EKOLOGIJA. 2009. Vol. 55. No. 3–4. P. 220–225
DOI: 10.2478/v10055-009-0029-1

Influence of substrate on biological removal of phosphorus

Regimantas Dauknys,

Giedrė Vabolienė,

Marina Valentukevičienė*,

Mindaugas Rimeika

Department of Water Management,
Vilnius Gediminas Technical University,
Saulėtekio al. 11, AIF,
LT-10223 Vilnius,
Lithuania

Nitrogen and phosphorus removal from wastewater is a way to avoid eutrophication of water bodies. The most often used method for phosphorus removal from wastewater is biological treatment. Under anaerobic conditions, phosphorus-accumulating organisms can accumulate more phosphate than is required for their metabolism processes and cell synthesis. The composition of wastewater may have an important influence on phosphorus biological removal from wastewater. In this study, the composition of wastewater in respect of the readily biodegradable chemical oxygen demand (rbCOD) was investigated. The rbCOD characterises the amount of volatile fatty acids (VFAs) and a readily biodegradable substrate in wastewater, which could be rapidly fermented into VFAs and other fermentation products.

Key words: wastewater treatment, readily biodegradable COD, activated sludge, biological phosphorus removal

INTRODUCTION

According to the EC Directive and national legislation, the removal of phosphorus from wastewater should be implemented (Council Directive, 1999). The most often used method for phosphorus removal from wastewater is biological treatment. During this process, wastewater is supplied to the anaerobic zone, and the treatment is continued in the aerobic zone. Finally, after the sludge settles in the sedimentation tank, part of it is returned to the anaerobic zone. During the above-mentioned process, a positive existence medium is created for special species of bacteria generally known as phosphorus-accumulating organisms (PAOs). Under aerobic conditions, PAOs can accumulate more phosphate than is required for their metabolism processes and cell synthesis.

Under anaerobic conditions, PAOs can assimilate a substrate from wastewater. The main substrate for PAOs is simple volatile fatty acids (VFAs), such as acetic acid (Beccari et al., 2002). By assimilating and accumulating organic matter for energy needs, under anaerobic conditions these organisms can successfully compete with other species of bacteria (Henze et al., 2001). Hence, the quantity of simple VFAs in the anaerobic zone is a decisive parameter for the biological removal of phosphorus (Hood, Randall, 2001; Janssen et al., 2002).

The quantity of VFAs comprises about 2–10% of the total chemical oxygen demand (COD) in primary settled wastewater (Henze et al., 2001). But municipal wastewater contains a readily biodegradable substrate which can be fermented into VFAs and other products. The quantity of the substrate usually varies between 10% and 20% of the total COD (Henze et al., 2001). The VFAs and the above-mentioned readily biodegradable substrate comprise the readily biodegradable organic matter (Mathieu, Etienne, 2000) characterized as soluble readily biodegradable COD (rbCOD). Thus, rbCOD in primary settled wastewater comprises 10–35% of the total COD (Henze et al., 2001; Orhon et al., 2002). However, some authors argue that rbCOD in settled wastewater can reach up to 56% of the total COD (Henze, 1992; Dauknys, Matuzevičius, 1999).

Slowly biodegradable organic matter is also present in wastewater. It comprises the remaining part of the carbon source. Slowly biodegradable COD comprises 30–60% of the total COD (Henze et al., 2001). PAOs cannot directly assimilate slowly biodegradable organic matter such as proteins, hydrocarbons and lipids (Tchobanoglous et al., 2003). It is degraded by enzymes into more simple compounds such as amino acid, glycerol and monosaccharide. The products of hydrolysis, as well as the readily biodegradable substrate, can be fermented into VFAs. However, the wastewater retention time in the anaerobic zone is too short to ensure the hydrolysis of slowly biodegradable COD (Henze et al., 2001; Jun, Shin, 1997). Therefore, the main source of substrate for PAOs is readily biodegradable organic matter. The slowly biodegradable organic matter is assimilated by other micro-organisms in the aerobic zone (Kong et al., 2005).

* Corresponding author. E-mail: marina.valentukeviciene@ap.vgtu.lt

The assimilation rate of readily biodegradable organic matter depends on the food and micro-organisms (F/M) ratio, where F/M is the ratio between the total COD and mixed liquor volatile suspended solids. Its value is important for the exact determination of the concentration of readily biodegradable organic matter. Preliminarily, the F/M ratio can be selected according to sludge age.

Besides, activated sludge needs to be adapted to the wastewater under analysis. If wastewater contains compounds that are not adapted by activated sludge, PAOs cannot assimilate the organic matter immediately, even if it is readily biodegradable (Ekama et al., 1986). In this case, the rbCOD can be determined incorrectly.

In order to ensure biological phosphorus removal together with the processes of nitrification and denitrification, the concentration of the rbCOD in the anaerobic zone should not be less than 25 mg/l (Mathieu, Etienne, 2000). Approximately 10 mg of rbCOD as acetic acid or similar simple organic compounds are consumed to remove 1 mg of soluble phosphorus (Henze et al., 2001; Mulkerrinsa et al., 2004). Nitrate in returned activated sludge decreases the concentration of readily biodegradable organic matter in the anaerobic zone. For this reason, the effectiveness of phosphorus removal also decreases. Hence, it should be taken into account that 8.6 mg of COD (Tchobanoglous et al., 2003) or 4–6 mg of rbCOD (Henze et al., 2001) is consumed for the denitrification of 1 mg of nitrate.

The aim of the study was to determine changes in the composition of wastewater in respect to rbCOD and to find a relationship between rbCOD and total COD in primary settled wastewater.

MATERIALS AND METHODS

An aerobic batch test was used to determine the concentrations of rbCOD in primary settled wastewater from two different wastewater treatment plants (WWTPs). For the investigation of rbCOD, wastewater samples were taken from Vilnius and Utena WWTPs after primary treatment, and samples of mixed liquid were taken from the aeration tank. Before each experiment, the following parameters were determined: total COD (LAND 83-2006), the concentration of mixed liquid suspended solids (MLSS) and the concentration of mixed liquid volatile suspended solids (MLVSS) (LAND 46-2002).

During the experiment, wastewater is mixed with activated sludge in the batch reactor, and this mixture is continuously aerated. Every 10 minutes, part of the mixture with the oxygen concentration of 5.5–8.0 mg O_2/l is poured into a 250 ml flask. This flask is closed with a rubber cork in such a manner that no air remains in the flask. The concentration of dissolved oxygen (DO) is measured with an oxygen meter (WTW OXI 330) and inserting an oxygen sensor into the flask. Data from the meter are fixed every minute. To ensure an accurate determination of DO concentrations, the mixture was mixed continuously in the flask with an electromagnetic stirrer. After 10 minutes of measuring, the new oxygen-enriched sample of the mixture is taken from the batch reactor, and the previous sample is poured back repeatedly into the batch reactor for saturation. The measuring lasts 2–3 hours.

According to the obtained data, the DO concentration curves are shown in Fig. 1. From these curves, the values of oxygen uptake rate (OUR) are calculated according to the following equation:

$$OUR = \frac{\mathrm{dC}}{\mathrm{dt}} \cdot 60, \text{ mg } O_2/\text{l/h}, \quad (1)$$

where dt is the time of measuring of oxygen decrease, min, and dC is the difference between the concentrations of dissolved oxygen during the time of measuring, mg O_2/l.

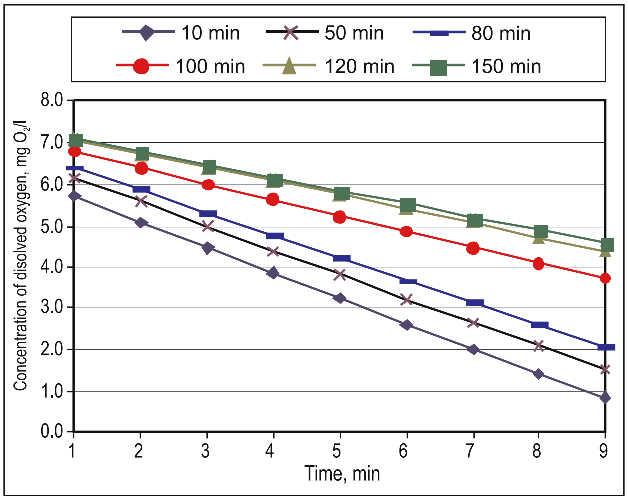

Fig. 1. Example of response of dissolved oxygen concentration

The calculated values of OUR are presented in the time diagram in Fig. 2.

In the first stage, the oxygen is utilized because of the consumption of the rbCOD. When the rbCOD is consumed, the curve of OUR drops and becomes constant in the second stage. At this stage, the oxygen is consumed by nitrification, degradation of slowly biodegradable organic matter and endogenous respiration (Ekama et al., 1986; Kappeler, Gujer, 1992; Tchobanoglous et al., 2003). Hence, the quantity of the consumed oxygen depends on the concentration of rbCOD, which is proportional to the area between the curve of the first stage of OUR and the horizontal line in the second stage of OUR area 1 in Fig. 2. The concentration of rbCOD (S_s) is calculated by applying the following equation:

$$S_s = \frac{\int r_{o_2,b} - \int r_{o_2,h-k}}{1 - Y_H} \frac{V_{as} + V_w}{V_w}, \text{ mg COD/l}, \quad (2)$$

where: $\int r_{o_2,b}$ – oxygen consumption for degradation of rbCOD, mg O_2/l; $\int r_{o_2,h-k}$ – oxygen consumption for nitrification, degradation of slowly biodegradable COD and endogenous respiration, mg O_2/l; Y_H – yield coefficient for heterotrophic bacteria, mg cell COD/mg COD used. This coefficient

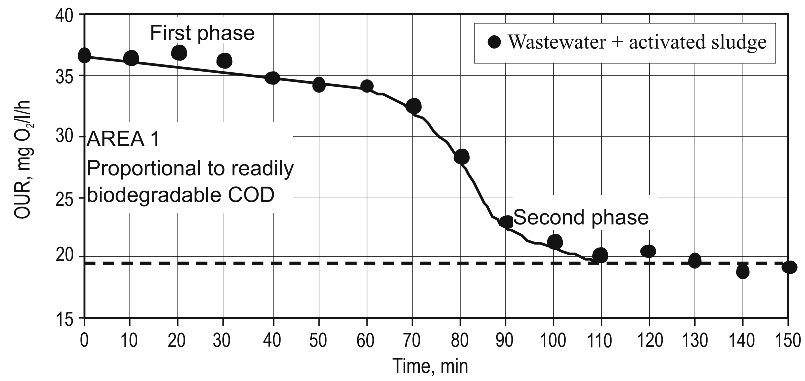

Fig. 2. Example of response of oxygen uptake rate

is kept equal to 0.67 (Ekama et al., 1986; Kappeler, Gujer, 1992; Tchobanoglous et al., 2003) and it does not depend on a temperature between 10 °C and 20 °C (Kappeler, Gujer, 1992); V_{as} – volume of the activated sludge used in the experiment, ml; V_w – volume of wastewater sample, ml.

Value of F/M was selected so that the OUR will remain high for 1–1.5 hours from the start of the experiment. Activated sludge that is used in the investigations was adapted to the wastewater to be investigated, and so an acclimatization period was not required. All experiments were performed under temperatures between 19–20 °C. The nitrification had no influence on measurements of the concentration of rbCOD, because the nitrification OUR remains constant for a longer period compared to the period that is required to consume all readily biodegradable organic matter.

The concentrations of biologically removed total phosphorus and of the total phosphorus that should be removed were calculated using equations (3) and (4), respectively:

$$P_{removed} = P_m - P_v + P_{vn}, \text{ mg/l}, \quad (3)$$

$$P_{rr} = P_m - (1.0 - C_v \cdot P_{in\,sludge}), \text{ mg/l}, \quad (4)$$

where $P_{removed}$ is the concentration of total phosphorus removed during biological treatment process, mg/l; P_m is the concentration of total phosphorus before biological treatment, mg/l; P_v is the concentration of total phosphorus in the effluent, mg/l; $P_{vn,}$ is the concentration of total phosphorus in suspended solids of the effluent, mg/l; P_{rr} is the concentration of total phosphorus that should be removed, mg/l; C_v is the concentration of suspended solids in the effluent, mg/l; $P_{in\,sludge}$ is the content of total phosphorus in MLSS, %.

The above-mentioned concentrations were applied to determine the amount of rbCOD required to remove 1 mg of total phosphorus.

RESULTS AND DISCUSSION

Thirteen experiments on the concentration of readily biodegradable organic matter were performed in July 2005 at the Vilnius WWTP and ten experiments in 2006–2007 at the Utena WWTP. According to statistical equations, selecting a reliability interval of 95%, the unreliable values of data were rejected. Results of a statistical estimation of the data are presented in Table 1.

Selected values of F/M varied between 366 and 762 mg COD/g MLVSS the (average value 541 mg COD/g MLVSS) at the Vilnius WWTP and between 444 and 945 mg COD/g MLVSS (the average value 605 mg COD/g MLVSS) at the Utena WWTP in separate experiments. At such a range of F/M values, the OUR curve drops to the second phase after 50–120 minutes from the start of the experiment. In an ideal case, the OUR curve should decrease after 1–2 hours from starting the experiment (Ekama et al., 1986). Hence, applying the above-mentioned range of F/M values, it is possible to estimate Area 1 exactly (Fig. 2). According to this area the concentrations of readily biodegradable organic matter were calculated. In Vilnius wastewater, the rbCOD ranged between 7.8–16.7% of total COD with an average value of 12.5% of the total COD, and in Utena wastewater it ranged between

Table 1. **Results of statistical processing of the obtained data**

	S_b mg/l	S_s mg/l	F / M mg COD/g MLVSS	S_s/S_b %
Vilnius WWTP				
Minimum value	373	58	366	7.8
Maximum value	822	89	762	16.7
Average	578	76	541	12.5
Median	550	77	529	12.9
Amplitude	449	32	396	8.8
Dispersion	17554	105	16132	7.0
Standard deviation	132	10	127	2.6
Utena WWTP				
Minimum value	391	64	444	15.4
Maximum value	710	131	945	19.4
Average	487	88	605	18.0
Median	467	83	578	18.4
Amplitude	319	67	501	4.0
Dispersion	7914	331	1622	1.6
Standard deviation	88.9	18.2	127.4	1.3

Note. S_b – total COD, S_s – readily biodegradable COD, F/M – ratio between food and micro-organisms.

15.4–19.4% of total COD with an average value of 18% of the total COD (Table 1). Thus, in Utena wastewater the rbCOD was 1.5 times higher than in Vilnius wastewater. The obtained results are in compliance with typical referred ranges of rbCOD in primary settled wastewater, which are equal to 10–35% of the total COD (Henze et al., 2001; Orhon et al., 2002).

To evaluate the relationship between the total COD and rbCOD values, a regressive data analysis was performed. The linear regression equations characterize the above-mentioned parameters of Vilnius and Utena wastewater respectively:

$$S_s = 40.1 + 0.0616 \cdot S_b, \qquad (5)$$

$$S_s = 0.194 \cdot S_b - 6.65, \qquad (6)$$

where S_s is readily biodegradable COD in primary settled wastewater, mg/l, and S_b is the total COD in primary settled wastewater, mg/l.

The diagram of the linear equation shows that the concentration of rbCOD increases with an increase of the total COD (Figs. 3 and 4). A reliable correlation between the rbCOD values and the total COD was determined: the coefficient of correlation was 0.87 for wastewater of Vilnius and 0.95 for wastewater of Utena. The prediction of the rbCOD values could be performed according to the selected regression equations (5) and (6). Hence, by measuring the total COD in primary settled wastewater it is possible to obtain a reliable information on the rbCOD in primary settled wastewater. This information can be useful for the assessment of phosphorus removal possibilities during the further improvement of WWTPs.

The concentrations of biologically removed total phosphorus, which were calculated according to equation (3), fluctuated from 4.7 mg/l to 14.5 mg/l (average 10.2 mg/l) at the Vilnius WWTP and from 4.1 mg/l to 13.1 mg/l (average 8.4 mg/l) at the Utena WWTP. Upon calculating the rbCOD values according to equation (5), the ratio between the rbCOD and the concentration of removed total phosphorus were determined. The investigation showed that 4.3–14.2 mg of rbCOD is consumed to remove 1 mg of total phosphorus (average 7.2 mg rbCOD/mg $TP_{removed}$) at the Vilnius WWTP and 5.1–20 mg of rbCOD (average 8.8 mg rbCOD/mg $TP_{removed}$) at the Utena WWTP (Table 2). A weak correlation between rbCOD and the concentrations of total phosphorus removed applying biological treatment was determined. The obtained average values of rbCOD consumption for total phosphorus removal (7.2 and 8.8 mg rbCOD/mg $TP_{removed}$) are lower than those

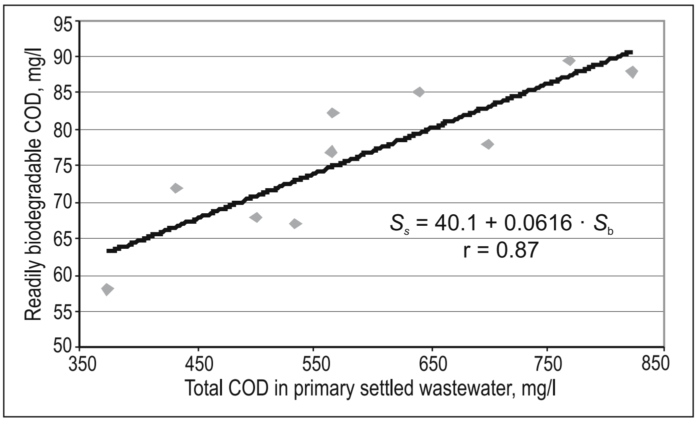

Fig. 3. Correlation between readily biodegradable COD and total COD in primary settled wastewater from Vilnius

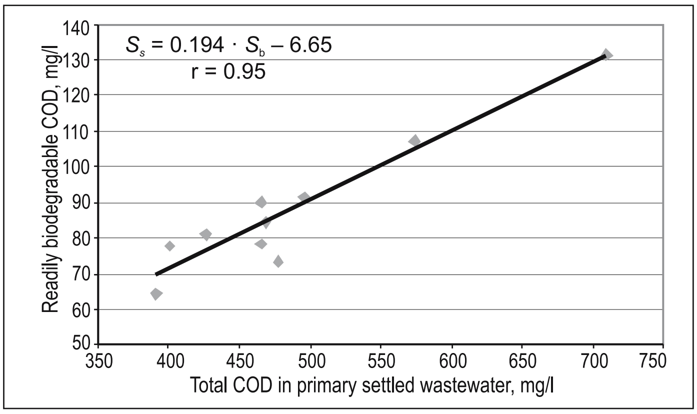

Fig. 4. Correlation between readily biodegradable COD and total COD in primary settled wastewater from Utena

Table 2. **Results of statistical processing of data, 2004–2006**

	S_b mg/l	S_s mg/l	P_m mg/l	P_v mg/l	$P_{removed}$ mg/l	$S_s / P_{removed}$ mg COD/mg $TP_{removed}$
Vilnius WWTP						
Minimum value	336	56	5.0	0.1	4.7	4.3
Maximum value	736	85	15.0	4.3	14.5	14.2
Average	505	71	11.1	1.2	10.2	7.2
Median	503	71	11.5	0.9	10.6	6.9
Amplitude	400	29	10.0	4.2	9.8	9.8
Dispersion	8107	44	4.1	0.9	4.4	2.9
Standard deviation	90	7	2.0	1.0	2.1	1.7
Utena WWTP						
Minimum value	274	47	5.2	0.2	4.1	5.1
Maximum value	540	98	14.0	1.3	13.1	20
Average	396	70	9.5	0.7	8.4	8.8
Median	394	70	9.7	0.7	5.6	8.5
Amplitude	266	51	8.8	1.1	9.0	15.2
Dispersion	2582	97	3.5	0.1	3.5	6.0
Standard deviation	51	10	1.8	0.3	1.9	2.5

Note. S_b – total COD, mg/l; S_s – readily biodegradable COD; P_m – concentration of total phosphorus after mechanical treatment; P_v – concentration of total phosphorus in effluent; $P_{removed}$ – concentration of total phosphorus removed applying biological treatment.

reported by other investigators (Henze et al., 2001) (approximately 10 mg of rbCOD/1 mg of soluble phosphorus). The main reason for the difference in the values could be the different parameters used: we applied the parameter of total phosphorus while other investigators used the parameter of soluble phosphorus.

CONCLUSIONS

1. The content of rbCOD differed in wastewater from two different sources. On the average, rbCOD was equal to 12.5% and 18.0% of total COD. In Utena wastewater, the rbCOD was 1.5 times higher than in Vilnius wastewater.

2. The difference may occur due to the influence of industrial wastewater. In Utena, the major part of wastewater (about 60%) is conveyed to the WWTP from industry (mainly food industry). It is presumable that wastewater from food industry contains bigger amounts of readily biodegradable organic matter than does municipal wastewater.

3. Each source of wastewater should be investigated individually to get reliable data on rbCOD concentrations.

4. On average, 7.2 mg of rbCOD at the Vilnius WWTP and 8.8 mg of rbCOD at the Utena WWTP was consumed to remove 1 mg of total phosphorus. Also, there was a weak correlation between rbCOD and the concentration of total phosphorus removed applying biological treatment.

Received 20 March 2009
Accepted 30 October 2009

References

1. Beccari M., Dionisi D., Giuliani A., Majone M., Ramodari R. 2002. Effect of different carbon sources on aerobic storage by activated sludge. *Water Science & Technology.* Vol. 45(6). P. 157–168.
2. Council Directive of 21 May 1991 Concerning Urban Waste Water Treatment (91 / 271 / EEC) (OJ L 135, 30.5.1991, p. 40). 1999. *Official Journal.* L 284 1 31.10.2003 Corrected by: C1 Corrigendum, OJ L 139, 2.6., (98 / 15 / EC), 34 p.
3. Dauknys R., Matuzevičius A. 1999. Experimental investigation of determining the concentration of readily biodegradable organic matter. *Aplinkos inžinerija.* T. VII. Nr. 3. P. 157–164.
4. Ekama G. A., Dold P. L., Marais G. V. R. 1986. Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Water Science & Technology.* Vol. 18. P. 91–114.
5. Henze M. P., Harremoes J. la C., Jansen E. Arvin. 2001. *Wastewater Treatment. Biological and Chemical Processes.* 3rd edn. Berlin: Springer Verlag. P. 436.
6. Henze M. 1992. Characterization of wastewater for modelling of activated sludge processes. *Water Science & Technology.* Vol. 25. No. 6. P. 1–16.
7. Hood C. R., Randall A. A. 2001. A biochemical hypothesis explaining the response of enhanced biological phosphorus removal biomass to organic substrates. *Water Research.* Vol. 35(11). P. 2758–2766.
8. Janssen P. M. J., Stowa K., Meinema H. F., van der Roes T. 2002. *Biological Phosphorus Removal Manual for Design and Operation.* IWA Publishing. P. 224.
9. Jun H., Shin H. 1997. Substrates transformation in a biological excess phosphorus removal system. *Water Research.* Vol. 31(4). P. 893–899.
10. Kappeler J., Gujer W. 1992. Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modeling. *Water Science & Technology.* Vol. 25(6). P. 125–139.
11. Kong Y., Nielsen J. L., Nielsen P. H. 2005. Identity and ecophysiology of uncultured actinobacterial polyphosphate-accumulating organisms in full-scale enhanced biological phosphorus removal plants. *Applied and Environmental Microbiology.* Vol. 71(7). P. 4076–4085.
12. LAND 46–2002. Vandens kokybė. Skendinčių medžiagų nustatymas. Košimo pro stiklo pluošto koštuvą metodas. LR aplinkos ministro 2002 08 08 įsakymas Nr. 406.
13. LAND 83–2006. Vandens kokybė. Cheminio deguonies suvartojimo nustatymas. LR aplinkos ministro 2006 12 08 įsakymas Nr. D1-579.
14. Mathieu S., Etienne P. 2000. Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various So / Xo ratios. *Water Research.* Vol. 34(9). P. 2627.
15. Mulkerrins D., Dobson A. D., Colleran E. 2004. Parameters affecting biological phosphate removal from wastewaters. *Environment International.* Vol. 30(2). P. 249–259.
16. Orhon D., Okutman D., Insel G. 2002. Characterisation and biodegradation of settleable organic matter for domestic wastewater. *Water SA.* Vol. 28(3). P. 299–305.
17. Tchobanoglous G., Burton F. L., Stensel H. D. 2003. *Wastewater Engineering: Treatment and Reuse.* McGraw-Hill Professional. 1848 p.

Regimantas Dauknys, Giedrė Vabolienė,
Marina Valentukevičienė, Mindaugas Rimeika

SUBSTRATO ĮTAKOS FOSFORO BIOLOGINIAM ŠALINIMUI ANALIZĖ

Santrauka

Biogeninių medžiagų perteklius paviršinio vandens telkiniuose sukelia eutrofikaciją – masinį fitoplanktono vystymąsi ir aukštesniųjų vandens augalų augimą. Dėl to pablogėja organoleptinės vandens savybės, nyksta kai kurios žuvys, mažėja biologinė įvairovė. Vandens telkinių eutrofikacijos galima išvengti iš nuotekų šalinant azotą bei fosforą. Šioms biogeninėms medžiagoms iš nuotekų šalinti pasaulyje plačiai naudojamos biologinės nuotekų valymo technologijos. Biologinis fosforo šalinimas pagrįstas bakterijų, vadinamų polifosfatus kaupiančiais organizmais, veikla. Šios bakterijos tam tikromis sąlygomis geba sukaupti fosforo daugiau nei jo reikia ląstelių sintezei, todėl kintant anaerobinėms / aerobinėms sąlygoms fosforas sukaupiamas dumble ir iš sistemos pašalinamas su pertekliniu veikliuoju dumblu. Vienas pagrindinių veiksnių, turinčių įtakos fosforo biologiniam šalinimui, yra į nuotekų valymo įrenginius atitekančių nuotekų sudėtis.

Šio darbo tikslas buvo ištirti Vilniaus ir Utenos nuotekų sudėtį lengvai skaidomų organinių medžiagų atžvilgiu bei nustatyti lengvai biologiškai skaidomos cheminio deguonies suvartojimo (ChDS) dalies ir bendrojo ChDS nuotekose priklausomumą. Paprastai lengvai biologiškai skaidomos organinės medžiagos yra išreiškiamos kaip dalis nuo bendrojo ChDS. Tyrimams naudotos nuotekos po pirminių nusodintuvų, t. y. nuotekos, atitekančios į biologinio valymo įrenginius. Nustatyta, kad Vilniaus miesto nuotekose lengvai skaidomos organinės medžiagos sudaro vidutiniškai 12,5 % nuo bendrojo ChDS, o Utenos miesto nuotekose – 18 %.

Straipsnyje pateikiamos lengvai skaidomų organinių medžiagų ir bendrojo cheminio deguonies suvartojimo regresinės priklausomybės, iš kurių galima prognozuoti šių medžiagų tarpusavio kitimo tendencijas. Be to, nustatytas lengvai biologiškai skaidomų organinių teršalų poreikis pašalinti 1 mg bendrojo fosforo, kurio vidutinė reikšmė yra 7,2 mg lengvai biologiškai skaidomos ChDS dalies Vilniaus nuotekų valykloje ir 8,8 mg lengvai biologiškai skaidomos ChDS dalies Utenos nuotekų valykloje.

Raktažodžiai: nuotekų valymas, lengvai biologiškai skaidoma ChDS dalis, veiklusis dumblas, biologinis fosforo šalinimas