Impact of temperature on biological phosphorus removal from wastewater in Lithuania

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Enhanced biological phosphorus removal (EBPR) is frequently efficient, but the process can be unreliable because it is influenced by many factors. Temperature is an important factor determining the outcome of competition between phosphate-accumulating organisms (PAOs) and an glycogen-accumulating organisms (GAOs) and thus the resultant stability of EBPR. At wastewater temperature of 20 °C and sufficient sludge age, GAOs start to grow and dominate in EBPR. These GAOs take up volatile fatty acids (VFAs) in competition with PAOs. However, GAOs do not accumulate polyphosphate and hence do not contribute to the excess phosphorus removal. The objective of this study was to evaluate the impact of wastewater temperature on EBPR in wastewater treatment plants (WWTPs) of Lithuania under different technological schemes of biological nitrogen and phosphorus removal.

Key words: enhanced biological phosphorus removal (EBPR), polyphosphate–accumulating organisms (PAOs), glycogen-accumulating organisms (GAOs), volatile fatty acids (VFAs)

INTRODUCTION

The effluents generated by industrial and civil activities need the pre-treatment before their discharge into rivers, lakes and seas in order to reduce contaminants to environmentally safe levels. Special attention is required to inorganic substances such as ammonium, nitrate and phosphate, which contribute to the eutrophication of water bodies (Lodi et al., 2003). Biological nitrogen and phosphorus removal processes have been developed and are being used as an economical and effective method of treating municipal wastewater. But this process is influenced by a lot of factors that should be evaluated in order to ensure an efficient process of biological phosphorus removal. The temperature of wastewater is one of these factors and has an indirect impact on the EBPR process.

In the last decade, scientists of the world have intensively investigated the impact of the temperature on the EBPR process. The results of investigations were quite contrary. Part of scientists stated that biological phosphorus removal was more efficient at higher temperatures (above 20 °C) (Jones et al., 1996, Brdjanovic et al., 1997, Choi et al., 1998). The other part of scientists have stated that the EBPR process is more efficient when the temperature of mixed liquor varied between 5 °C and 15 C (Brdjanovic et al., 1998, Garcia-Usach et al., 2005, Barnard et al., 1985, Erdal et al., 2003). The opposite results encouraged researchers to carry out a number of investigations in order to elucidate the impacts of temperature on micro-organisms that participate in the EBPR process. Investigating microorganisms in the system of activated sludge, firstly, there were identified and microscopically fixed special micro-organisms that in aerobic conditions can accumulate more polyphosphate than required for metabolism and cell synthesis (Fuhs et al., 1975). These micro-organisms were called phosphate accumulating organisms (PAOs). Later, about 1997, another group of bacteria that use a different source of energy was identified and called glycogen accumulating organisms (GAOs) (Liu et al., 1996, 1997, Pereira et al., 1996). Both species compete for the VFAs (e. g., acetate). However, consuming the VFAs, PAOs accumulate polyphosphate in their cells, whereas GAOs do not. GAOs use glycogen as a source of energy. Thus, when GAOs dominate in the system, phosphate is not being accumulated and the efficiency of phosphorus removal decreases significantly. The growth of GAOs in the EBPR process is affected by the following factors: (1) GAOs are mezophylic micro-organisms. So, their optimal growth temperature is 20–38 °C. However, PAOs are psychrophylic microorganisms (Helmer et al., 1997). So, when the temperature of wastewater decreases seasonally, the amount of GAOs decreases and the efficiency of phosphorus removal increases; (2) long age of sludge in the warm season. It was determined that at the wastewater temperature value of 20 °C phosphorus removal is efficient when sludge age is 10 days (Erdal et al., 2003, Fukase et al., 1984). GAOs grow rapidly and the phosphorus removal decreases significantly if the sludge age is more than 10 days (Whang et al., 2006); (3) the quantity of glucose in the influent stimulates the growth of GAO population because glucose is the main source of energy for their growth (Erdal et al., 2003). (4) a low P/C ratio in the influent also has an impact on the growth of GAOs (Liu et al., 1997); (5) low pH value. It has been stated that

the medium is favourable for the growth of PAOs at the pH equal to or less than 7.25 (Cokgor et al., 2004, Filipe et al., 2001).

Time is required in order to adapt the population of GAOs to environmental conditions such as pH, temperature, wastewater composition (Brock, 1987). This period is called the acclimatization period. In practice, the acclimatization period is equal to 3 sludge ages determined in laboratory models and full-scale WWTP (McClintock et al., 1991). Thus, when a season changes and the temperature of wastewater rises, the growth of GAOs intensifies, and during the acclimatization period of about three sludge ages they start to dominate in the system and disturb the process of phosphorus removal.

The aim of this work was to investigate the EBPR in Lithuania and to evaluate the impact of temperature on biological phosphorus removal.

Object and methods

To determine the influence of temperature on EBPR, data on the work of wastewater treatment plants (WWTPs) of the largest cities of Lithuania were collected and analysed. The wastewaters of Vilnius, Klaipėda, Alytus, Šiauliai and Utena were selected for the investigation. The Johannesburg (JHB) process, including an anaerobic selector, is applied at Vilnius and Alytus WWTPs for the biological removal of phosphorus and nitrogen from wastewater. The technological line contains a denitrification zone for return activated sludge, selector, anaerobic, denitrification and nitrification zones. The return sludge and 20% of primary treated wastewater are supplied to the denitrification zone for return activated sludge. The mixed liquor, together with the remaining amount of wastewater, flows to the selector and later to the anaerobic zone. Here, anaerobic conditions favourable for PAOs are maintained. Wastewater is supplied from the anaerobic zone to the denitrification (anoxic conditions) and nitrification (aerobic conditions) zones consecutively.

At Klaipėda and Šiauliai WWTPs, for the biological removal of phosphorus and nitrogen from wastewater the UCT process is applied. The process allows to reduce the concentration of nitrate in the anaerobic zone considerably. Applying the UCT process, the return activated sludge is supplied to the anaerobic zone through the anoxic zone. This allows to decrease the negative impact of nitrates on biological phosphorus removal. The amount of the return sludge is equal to 100% of influent.

The biological phosphorus removal and the intermittent denitrification process are applied at the Utena WWTP. The process is controlled using the Biobalance Symbio technology. The denitrification of return activated sludge is not carried out here.

The data of at least one-year period were collected at each WWTPs in order to get the view of all seasons. The data of 2003–2005 were analysed for the Vilnius WWTP and the data of 2006 for Klaipėda, Alytus, Šiauliai and Utena WWTPs. First, information on changes of wastewater temperature was collected. Later, the concentration of total phosphorus (P-tot) in the effluent and the BOD_7 and P-tot (BOD_7 / P-tot) ratio were analysed and sludge age was calculated. All analysis was carried out using standard methods (Lithuanian…, 1994).

A statistical processing of the obtained results was performed. Following the statistical equations, the values that do not fit into the interval of reliability of 95% were eliminated (Martinenas, 2004).

RESULTS AND DISCUSSION

First, data on wastewater temperature for a period of one year were analysed. The statistical processing of data is presented in Table 1. The statistically reliable data show that the mean temperature of wastewater was the highest in Utena and Klaipėda and the lowest in Šiauliai. The difference between the highest and lowest temperatures of wastewater was 28%. The maximum wastewater temperature was lower than 20 °C only in Šiauliai.

The temperature of the influent depends on the season and varies from 6 °C to 24 °C (Fig. 1). At the Vilnius WWTP, the temperature of wastewater varied from 7 °C to 22 °C in 2003–2005. The temperature of wastewater became higher than 20˚ C in June or July; it did not decrease below 20 °C 3–4 months and depended on the air temperature. In this period, the decrease of wastewater temperature could occur during rainfalls.

At the Klaipėda WWTP, the temperature of wastewater varied from 10 °C to 23 °C in 2006. In July, the temperature reached 20 °C and it remained above 20 °C for 4 months until November. The temperature of wastewater was above 20 °C until November 2006 at the Alytus WWTP as well, whereas at the Šiauliai WWTP it did not reach 20 °C and varied between 6 °C and 17.5 °C in 2006. At the Utena WWTP, the temperature of wastewater reached 20 °C in June and remained above 20 °C until October.

In the warm season, favourable conditions for the growth of the GAO population were formed at the Vilnius, Klaipėda, Alytus and Utena WWTPs because wastewater temperature was higher than 20 °C during a period of 3 to 5 months (June, July, August,

Table 1. **Results of statistical processing of data on wastewater temperature in influent**

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Vilnius	Vilnius	Vilnius	Klaipėda	Alytus	Šiauliai	Utena
2003	2004	2005	2006	2006	2006	2006
243	259	257	365	47	25	140
Temperature of wastewater in influent, °C						
21.0	22.0	21.0	23.2	24.0	17.5	23.5
10.0	10.0	7.0	10.6	10.0	6.0	11.6
16.0	15.5	15.1	17.1	16.6	12.8	17.7
15.0	16.0	15.0	16.5	16.0	13.0	17.6
11.0	12.0	14.0	12.6	14.0	11.5	11.9
9.2	8.9	16.7	13.8	13.9	12.8	12.8
3.0	3.0	4.1	3.7	3.7	3.6	3.6

Fig. 2. Change of wastewater temperature and P-tot in effluent at Vilnius WWTP, 2003–2005

September and October). In this period, the mean sludge age ranged from 16 to 30 days and was suitable for the growth of GAOs. Thus, when a season changes and the temperature of mixed liquor rises, GAOs may start to dominate in the system and disturb the process of biological phosphorus removal after an acclimatization period of about three sludge ages. To verify this assumption, analysis of phosphorus removal was carried out at the WWTPs.

The P-tot in the effluent was also analysed. Results of the statistical processing of data are presented in Table 2.

Analysing the technological parameters of the Vilnius WWTP in of 2003–2005, it was noted that P-tot in the effluent increased in the warm season. Figure 2 shows that in July and August of 2003 and during part of September, the P-tot in the effluent was higher in comparison to other months. At that time the temperature of the influent varied above 20 °C. The same tendency could be seen in June, July, August and September of 2004 and in July, August and September of 2005.

Analysing the technological parameter of 2006 at the Klaipėda WWTP, we found that the P-tot in the effluent increased within the warm season as well (Fig. 3). The mean monthly concentrations of total phosphorus in July, August, September and October differed much in comparison with other months.

At the Alytus WWTP, the increase of the P-tot was also determined within the warm season – in July, August and October, while in September there was no such tendency (Fig. 4). At the Utena WWTP, a significant increase of the P-tot in the

City	Vilnius	Vilnius	Vilnius	Klaipėda	Alytus	Šiauliai	Utena
Year	2003	2004	2005	2006	2006	2006	2006
Number of records, n	243	246	170	324	46	24	49
		P-tot in effluent, mg/l					
Maximum value, x_{max}	3.8	6.8	5.3	2.8	2.3	1.5	1.6
Minimum value, x_{min}	0.10	0.28	0.26	0.07	0.36	0.10	0.25
Arithmetical mean, x	0.93	1.97	1.59	0.69	0.79	0.56	0.83
Median	0.45	1.40	1.40	0.37	0.68	0.27	0.79
Amplitude	3.70	6.52	5.04	2.73	1.97	1.37	1.35
Dispersion, s^2	0.96	2.27	1.37	0.50	0.18	0.23	0.08
Standard deviation, s	0.98	1.51	1.17	0.70	0.42	0.48	0.28

Table 2. **Results of statistical processing of data of P-tot in effluent**

Fig. 3. Change of wastewater temperature and P-tot in effluent at Klaipėda WWTP, 2006

Fig. 4. Change of wastewater temperature and P-tot in effluent at Alytus WWTP, 2006

Fig. 5. Change of wastewater temperature and phosphate concentration in activated sludge tank at Utena WWTP, 2006

Fig. 6. Change of wastewater temperature and P-tot in effluent at Šiauliai WWTP, 2006

effluent was noted in August and September of 2006 (Fig. 5). At the Šiauliai WWTP, no increase of the P-tot in the effluent was observed in the warm season, and the highest temperature of wastewater reached 17.5 °C only (Fig. 6).

To determine the relationship between EBPR and the temperature of influent, a regression analysis of statistically reliable data was carried out.

At the Vilnius WWTP, statistically reliable data on the temperature and P-tot in the effluent were analysed separately for 2003, 2004 and 2005, as the temperature of wastewater is not a direct factor that can have an impact on the biological phosphorus removal. The temperature of wastewater influences the growth of GAOs. In case of the same temperature of wastewater, the number of GAOs can differ significantly depending on the age of sludge. For this reason, data of each year should be analysed separately. The correlation and regression analysis showed a moderately strong relationship between the temperature of wastewater and the P-tot in the effluent. The R-squared value varied around 0.7 (Table 3). The results of 2003 showed that the relationship between the temperature of wastewater and the P-tot in the effluent can be expressed applying an exponential model, and it could be expressed applying a linear model for the data of 2004 and 2005.

Table 3. **Results of correlation and regression analysis of data: wastewater temperature and P-tot in effluent at different WWTP in Lithuania**

WWTP at	Year	Model	R-squared
Vilnius	2003	P-tot = $0.03e^{0.2t}$	0.68
Vilnius	2004	$P-tot = 0.39t - 0.83$	0.69
Vilnius	2005	$P-tot = 0.15t-0.75$	0.65
Klaipėda	2006	$P-tot = 0.12t-1.27$	0.83
Alytus	2006	$P-tot = 0.1t-0.2$	0.5
Utena	2006	P-tot = $0.003e^{0.24t}$	0.51
Šiauliai	2006	$P-tot = 0.04t + 0.03$	0.14

P-tot – concentration of total phosphorus in effluent, t – wastewater temperature.

Regression analysis of data on the Klaipėda WWTP showed a strong relationship between wastewater temperature and P-tot in the effluent. The R-squared value was equal to 0.83, and a linear model was used to characterise the relationship between the abovementioned parameters (Table 3). Analysis of data on the Alytus and Utena WWTPs showed a weaker relationship between wastewater temperature and P-tot in the effluent. In this case, the R-squared value was equal to about 0.5, and the linear and exponential models were used to characterise the relationship between the above-mentioned parameters (Table 3). At the Šiauliai WWTP, results of regression analysis showed that the relationship between wastewater temperature and P-tot in the effluent was very weak. The R-squared value was equal to 0.14, possibly because the highest temperature in the influent was relatively low – less than 20 °C (17.5 °C), (Table 3).

However, there are factors that have a negative impact on the process of biological phosphorus removal. These factors are the composition of municipal wastewater, the supply of oxygen, the sludge load / sludge age and the concentration of nitrate. The composition of wastewater plays an important role in the process of biological phosphorus removal, especially products of organic fermentation that are a carbon source for PAOs. The bigger amount of VFAs in the anaerobic zone the higher release of phosphates is

Fig. 7. Relationship between BOD₇ / P-tot in influent and P-tot in effluent at Vilnius WWTP, 2003: a) in the warm season, b) in the cold season

obtained. VFAs could be produced from a readily biodegradable substrate by fermentation bacteria. So, the BOD_{7}/P -tot ratio is very important for biological phosphorus removal, especially in the warm season when there are suitable conditions for the growth of GAOs. In the warm season, GAOs propagate rapidly and consume VFAs competing with PAOs. So, we may assume that biological phosphorus removal is not disturbed, even if GAOs prevail in the system, if there is enough VFAs. Therefore, we hypothesised that within the warm period when GAOs prevail, the P-tot in the effluent could be predicted according to the $\text{BOD}_7/\text{P-tot}$ ratio in the influent. To prove this assumption, a correlation and regression analysis of statistically reliable data on the BOD_{7} / P-tot ratio in the influent and P-tot in the effluent was carried out. Data on the Vilnius, Klaipėda and Alytus WWTPs were used, and they were divided into two groups of the warm and the cold seasons. Analysis of data on the Šiauliai WWTP was inexpedient because the conditions did not favour the growth of GAOs because of the relatively low temperature of wastewater.

The results show that a relationship between the $BOD_{7}/$ P-tot in the influent and P-tot in the effluent is obtained only in the warm season at the Vilnius and Alytus WWTPs (Figs. 7a and 8a). The R-squared value varies around 0.7. In the cold season, the R-squared value of the above-mentioned relationship is 0.02 at the Vilnius WWTP (Fig. 7 b) and 0.39 at the Alytus WWTP (Fig. 8 b), showing a weak relationship between the parameters. To express the relationship between the BOD , \prime P-tot in the influent and P-tot in the effluent, a linear model is suitable. At the Klaipėda WWTP, no relationship between the study parameters was defined either in the warm or the cold season as the BOD_{7}/P -tot in the influent was rather steady. In the warm season, the BOD₇ / P-tot in the influent varied around 20 and versus about 24 in the cold season.

Fig. 8. Relationship between BOD₇ / P-tot in influent and P-tot in effluent at Alytus WWTP, 2006: a) in the warm season, b) in the cold season

This analysis allows a conclusion that in the warm season when GAOs prevail, the value of BOD_{7} / P-tot in the influent should be more than 30 to ensure the undisturbed biological phosphorus removal process, i. e. the amount of VFAs should be sufficient for both PAOs and GAOs. The results of regression analysis of the BOD_{7}/P -tot in the influent and P-tot in the effluent explain why the increase of P-tot in the effluent of the Alytus WWTP is observed in the warm season (July, August and October) but not in September. In September, the mean value of $BOD₇/P$ -tot in the influent was above 30, whereas in July, August and October it was equal to 24, 21 and 24, respectively. A bigger number of data is required for a more precise analysis as the $BOD₇/P$ -tot in the influent of the study WWTPs is determined once per week on the average. In some WWTPs, the above-mentioned relationships were not determined for the lack of data.

CONCLUSIONS

1. In Lithuania, in the warm season the temperature of wastewater reaches 20 °C in WWTPs. Such temperature remains for 3 to 4 months and the sludge age ranges within 16 to 30 days, thus favouring the growth of the GAO population.

2. In WWTPs of the largest cities of Lithuania, the concentrations of total phosphorus in the effluent increase above 20 °C in the warm season, except the Šiauliai WWTP where the highest temperature of wastewater does not reach 18 °C.

3. There is a moderately strong relationship between wastewater temperature and total phosphorus concentration in the effluent. The R-squared values vary between 0.5 and 0.8 in the analysed WWTPs where nitrogen and phosphorus are removed using different technologies.

4. In the warm season when GAOs prevail in the system, the $BOD₇$ / P-tot ratio in the influent is very important for the efficient removal of phosphorus. However, the concentration of total phosphorus can be predicted from the $BOD₇/P$ -tot ratio in the influent in the warm season only when the ratio increases above 30. A moderately strong relationship was found between the concentration of total phosphorus and the $BOD₇$ / P-tot ratio in the influent in the warm season and a weak relationship between the above parameters in the cold season.

5. To ensure an efficient biological phosphorus removal from wastewater in the warm season in Lithuania, it is necessary to investigate more thoroughly the impact of technological and maintenance parameters on biological phosphorus removal. Investigation of these parameters is the aim of further experiments.

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TEMPERATŪROS ĮTAKA BIOLOGINIAM FOSFORO ŠALINIMUI IŠ NUOTEKŲ LIETUVOJE

S a n t r a u k a

Siekiant išvengti eutrofikacijos vandens telkiniuose, būtina iš nuotekų šalinti azotą ir fosforą. Biologinis fosforo šalinimas (BPŠ) aktyviojo dumblo sistemoje yra ekonomiškas būdas šalinti fosforą iš nuotekų. Tačiau šiam procesui turi įtakos keletas veiksnių, kurie turi būti įvertinti, siekiant užtikrinti stabiliai efektyvų biologinio fosforo šalinimo procesą. Vienas veiksnių, netiesiogiai turintis įtakos BPŠ, yra valomų nuotekų temperatūra. Biologinį fosforą šalinina polifosfatus akumuliuojantys organizmai (PAO). Nagrinėjant mikroorganizmus fosforo

šalinimo veikliojo dumblo sistemoje, nustatyta mikroorganizmų rūšis – glikogeną kaupiantys organizmai (GAO), kurie konkuruoja dėl lakiųjų riebalų rūgščių (LRR), tačiau PAO naudodami LRR sukaupia polifosfatus savo ląstelėse, tuo tarpu GAO nekaupia polifosfatų, o kaip energijos šaltinį naudoja glikogeną. Taigi, kai sistemoje vyrauja GAO, fosfatai nekaupiami ir fosforo šalinimo efektyvumas smarkiai sumažėja. GAO augimui fosforo šalinimo veikliuoju dumblu sistemoje turi įtakos šie veiksniai: 1) GAO yra mezofiliniai mikroorganizmai, todėl optimali jų augimo temperatūra yra 20–38°C. Todėl sezoniškai krintant nuotekų temperatūrai, mažėja GAO ir padidėja fosforo šalinimo efektyvumas; 2) ilgas dumblo amžius šiltuoju metų laikotarpiu.

Šio darbo tikslas buvo išnagrinėti BFŠ iš nuotekų Lietuvoje, įvertinant temperatūros įtaką. Atlikus penkių nuotekų valyklų darbo analizę, nustatyta, kad Lietuvoje šiltuoju metų laikotarpiu valomų nuotekų temperatūra pasiekia 20°C ir išsilaiko 3–4 mėnesius, o palaikomas dumblo amžius kinta nuo 16 iki 30 parų, todėl susidaro palankios sąlygos GAO populiacijai augti. Lietuvos didžiųjų miestų nuotekų valyklose šiltuoju metų laikotarpiu padidėja BP koncentracija valytose nuotekose, išskyrus Šiaulių valyklą, kurioje aukščiausia nuotekų temperatūra nesiekia 18˚C. Nustatyta, kad egzistuoja vidutinis valomų nuotekų temperatūros ir BP koncentracijos valytose nuotekose koreliacijos ryšys, koreliacijos koeficiento kvadratas nagrinėjamose valyklose, kuriose azotas ir fosforas šalinami naudojant skirtingas technologijas, kinta nuo 0,5 iki 0,8. Nustatyta, kad šiltuoju metų laikotarpiu, kai sistemoje vyrauja GAO bakterijos, efektyviam BFŠ ypač svarbus BDS₇ / BP valomose nuotekose. Tačiau BP koncentraciją valytose nuotekose galima prognozuoti pagal BDS₇ / BP valomose nuotekose šiltuoju metų laikotarpiu tik tuo atveju, kai BDS₇ / BP pakyla >30. Nustatytas vidutinis BP koncentracijos valytose nuotekose ir BDS₇/BP valomose nuotekose koreliacijos ryšys šiltuoju metų laikotarpiu, tuo tarpu šaltuoju metu minėtas ryšys nenustatytas.

Raktažodžiai: biologinis fosforo šalinimas, polifosfatus akumuliuojantys organizmai, glikogeną akumuliuojantys organizmai, lakiosios riebalų rūgštys