removal from wastewater is an effective approach for prevention of eutrophication in closed water systems. If present in excess amounts they can lead to many water quality problems including e.g.: increase of treatment cost, lowering the recreational values, loss of live-stock and possible sub-lethal effects of algal [1]. Traditionally phosphate removal from wastewater has been achieved mainly through chemical precipitation. Chemical precipitation is a relatively easy process, but this technique is rather expensive and primarily results in production of large quantities of sludge. The quantity of sludge to be disposed can increase in volume of up to 30%. Also the sludge can be contaminated with heavy metals.

An alternative approach to phosphate-removal is that of biological phosphate removal. Many microorganisms (e.g., Acinetobacter, Pseudomonas, Aerobacter, Moraxella, E.coli Mycobacterium, Corynebacterium) assimilate phosphorus, which enters into composition of several macromolecules in the cell [2, 3]. Same microorganisms have the ability to store phosphorus as polyphosphates in volutin granules. Polyphosphate is a linear polymer of residues linked together by high-energy phosphoanhydride bonds and may account for up to 10 – 20% of the cellular dry weight [4, 5]. Polyphosphate can be observed under bright-field or phase-contrast microscopy. 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 was often confirmed.
So far however, the implication of filamentous microorganisms present in the biological wastewater treatment processes on phosphorous removal was not evaluated. The filamentous microorganisms are responsible for foam formation and activated sludge bulking [8]. Foaming is a common problem encountered in many wastewater treatment plants world wide [9 – 13]. The importance and/or contribution of filamentous organisms in phosphorus uptake were not studied so far.

Microorganisms require also various cations. Cations as potassium, magnesium, calcium and iron must be present above critical concentrations in culture media.

The potassium and magnesium concentrations in the influent of biological wastewater treatment plants systems are responsible for specific requirements and activate of microorganisms involved in these processes. Potassium and magnesium play role principal inorganic cations in cells. Constitutes approximately 1% of the dry weight of the microbial cell, as the cofactors for same enzymes, potassium and magnesium stimulates enzyme reactions associated with a synthesis of cell materials [14]. The importance of potassium and magnesium for microorganisms responsible for biological phosphorus removal is even more significant. Potassium defines cell membrane permeability and play a major role in the phosphate transport between surrounding environment and cell [15]. Moreover this cation is an essential counterion for polyphosphate in the cell, and is general an important factor in the cells energy generation [16, 17]

An enzyme, polyphosphate kinase, catalyzes polyphosphate biosynthesis in presence of magnesium ions by transferring the terminal phosphoryl group from ATP to polyphosphate chain. Polyphosphate degradation is driven by several enzymes which depend on inorganic cations. Magnesium acts as an important counterion of polyphosphates too. It is taken up and released simultaneously with phosphate. Consequently these cations (potassium and magnesium) are necessary for polyphosphate accumulation in biological phosphorus removal.

MATERIALS AND METHODS
The aim of the research was to compare the phosphorus uptake by filamentous microorganisms present in the foam and that by activated sludge bacteria as well as the importance of potassium and magnesium ions in biological phosphorus removal from wastewater. Activated sludge and foam samples were taken from 3 different municipal treatment plants in Poland. Activated sludge and foam samples were analysed chemically and microscopically. Concentration of phosphates in liquid and total phosphorus in activated sludge biomass and foam were defined in accordance to Standards Methods procedures, using a spectrophotometer HACH DR 4000. Microscopic investigation were carried out using a microscope of bright field and contrast phase coupled with a camera. Results were analysed by a painting processing computer programme LUCIA. Samples for microscopic investigations were stained according to the Neisser method.

Concentration of potassium and magnesium ions were determined by atomic absorption analysis AAnalyst 100 Perkin Elmer.

RESULTS AND DISCUSSION
The results obtained indicated that filamentous microorganisms dominantly present in the scum have ability of phosphorus uptake and accumulation at a rate similar to that of activated sludge bacteria. The determined removal of phosphorus from sewage was supported with the results of microscopic observations of samples stained according to Neisser method (Plate 1 and 2).
It was found that phosphorus uptake by filamentous organisms was above the required for the biomass synthesis. A substantial part of phosphorus is stored within the microorganisms cells as large polyphosphates polymers. That ability of phosphorus uptake was so far mainly attributed to bacteria present in the activated sludge flocks. Under aerobic conditions an increase of stored phosphorus by microorganisms was measured in parallel to phosphates decrease in the liquor (Fig. 1, 2).
Figure 1. Changes of phosphates concentration as a result of removal by activated sludge biomass and filamentous microorganisms of foam (aerobic conditions).

Figure 2. Changes of phosphorus content in activated sludge biomass and foam filamentous microorganisms (aerobic conditions).
The maximum content of phosphorus rating 26.2 g P/kg dry solids (as an average of 3 series of experiments showed) for activated sludge was found after 4 hours of aeration (Fig. 2). Simultaneously after 4 hours of aeration the lowest concentration of phosphates was measured in the liquor. The phosphates concentration in the liquor have decreased in average from 34.8 mg PO₄/l, at the beginning, to 6.3 mg PO₄/l at the end of the aforementioned period of aeration (Fig. 1). Surprisingly, to some extent, continuation of aeration resulted in phosphates release from the activated sludge flocks.

In contrast, the filamentous microorganisms have shown a capacity of prolonged phosphates uptake within the entire period of 24 hours of the experiments, and store them in the form of polyphosphates. The average total phosphorous content of 19.2 g P/kg dry solids present in the filamentous at the beginning of the experiments, after 24 hours under aerobic conditions has increased to 40.2 g P/kg dry solids (Fig. 2).

Obviously the inclusion of polyphosphates in the microorganisms cells, resulted in decrease of phosphates in the liquor from 78.2 (in average) to 3.8 mg PO₄/l (Fig. 1).

The rate of phosphates uptake was certainly related to the initial concentration in the liquid associated to the activated sludge flocks or the foam. The higher initial PO₄³⁻ concentration of phosphates in the foam liquid was probable the effects of dissolved oxygen depletion, (no direct oxygen supply to the floating scum) approaching anaerobic conditions.

The results obtained in this study showed that uptake and accumulation of phosphates in aerobic condition dependent on potassium and magnesium ions concentrations in foam liquid (Fig. 3 and 4). Potassium concentrations in the liquid decreased from 45.0 mg K⁺/l to 24.0 mg K⁺/l, and magnesium concentrations decreased from 17.9 mg Mg⁺/l to 10.2 mg Mg⁺/l.

![Figure 3](image-url)  
**Figure 3.** Phosphates uptake by filamentous microorganisms dependent on potassium concentration with aeration time.
In anaerobic condition potassium and magnesium were released together with phosphates. The studies have shown that the release of phosphates from biomasses foam microorganisms was connected to the concentrations increase of potassium and magnesium in scum liquid (Fig. 5 and 6). Potassium concentrations in the liquid increased from 23.8 mg K⁺/dm³ to 97.7 mg K⁺/dm³, and magnesium concentrations increased from 15.3 mg Mg⁺/dm³ to 20.8 mg Mg⁺/dm³.

Comparing the amount of phosphorus uptake in ratio to the elementary dry weigh of filamentous microorganisms present in the foam with the suitable amount of potassium or magnesium taken it could be possible to calculate of ratio K/P and Mg/P (Fig. 7 and 8). The research done in the laboratory scale evaluated ratio K/P contained between 1.8 and 19.4 however relation was Mg/P from 0.7 to 8.2.

In anaerobic conditions comparing the effects of phosphates release to the effects of potassium and magnesium release it can be draw the graph possibility of K/P and Mg/P dependent on time (Fig. 9 and 10).

The evaluated ratio K/P was contained between 2.39 and 1.32 however the ratio of Mg/P was from 0.68 to 0.28.

In literature, so far there has been no information concerning the value of ratio K/P and Mg/P for foam in aerobic and anaerobic conditions. Only the values of this ratio referring to the activated sludge can be found [18, 19].
Figure 5. Potassium release together with phosphates (anaerobic conditions).

Figure 6. Magnesium release together with phosphates (anaerobic conditions).
Figure 7. Changes of ratio K/P - filamentous microorganisms (aerobic conditions).

Figure 8. Changes of ratio Mg/P - filamentous microorganisms (aerobic conditions).

Figure 9. Changes of ratio K/P - filamentous microorganisms (anaerobic conditions).
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CONCLUSIONS
1. Filamentous microorganisms dominantly present in the floating scum over the bioreactors liquid surface, have a capacity of phosphorus uptake and accumulation at a rate similar to that of activated sludge biomass.
2. The measured uptake of phosphorus from wastewater by filamentous micro-organisms was supported with microscopic observations of polyphosphates granules growth within the bacteria cells.
3. The effects of biological phosphorus uptake depend on the presence of potassium and magnesium ions.
4. The variable in time rate of potassium and magnesium release in comparison to the release of phosphates (expressed as K/P an Mg/P rates), are confirming that polyphosphates are present in different forms and also the probability of simultaneous forming of intercellular and external polymers.
5. Change of rate potassium and magnesium release (ratio K/P and Mg/P) in time, confirm occur different form polyphosphates and probability simultaneous of formed inside and outside cells.
6. Potassium and magnesium polyphosphates are unstable and are very fast released under anaerobic conditions.

REFERENCES

Figure 10. Changes of ratio Mg/P - filamentous microorganisms (anaerobic conditions).


