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Analysis of the Effect of Temperature Cycling on Phosphorus Fractionation in Activated Sludge

Elżbieta Bezak-Mazur, Renata Stoińska, Bartosz Szeląg
Kielce University of Technology

1. Introduction

Sewage treatment by the activated sludge method consists in the mineralization of organic pollutants by microorganisms in aerated bioreactors. Decomposing organic matter provides microbes with essential nutrients necessary for life, i.e. mineral forms of carbon, phosphorus, sulfur and nitrogen. Temperature is an important parameter affecting wastewater treatment since its increase accelerates the decomposition of organic compounds. The optimum temperature of this process falls within the range of 18-22°C (Bugajski et al., 2012). As far as the biological phosphorus removal is concerned, reference sources provide conflicting reports concerning the optimum temperature. According to Converti et al. (Converti et al., 1995) the biological phosphorus removal is most efficiently performed at the temperatures from 20 to 37°C, whereas Florentz M. et al. think a different range of temperatures is optimum for this process, i.e. 5-15°C (Florentz et al., 1987). The latter maintain it is associated with the metabolism of phosphorus bacteria since these microorganisms are believed to belong mainly to a psychrophilic group and can already grow at temperatures slightly above 5°C (Kaczor 2008). Similarly, Xiong Liu et al. (Xiong Liu et al. 2014) regard phosphorus accumulating bacteria as primarily psychrophilic, which accounts for the reduction in phosphorus removal at temperatures above 20°C. The studies of Bassin et al. (Bassin et al. 2012) also investigated the process of removing phosphorus from wastewater. Their research revealed that the efficiency of phosphorus removal at 20°C (> 90%) was higher than that at 30°C (60%). Piaskowski et al. (Piaskowski et al., 2005) examined the effect of temperature on biological phosphorus removal in a wider temperature range (5-45°C). They demonstrated that in anaerobic conditions the rise in temperature from 5°C to 30°C was accompanied by the increase in the amount of the released orthophosphates from 13.25 to 27.4 mg P/dm^3. A similar relation was also observed during the accumulation of orthophosphates under aerobic conditions, with the highest intensity being registered for the range of 25-30°C. These authors point to a direct effect of wastewater temperature on the rate of enzymatic reaction involving PAO bacteria. The rate of the phosphorus removal reaction in the function of temperature observed by the authors is in accordance with van't Hoff's rule, determining a 2-3-fold increase in the rate of enzymatic reactions for each rise in temperature by 10°C within the range of 0-30°C and a reduction in the rate at temperatures above 30°C (Kaczkowska 2002; Rheinheimer 1977). Since there are discrepancies in reference sources with regard to the effect of sewage sludge temperature on the phosphorus removal process, this research paper presents the analysis results of phosphorus speciation in activated sludge in an annual cycle (in temperate climate) in which the sewage sludge temperature can range from 5°C to 25°C. The tests examined samples of sewage sludge from oxygenated zone which represents the first stage of phosphorus accumulation by rapidly multiplying PAO microbes.

2. Research methodology

In order to determine the effect of cyclic temperature changes on the phosphorus removal process, the investigation involved monthly analyses of phosphorus fractionation in activated sludge samples taken from six sewage treatment plants (Table 1).

The content of different phosphorus fractions was determined in the collected sludge samples using fractionation scheme presented by Golterman (Golterman 1996). This is a sequential extraction scheme method where each extraction stage is followed by permeating the sample and treating it with another reagent of increasing extraction force (Tab. 2) (Bezak Mazur et al.,2014). Each determination of phosphorus fractions was repeated three times.

Table 1. Sewage treatment plants that provided the activated sludge samples
Tabela 1. Prezentacja oczyszczalni z których pobierano osady czynne do badań

Type of plant		Treatment method	Nominal capacity
A	biological	three-stage activated sludge method	900 m^3/d
B	biological	three-stage activated sludge method	1200 m^3/d
C	biological	three-stage activated sludge method with a sequencing batch reactor operating in cycles	300 m^3/d
D	biological	three-stage activated sludge and the rotating biological bed methods	500 m^3/d
E	biological-chemical	three-stage activated sludge method and chemical precipitation with lime	500 m^3/d
F	biological-chemical	three-stage activated sludge method and chemical precipitation with the PIX coagulant	72000 m^3/d

Table 2. Modified extraction scheme according to Golterman (Golterman 1996)
Tabela 2. Zmodyfikowany schemat ekstrakcji wg Goltermana (Golterman 1996)

Stage	Extraction conditions	Fraction	Phosphorus fraction – description
1	4h, 0.05 M $CaNa_2$-EDTA	Ca-EDTA	phosphorus associated with oxides and hydroxides of iron, aluminum and manganese
2	18h, 0.1 M Na_2-EDTA	Na-EDTA	phosphorus associated with carbonates
3	2h, 0.5 M H_2SO_4	H_2SO_4	phosphorus found in soluble combinations with organic matter
4	2 M NaOH, 2h	NaOH	The remaining forms, including phosphorus bound with aluminosilicates and contained in organic matter in the form of combinations not affected by the sulfuric acid activity in stage 3.

The concentration of total phosphorus in the obtained permeates was determined by spectrophotometry using a UV-VIS Lambda 25 PERKIN ELMER spectrophotometer. During sampling the temperature of the activated sludge was measured by means of a portable thermometer.

3. Results analysis

Wastewater treatment is predominantly conducted in a continuous manner, which results in a constant supply of organic matter. Activated sludge is therefore a mixture of substances already mineralized (flowing out of the bioreactor as an excess sludge) and organic substances flowing into the bioreactor with raw sewage. This diversity of organic and inorganic matter in the activated sludge is also reflected in the results of the speciation analysis of phosphorus in the sludge. Phosphorus is a mineral contained both in organic and inorganic substances.

Fractionation analysis performed by Golterman method allows for distinguishing mineral fractions of phosphorus adsorbed on the sewage particle surface. These fractions are considered as the most mobile (Table 2), bioavailable (Ca-EDTA and Na-EDTA fractions). This method involves also the extraction of phosphorus bound in strong combinations with aluminosilicates (NaOH fraction) and phosphorus identified primarily with organic forms (Table 2), or phosphorus fractions found in soluble combinations with organic matter (H_2SO_4 fraction) and those contained in the organic matter (NaOH fraction). The fractions of phosphorus bound in combinations with aluminosilicates and identified with organic fractions are defined by Golterman as fractions difficult to access (H_2SO_4 and NaOH fractions).

Fractionation analysis showed that in an annual cycle the shares of phosphorus fractions in the examined specimens vary considerably. It is due, among others, to the constantly changing composition of raw waste flowing into the reactor and to the sludge temperature fluctuations in an annual cycle resulting from varying air temperature in the temperate climate. The samples of the analysis results are presented in Figure 1.

The percentage shares of different phosphorus fractions in the activated sludge demonstrate high variability of values, however, in the case of their classification into one of the two groups on the basis of bioavailability (form: mobile and difficult to access), one can notice a pattern occurring in the annual cycle. Figure 2 shows the percentage shares of phosphorus mobile fractions (the sum of the Ca-EDTA and Na-EDTA fractions shares) and the temperature of the activated sludge in particular months. The increase or decrease in the sludge temperature is noticeably accompanied by a rise or fall in the percentage share of phosphorus mobile fractions.

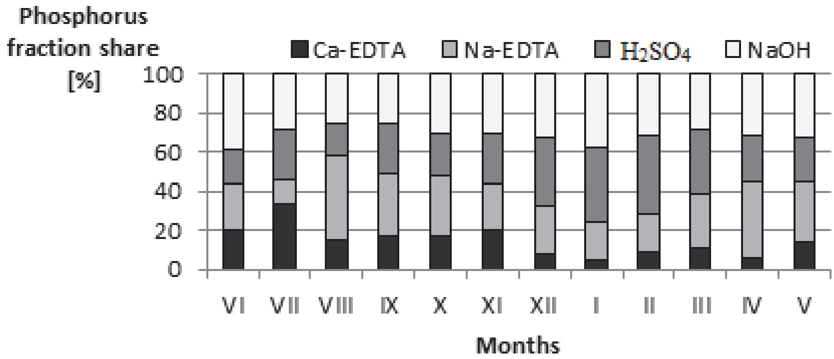

Fig. 1. Shares of different phosphorus fractions in the activated sludge collected at the *F* wastewater treatment plant
Rys. 1. Udział poszczególnych frakcji fosforu w osadach czynnych pochodzących z oczyszczalni F

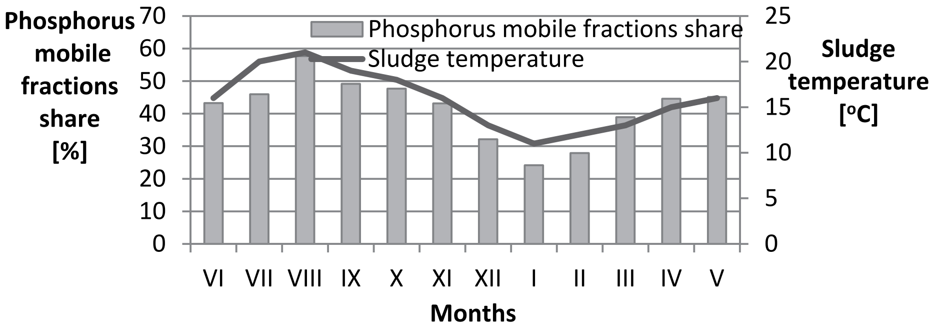

Fig. 2. Change dynamics in percentage shares of phosphorus mobile form and temperature in activated sludge collected at the *F* wastewater treatment plant
Rys. 2. Dynamika zmian udziałów procentowych frakcji fosforu oraz temperatury w osadzie czynnych pochodzącym z oczyszczalni *F*

In August, when the temperature of the sludge was 21°C, the share of phosphorus mobile fractions was the highest and amounted to 58%. In winter there occurs a decrease in the shares of mobile fractions and an increase in the shares of hard-accessible ones. In January, when the temperature of the activated sludge was the lowest at 11°C, the share of mobile phosphorus fractions was only 24%. Similar results were also obtained in the remaining treatment plants. Figure 3 presents the box graphs of the mobile phosphorus fractions shares in the activated sludge collected at the six sewage treatment plants discussed in this paper. The ends of the box charts' whiskers represent the minimum and maximum values of the mobile fractions shares. In all the analyzed facilities the extreme values of the mobile fractions shares correspond to the extreme values of the activated sludge temperatures. Analyzing the six treatment plants it can be said that the mobile fractions shares varied within the range of 19-64%.

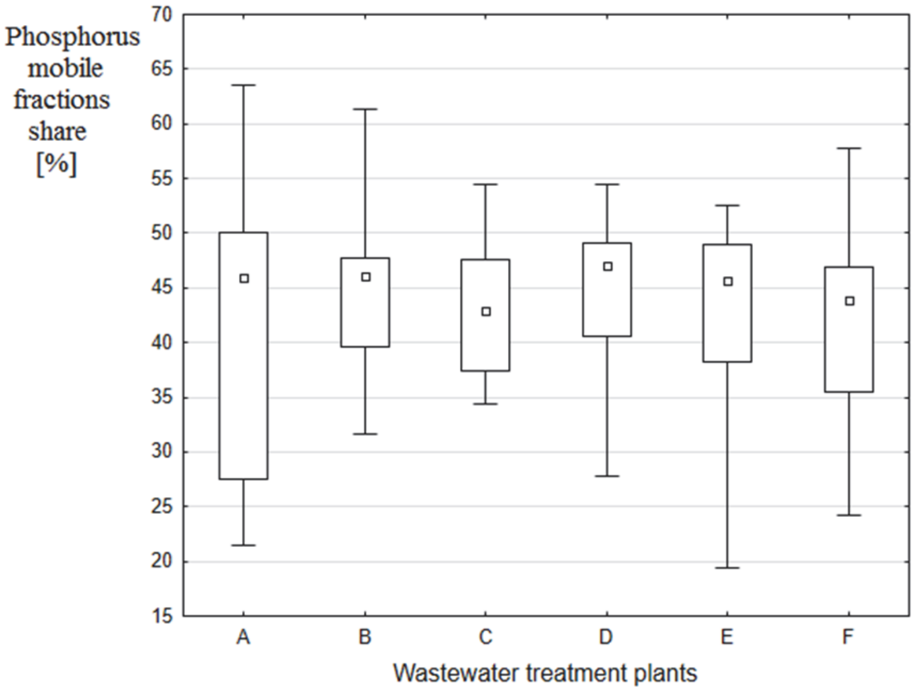

Fig. 3. Shares of phosphorus mobile fractions in activated sludge collected at *A, B, C, D, E* and *F* treatment plants
Rys. 3. Wartości udziałów form mobilnych fosforu w osadach czynnych pochodzących z oczyszczalni *A*, *B*, *C*, *D*, *E* i *F*

The values of the phosphorus mobile fractions shares (p.m.f.s) changing together with the temperature in an annual cycle (Figure 2 and 3) indicate the effect of temperature on phosphorus removal from wastewater. Therefore, in order to approximate the experimental relations with empirical formulas, a separate regression model was developed for the analyzed parameters of the activated sludge collected at each treatment plant.

Prior to statistical analyses the Shapiro-Wilk test was applied, primarily to confirm the compliance of the statistical distributions of the analyzed sludge temperature sequences and the mobile form shares with the normal distribution. The probability value for all the analyzed parameters was higher than $\alpha=0.05$, which means that the investigated variables have a normal distribution and it is possible to perform further statistical analyses.

The next analysis was aimed at establishing similarities and differences between the treatment plants under consideration. The cluster analysis was applied for this purpose. This method involves defining groups (clusters) of objects similar to each other on the basis of variables depicting the analyzed objects and showing how much one cluster differs from another. This methodology is discussed in detail in scientific literature (Licznar and Szeląg 2014). To determine the Euclidean distance (bond distance) between the emerging clusters, a popular single-bond method was applied. The results of the conducted calculations are presented in Figure 4.

On the basis of the dendrogram shown in Fig. 4 it can be concluded that the *F* and *E* treatment plants are most similar to each in terms of the analyzed variables, which may be associated with chemical dephosphatation used in these facilities. *C, B, D, F* and *E* treatment plants are similar with regard to the analyzed parameters. The bond distance for these facilities does not exceed 0.16, therefore they form a large cluster. Forming a cluster comprising five out of six investigated facilities may indicate that the joint analysis of parameters such as mobile forms share and activated sludge temperature is justified owing to the similarity of results obtained in the facilities. The sewage treatment plant *A* deviates most from the other plants presented in the dendrogram, which may be associated with the quality of the activated sludge in this facility. In the spring and summer filamentous microorganisms from the *Actinomycetes* group multiply very intensely in the bioreactors of this sewage treatment plant. These organisms have hydrophobic cell walls owing to which they stick to air bubbles and are carried up together with the sludge flocs to the surface of the reactor. They disrupt the homeostasis of the environment and thus the biochemical processes occurring in the activated sludge chamber.

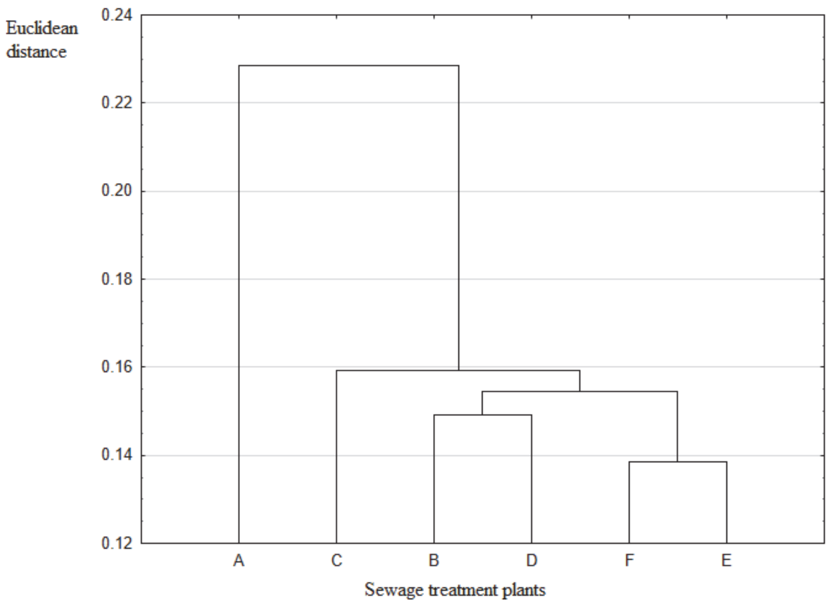

Fig. 4. Dendrogram determined for the investigated treatment plants based on the variable shares of the phosphorus mobile forms and the activated sludge temperature
Rys. 4. Dendrogram wyznaczony dla analizowanych oczyszczalni oparte na zmiennych udziałach form mobilnych i temperatury osadu czynnego

In the next stage an attempt was made to develop a mathematical model describing the dependence of the phosphorus mobile fractions share (p.m.f.s.) on temperature. Considering the observations made during the investigation, this relation was expressed as follows by the segmented regression (Seber et al. 2003):

$$p.m.f.s. = \begin{cases} \alpha_0 + \alpha_1 \cdot T \quad dla \quad T < T_{gr} & (1) \\ \alpha_0 - \alpha_2 \cdot T_{gr} + (\alpha_1 + \alpha_2) \cdot T \quad dla \quad T > T_{gr} & (2) \end{cases}$$

where α_0, α_1, α_2 – empirical model parameters estimated by Levenberg-Marquardt method, T_{gr} – limit temperature corresponding to the change of the dependence p.m.f.s. = f(T). It can be concluded on the basis of the performed calculations that, in the case of the *A, B, C, D* and *E* treatment plants, the p.m.f.s. = f (T) relation had a linear character and was described by equation (1), whereas for the *F* plant the dependence p.m.f.s. = f (T) was expressed by equation (2). The values of the determined parameters α_i and determination coefficients are given in Table 3.

Table 3. Parameter values of the m.f.s. = f(T) equation for the analyzed facilities
Tabela 3. Zestawienie wartości parametrów równania u.f.m. = f(T) dla analizowanych obiektów

Facility	Parameters				
	α_0	α_1	α_2	T_{gr}	R^2
A	0.029±(0.002)	0.0299±(0.003)			0.903
B	0.237±(0.039)	0.0166±(0.003)			0.771
C	0.279±(0.027)	0.0122±(0.002)			0.808
D	0.226±(0.036)	0.0169±(0.002)			0.778
E	0.013±(0.033)	0.0270±(0.004)			0.832
F	-0.206±(0.022)	0.080±(0.020)	0.072±(0.020)	7.77	0.906

In addition, Figure 5 shows a sample of a graphical representation of the p.m.f.s. = f (T) calculations for the *A* and *F* treatment plants. Based on the above, it can be stated that there is a statistically significant relationship between the activated sludge temperature and mobile fractions shares. In the case of the *A,B, C, D, E* treatment plants this relationship is linear. Based on the regression curves one can estimate how the mobile fractions share will change in the case of temperature alteration. The analysis of the parameters α_1 (Table 3) in equation (1) shows that in the case of the *A, B, C, D* and *E* treatment plants, the increase in the temperature of the activated sludge by 1°C will cause the rise in the share of mobile phosphorus fractions from 1.22 to 2.99%. It is a considerable change, because the annual amplitude of the activated sludge temperature is even 10°C. A piecewise regression curve was constructed for the sewage treatment plant *F* (Fig. 5).

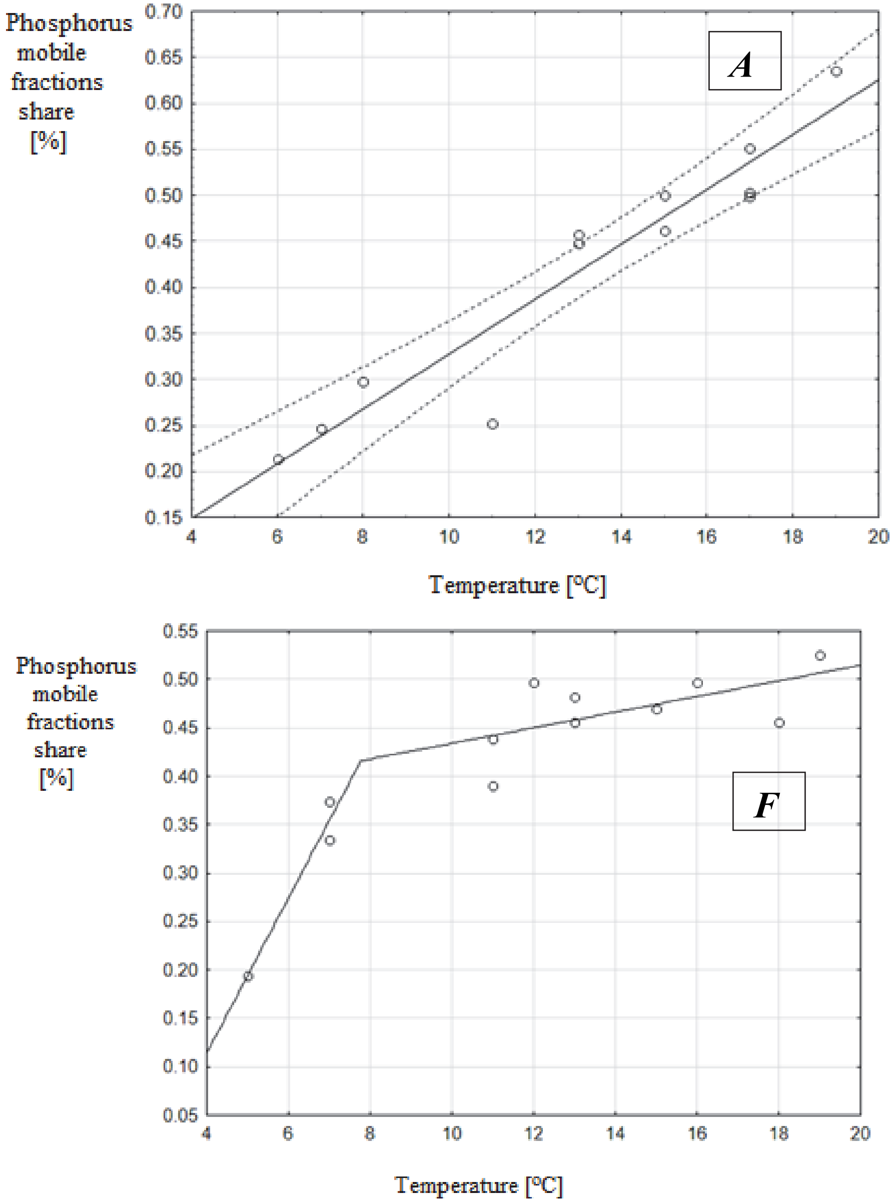

Fig. 5. Effect of activated sludge temperature on the share of mobile forms in wastewater treatment plants *A* and *F*
Rys. 5. Wpływ temperatury osadu czynnego na udział form mobilnych w oczyszczalniach *A* i *F*

The examination of the calculated parameters α_1 and α_2 in the regression model described by formula (2) leads to the conclusion that for the estimated limit temperature (T_{gr}) of 7.7 ^{o}C (Tab. 3) the increase in the temperature by 1°C causes the rise in the mobile fractions shares of phosphorus by 8%. However, above T_{gr} = 7.7°C the rise in temperature by 1°C leads to the increase in the mobile fractions shares of phosphorus merely by 7.2%.

4. Conclusions

The analysis of the above study provides ground for the conclusion that there is a statistically significant effect of temperature on the shares of phosphorus fractions in activated sludge. The increase in temperature is accompanied by the rise in the shares of phosphorus mobile fractions (Ca-EDTA and Na-EDTA) that can be identified with mineral fractions of this element adsorbed on the surface of sludge particles. The increase in the mineral fractions share under the influence of temperature may indicate the direct effect of the activated sludge temperature on the rate of enzymatic reactions and thus on the microbial mineralization of phosphorus contained in the organic matter reaching the bioreactor. This may mean that the rate of phosphorous bacteria metabolism increases together with the temperature in the analyzed range. Based on the estimated regression models obtained in this study it was found that the increase in the activated sludge temperature by 10°C can cause an increase in the mobile (mineral) phosphorus fractions share from 12% to 30%.

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Analiza wpływu cyklicznych zmian temperatury na specjacje fosforu w osadach czynnych

Abstract

Temperature is a significant parameter affecting wastewater treatment , because its increase accelerates decomposition of organic compounds. The optimum temperature of this process falls within the range of 18-22°C (Bugajski et al., 2012). As far as the biological phosphorus removal process is concerned, there are conflicting reports in reference sources with regard to the temperature optimum. Due to the existing discrepancies in the relevant literature on the effect of the sewage sludge temperature on the phosphorus removal processes, this study attempts to provide a speciation analysis of phosphorus in activated sludge in an annual cycle (in temperate climate), in which the sewage sludge temperature may range from 5°C to 25°C. The studies conducted to evaluate the effect of temperature cycling on the phosphorus speciation in activated sludge involved performing monthly speciation analyses of phosphorus in activated sludge collected at six sewage treatment plants. The content of different phosphorus fractions was determined in the sewage specimens using the fractionation scheme proposed by Golterman. The speciation analysis performed by this method allows to distinguish mineral phosphorus forms adsorbed on the surface of the sludge particles. These forms are considered as the most mobile, bioavailable (fractions of Ca-EDTA and Na-EDTA). This method is also used to extract phosphorus bound in strong combinations with aluminosilicates (NaOH

fraction) and phosphorus identified primarily with organic forms, i.e. forms of phosphorus found in soluble combinations with organic matter (H_2SO_4fraction) as well as the forms of phosphorus contained in organic matter (NaOH). Forms of phosphorous bound in combinations with aluminosilicates and identified with organic forms are distinguished by Golterman as forms difficult to access (H_2SO_4 and NaOH fractions). The results presented in the study show that the shares of different phosphorus fractions in activated sludge demonstrate high variability in value, however, in the case of their classification into one of the two groups on the basis of bioavailability (fractions: mobile and difficult to access), one can notice a pattern occurring in the annual cycle. There is a noticeable increase in the share of mobile (mineral) phosphorus forms which accompanies a rise in the sludge temperature (with the temperature increase by 1°C the mobile forms shares grow from 1.2% to 3%). The increase in the mineral forms share under the influence of temperature may indicate the direct effect of the activated sludge temperature on the rate of enzymatic reactions. The fact that the share of phosphorus forms difficult to access (H_2SO_4 and NaOH fractions), identified primarily with organic forms, is dominant in winter while the share of mobile forms is minor (24%), confirms the reduction of phosphorus removal at a low sludge temperature.

Streszczenie

Istotnym parametrem wpływającym na proces oczyszczania ścieków jest temperatura, ponieważ jej wzrost przyspiesza procesy rozkładu związków organicznych. Optymalna temperatura tych procesów mieści się w granicy 18-22°C (Bugajski i in. 2012). Z kolei w przypadku procesu defosfatacji biologicznej istnieją sprzeczne doniesienia literaturowe dotyczące optimum temperaturowego. Ze względu na występujące rozbieżności w doniesieniach literaturowych dotyczących wpływu temperatury osadu ściekowego na procesy defosfatacji, w niniejszej pracy podjęto się wykonania analizy specjacyjnej fosforu w osadach czynnych w cyklu rocznym (w klimacie umiarkowanym), w którym temperatura osadów ściekowych może wahać się od 5°C do 25°C. W celu oceny wpływu cyklicznych zmian temperatury na specjacje fosforu w osadach czynnych zostały wykonane badania, które polegały na wykonaniu comiesięcznych analiz specjacyjnych fosforu w osadach czynnych pochodzących z sześciu oczyszczalni ścieków. W pobranych próbach osadu oznaczano zawartość poszczególnych frakcji fosforu wykorzystując schemat frakcjonowania zaproponowany przez Golterman'a. Analiza specjacyjna wykonana tą metodą pozwala wyróżnić formy mineralne fosforu, zaadsorbowane na powierzchni cząstek osadów. Formy te uważane są jako najbardziej mobilne, biodostępne (frakcje Ca-EDTA i Na-EDTA). W metodzie tej ekstrahuje się również fosfor związany

w silnych połączeniach z glinokrzemianami(frakcja NaOH) oraz fosfor utożsamiany przede wszystkim z formami organicznymi, czyli formami fosforu występującymi w rozpuszczalnych połączeniach z materią organiczną (frakcja H_2SO_4) oraz formami fosforu zawartymi w materii organicznej (NaOH). Formy fosforu związanego w połączeniach z glinokrzemianami oraz utożsamiany z formami organicznymi Golterman wyróżnia jako formy trudnodostępne (frakcje H_2SO_4 i NaOH). Przedstawione w pracy wyniki świadczą iż wartości udziałów poszczególnych frakcji fosforu w osadach czynnych wykazują dużą zmienność, jednak w przypadku ich przyporządkowania do dwóch grup pod względem biodostępności (frakcje mobilne i trudnodostępne) można dostrzec występowanie tendencji w cyklu rocznym. Zauważalny jest wzrost udziału form mobilnych(mineralnych) fosforu wraz ze wzrostem temperatury osadu (przy wzroście temperatury o 1°C udziały form mobilnych wzrastają od 1,2% do 3%). Zwiększenie udziałów form mineralnych pod wpływem temperatury może świadczyć o bezpośrednim wpływie temperatury osadów czynnych na szybkość reakcji enzymatycznych. Dominujący w okresie zimowym udział form trudnodostępnych fosforu (frakcji H_2SO_4 i NaOH), utożsamianych przede wszystkim z formami organicznymi oraz niewielki udział form mobilnych (24%) potwierdza obniżenie procesów defosfatacji w przypadku niskiej temperatury osadu.

Słowa kluczowe:
formy specjacyjne fosforu, osad czynny, temperatura

Key words:
speciation forms of phosphorus, active sludge, temperature