Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

JOURNAL OF ENVIRONMENTAL SCIENCES
www.jesc.ac.cn

Effect of the C:N:P ratio on the denitrifying dephosphatation in a sequencing batch biofilm reactor (SBBR)

Artur Mielcarek[1], *Joanna Rodziewicz*[1,*], *Wojciech Janczukowicz*[1], *Arthur J. Thornton*[2], *Tomasz Jóźwiak*[1], *Paula Szymczyk*[1]

1. *University of Warmia and Mazury in Olsztyn, Department of Environment Engineering, ul. Warszawska 117a, Olsztyn 10-719, Poland. E-mail:* artur.mielcarek@uwm.edu.pl
2. *Atkins, Woodcote Grove, Ashley Road, Epsom KT18, United Kingdom*

ARTICLE INFO

Article history:
Received 16 January 2015
Revised 14 April 2015
Accepted 4 May 2015
Available online 23 July 2015

Keywords:
Wastewater treatment
Sequencing batch biofilm reactor
Nitrogen and phosphorus removal
Enhanced intracellular polyphosphate accumulation
Denitrifying dephosphatation
Denitrification and dephosphatation rates

ABSTRACT

A series of investigations were conducted using sequencing batch biofilm reactor (SBBR) to explore the influence of C:N:P ratio on biological dephosphatation including the denitrifying dephosphatation and the denitrification process. Biomass in the reactor occurred mainly in the form of a biofilm attached to completely submerged disks. Acetic acid was used as the source of organic carbon. C:N:P ratios have had a significant effect on the profiles of phosphate release and phosphate uptake and nitrogen removal. The highest rates of phosphate release and phosphate uptake were recorded at the C:N:P ratio of 140:70:7. The C:N ratio of 2.5:1 ensured complete denitrification. The highest rate of denitrification was achieved at the C:N:P ratio of 140:35:7. The increase of nitrogen load caused an increase in phosphates removal until a ratio C:N:P of 140:140:7. Bacteria of the biofilm exposed to alternate conditions of mixing and aeration exhibited enhanced intracellular accumulation of polyphosphates. Also, the structure of the biofilm encouraged anaerobic–aerobic as well as anoxic–anaerobic and absolutely anaerobic conditions in a SBBR. These heterogeneous conditions in the presence of nitrates may be a significant factor determining the promotion of denitrifying polyphosphate accumulating organism (DNPAO) development.

© 2015 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

Introduction

The reduction of eutrophication in the aquatic environment is often achieved by the co-removal of phosphorus and nitrogen compounds from wastewater using activated sludge systems. Many studies demonstrate that the organic demand required for denitrifying phosphorus removal depends not only on the type of a chemical compound, acceptors of electrons (nitrates or nitrites), but also on the type of reactor. The use of biofilm systems for integrated removal of C, N and P compounds may have many advantages. They display greater resistance to changes in the influent contaminant loads or to the presence of toxic compounds, they are characterized by smaller process foot-print and less growth of excess biomass, and finally require lower operational costs (Helness and Ødegaard, 2000; Mielcarek et al., 2015).

In addition, the morphological structure of biofilm may also enable the occurrence of anaerobic, anoxic and anaerobic zones in the biofilm's profile at the same time (Meyer et al., 2005).

Under certain conditions the presence of the intermediate products of nitrogen bio-removal, nitrate and nitrite, may

* *Corresponding author*. E-mail: joanna.rodziewicz@uwm.edu.pl (Joanna Rodziewicz).

http://dx.doi.org/10.1016/j.jes.2015.05.018
1001-0742/© 2015 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

inhibit the bio-processes of phosphorus removal in activated sludge systems (Kerrn-Jespersen and Henze, 1993; Patel and Nakhla, 2006).

According to the literature, enhanced biological phosphorus removal may proceed in the presence of oxidized forms of nitrogen. Apart from polyphosphate-accumulating organisms (PAOs), such conditions also facilitate the occurrence of denitrifying polyphosphate accumulating organisms (DNPAOs). The PAOs use only oxygen as an acceptor of electrons, whereas the DNPAOs use both oxygen and oxidized forms of nitrogen (Hu et al., 2003; Zhang et al., 2010). Under anaerobic conditions, in systems where the removal of nitrogen and phosphorus occurs concurrently, the occurrence of oxidized nitrogen forms, acting as electrons acceptors, may contribute to a reduced demand for organic carbon and oxygen.

A series of investigations were conducted using a sequencing batch biofilm reactor (SBBR) type reactor to explore the influence of C:N:P ratio on biological dephosphatation including the denitrifying dephosphatation and the denitrification process. Biomass in the reactor occurred mainly in the form of a biofilm attached to completely submerged disks, whereas the contribution of suspended biomass in the treatment process was marginal. Acetic acid was used as the source of carbon. According to reported studies, it is the best source of organic carbon for both the denitrifying and dephosphatating bacteria in the activated sludge system (Elefsiniotis et al., 2004; Janczukowicz et al., 2013). The organic carbon load remained unchanged, whereas the total nitrogen load was changed to create conditions in which nitrates are used as the electron acceptors in denitrifying dephosphatation.

1. Experimental

The experiments were divided into 4 series treating wastewater with differing C:N:P ratios and were performed in two replications.

1.1. Reactors

The experiments were conducted using two SBBRs in the shape of a cylinder with an internal diameter of 0.14 m and height of 0.5 m, made of plexiglass. The volume of each reactor was 5 L. The media construction comprised a package of completely submerged 8 disks made of stainless steel with diameter of 0.1 m. The disks were mounted coaxially, at 30° angle to the vertical axis of the reactor, and rotated by an electrical engine with the speed of 60 r/min. The distance between disks was 20 mm (Fig. 1).

1.2. Synthetic wastewater

The synthetic wastewater used in the experiments was typical of effluent from the nitrification process. Wastewaters were prepared using KH_2PO_4, $NaNO_3$ and from $MgSO_4 \cdot 7H_2O$ (0.308 g/L), KCl (0.021 g/L), $CaCl_2$ (0.021 g/L), enriched broth (0.02 g/L) and tap water. Influent redox potential was 271.0 mV, pH — 7.6. Total phosphorus and nitrogen (in the form of nitrates) influent concentrations were 7.0 mg P/L and 7.0–140.0 mg N/L respectively, depending on to assumptions of a given series. The only source of organic carbon was acetic acid. Based on preliminary study, the initial content of total organic carbon (TOC) in wastewater was adopted at 140 mg C/L. Investigation were conducted for different C:N:P ratios, *i.e.*, 140:7:7 — series 1, 140:35:7 — series 2, 140:70:7 — series 3, and 140:140:7 — series 4.

Fig. 1 – Filling of sequencing batch biofilm reactor (SBBR) with biofilm.

Activated sludge from the anoxic tank and anaerobic tank of the Municipal Wastewater Treatment Plant in Olsztyn was used as the inoculum.

1.3. SBBR operation

The operating cycle of a reactor was 12 hr (mixing (6 hr) and aeration (6 hr)). The reactors were aerated with compressed air. The sedimentation was not included, because the reactor was emptied after completion of the cycle by removing wastewater and the sloughed biofilm, only the biomass attached to disks remained in the reactor. The exception to this method was during the adaptation period (1–60 cycles), when 50% of reactor volume was discharged and part of suspended biomass was left. This was to facilitate the rapid formation of the stable biofilm.

The reactors operated at room temperature (20–22°C). In each operation, 5 L of wastewater was fed to the reactor at the beginning of the cycle. During the kinetic studies, samples of wastewaters were collected every 0.25 hr for the first hour of the cycle and then every hour. The first series of analyses was conducted after 90 completed cycles. This facilitated the stable operation of the reactors for at least 30 cycles under the defined experimental conditions. In successive series, the analysis was begun after 60 cycles post the change in the experimental parameters.

The following physicochemical parameters were monitored: pH value and temperature using a CP-105 waterproof

pH-meter (Elmetron, Zabrze, Poland); redox potential using pH211 Microprocessor (Hanna Instruments, Eibar, Spain); dissolved oxygen using Oxi 330i/set (WTW, Weilheim in Oberbayern, Germany). Treated wastewaters filtrated through a medium filter were analyzed for TOC using TOC analyzer (TOC-L CPH/CPN, Shimadzu Corporation, Kyoto, Japan) with the "oxidative combustion-analysis in infrared" method. Total nitrogen (TN) was determined using TOC analyzer (TOC-L CPH/CPN with TNM-L device, Shimadzu Corporation, Kyoto, Japan) with the "oxidative combustion-chemiluminescence" method. Total phosphorus (TP) was determined using UV-VIS 5000 DR spectrophotometer (HACH Lange, Dusseldorf, Germany) with the HACH Lange LCK 348-350 method. Wastewaters were also analyzed for ammonia nitrogen, nitrites, nitrates, and orthophosphate according to APHA-AWWA-WEF (1992).

2. Results and discussion

The consumption of TOC as well as changes in concentrations of nitrogen and phosphorus compounds were monitored in a SBBR at various C:N:P ratios. It was found that, the profiles of nitrogen and phosphorus removal and organic carbon consumption were affected by the C:N:P ratio. The organic load at the beginning of the cycle was at 11,200 mg C/(m^2·day) (280,000 mg C/(m^3·day)) (Table 1).

It was observed that the thickness of biofilm ranged from 0.5 mm at the edges of disks to 20 mm at center, due to the various effects of abrasive forces (Fig. 1). The color of film ranged from milk-like to dark brown, however it showed clear stratification.

In order to investigate the changes in the mechanisms of organic carbon consumption, denitrification and biodephosphatation, the operating cycle of SBBR was divided into shorter periods depending on the factors likely to have an influence on these processes. The rate of concentration change was determined by using the zero order reaction equation.

The profiles of changes in the concentrations of C, N and P were shown to be highly variable, which most likely resulted from the many concurrent processes within one cycle.

In each series, the concentration of organic carbon at the end of the cycle was below 1 mg C/L, however the profiles of organic compound consumption depended on the C:N:P ratio.

Under anaerobic conditions, the PAOs uptake organic compounds from wastewater for the synthesis of intracellular polyhydroxy acids (PHA), and derive the necessary energy from degradation of intracellular polyphosphates. Such a phenomenon was the most likely reason of the presence of the highest amount of phosphates in the first hour of the cycle in series 1.

At the same time, organic compounds were also utilized in the denitrification process which proceeded at a rate of 12.57 mg N/(L·hr). It confirms the fact that after 0.5 hr of the cycle, the concentration of nitrates was below 1 mg N/L. The rate of the consumption of organic compounds during the release of phosphates and denitrification reached 18.97 mg C/(L·hr) (Fig. 2). Under anaerobic conditions and with the lack of electron acceptors in the form of nitrates, sulfates or carbonates, the consumption of organic compounds may be reduced.

In each series, the concentration of dissolved oxygen at the mixing stage was in the range of 0.2–0.4 mg O_2/L. In series 1, at the C:N:P ratio of 140:7:7, the redox potential (ORP) after the mixing stage reached -167.4 ± 33 mV, and the consumption of acetic acid, after the initial period, was significantly impaired and proceeded with the rate of 2.91 mg C/(L·hr). Only after introduction of electron acceptors in the form of oxygen during the aeration stage was the rate of organic carbon consumption observed to increase to 19.41 mg C/(L·hr). In each series, the concentration of dissolved oxygen at the aeration stage was 7.1–7.8 mg O_2/L (Fig. 2).

Aerobic conditions may facilitate the degradation process of earlier synthesized intracellular PHAs, and the energy derived from this process is retained in the cell owing to the synthesis of intracellular polyphosphates. This resulted in the phosphate concentration decreasing in wastewater during the aeration stage with the rate of 0.18 mg P/(L·hr) (Fig. 2).

The higher concentration of oxidized forms of nitrogen at the mixing stage in the successive series enabled more effective use of organic compounds under anaerobic conditions. In series 2, organic substrate utilization in the presence of nitrates accounted for 24.8 mg C/(L·hr), whereas in the absence of nitrates — for 2.9 mg C/(L·hr). After oxygen feeding to reactors, the rate of acetic acid consumption reached 23.8 mg C/(L·hr) (Fig. 3).

In the mixing stage of series 2, the rate of phosphate removal reached 0.10 mg P/(L·hr), and the profile of phosphorus

Table 1 – Pollutant removal efficiencies in SBBR (sequencing batch biofilm reactor).

Series		Load		Average load of pollutants removed		Pollutant removal efficiencies (%)
		(mg/(m^3·day))	(mg/(m^2·day))	(mg/(m^3·day))	(mg/(m^2·day))	
1	N	14000	560	13320 ± 141.42	532.8 ± 5.66	95.14 ± 1.01
	P	14000	560	430 ± 70.71	17.2 ± 2.83	3.07 ± 0.51
	C	280000	11200	278610 ± 70.71	11144.4 ± 2.83	99.50 ± 0.03
2	N	70000	2800	69320 ± 141.42	2772.8 ± 5.66	99.03 ± 0.20
	P	14000	560	2790 ± 749.53	111.6 ± 29.98	19.93 ± 5.35
	C	280000	11200	279010 ± 212.13	11160.4 ± 8.49	99.65 ± 0.08
3	N	140000	5600	139410 ± 14.14	5576.4 ± 0.57	99.58 ± 0.01
	P	14000	560	4540 ± 226.27	181.6 ± 9.05	32.43 ± 1.62
	C	280000	11200	279640 ± 84.85	11185,6 ± 3.39	99.87 ± 0.03
4	N	280000	11200	110787 ± 8333.96	4431.48 ± 333.36	39.57 ± 2.98
	P	14000	560	4160 ± 480.83	166.4 ± 19.23	29.71 ± 3.43
	C	280000	11200	277758 ± 115.97	11110 ± 4.64	99.20 ± 0.04

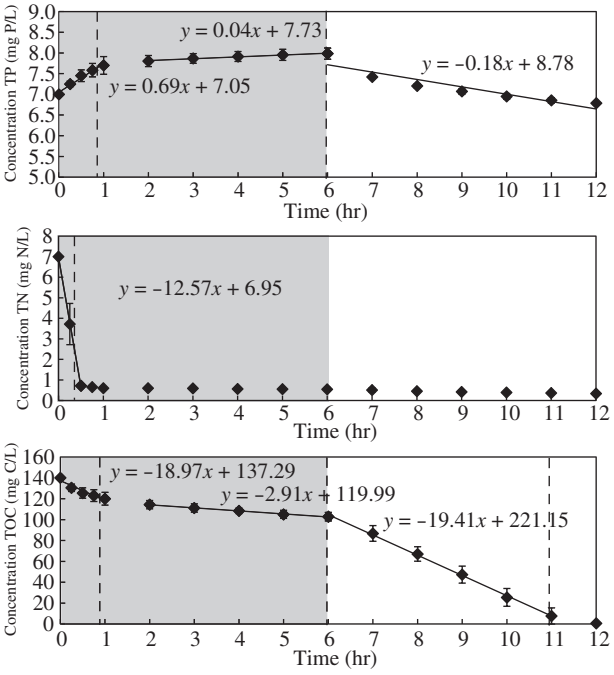

Fig. 2 – Changes of TOC (total organic carbon), TN (total nitrogen) and TP (total phosphorus) in series 1 (the ratio of C:N:P 140:7:7).

concentration changes in wastewater differed significantly compared to series 1. The reduction in the release of phosphates could result from too high a concentration of nitrate nitrogen which, as demonstrated by many investigations with activated sludge, may result a decrease in both the phosphate release and phosphate uptake (Patel and Nakhla, 2006; Janczukowicz et al., 2011). The concentration of total phosphorus at the end of the mixing phase reached 7.56 ± 0.09 mg P/L. The morphological structure of a biofilm differs from that of activated sludge flocks. The characteristics of the biofilm structure might have determined the occurrence of aerobic–anaerobic conditions in its surface layers, as well as the occurrence of anoxic–anaerobic conditions (but not aerobic) in its deeper layers. This, when coupled with the presence of oxidized forms of nitrogen, could influence the denitrifying activity of bacteria of the PAOs group. It also means that the process of denitrifying dephosphatation could affect the profiles of phosphorus and nitrogen concentrations within a cycle. These conditions are not easily created in the activated sludge system which means that in such cases this method is less applicable. A review of the literature indicates many theories that explain the denitrifying activity of PAO group bacteria. Kerrn-Jespersen and Henze (1993) identified among PAOs the bacteria, which may use only oxygen as acceptors of electrons and other bacteria which may also use nitrate. Hu et al. (2003) additionally describe a third group of bacteria capable of using nitrite as the acceptor of electrons.

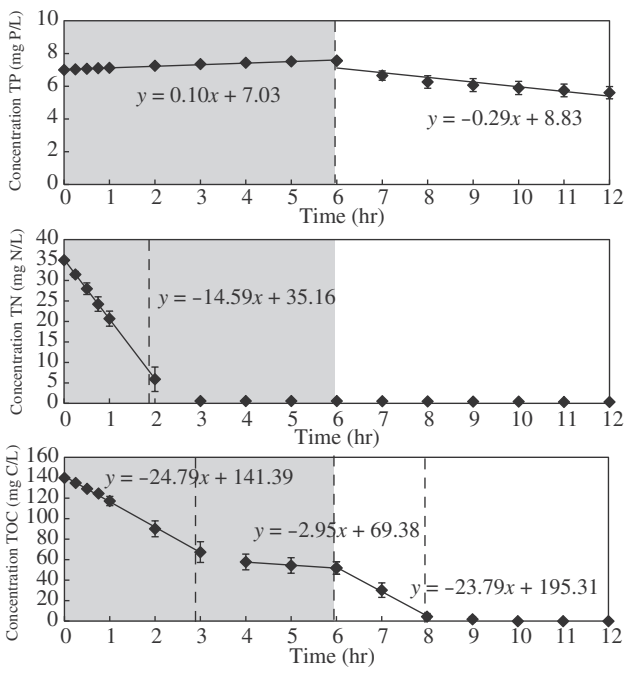

Fig. 3 – Changes of TOC (total organic carbon), TN (total nitrogen) and TP (total phosphorus) in series 2 (the ratio of C:N:P 140:35:7).

Wachtmeister et al. (1997) assume the existence of one PAO group in which, depending on conditions, denitrifying activity reaches different levels. Under anaerobic–aerobic conditions, the level of denitrifying activity is low or null, whereas under anaerobic–anoxic conditions (but never aerobic ones) the denitrifying activity is at a maximum.

An increase in the nitrate concentration caused changes in the profiles of phosphate release and phosphate uptake and in the profiles of nitrogen and organic carbon removal within the cycle. The presence of oxidized forms of nitrogen in series 2 may have contributed to the induction of denitrifying activity in PAOs. The reduction in the quantity of phosphates released at the mixing stage was most likely due to the utilization of nitrates as electron acceptors under anaerobic conditions and the simultaneous release and uptake of phosphates in the different layers of the biofilm.

However, the presence of simple organic compounds at the beginning of the aeration stage in this series could contribute to the promotion of denitrifying microorganisms other than the DNPAOs, because under anoxic conditions the available electron acceptors are used for the oxidation of organic substrate in wastewater and not of the oxidation of the PHAs accumulated within the cells.

Furthermore, both the nitrate concentration in the treated wastewater and morphological structure of a biofilm significantly influence the transport of the substrate within the biofilm. At higher concentration of nitrates it was possible to transport them into deeper layers according to Fick's first law. At low concentration of nitrates they could be transported only within the surface layers of the biofilm, where the activity of bacteria of the DNPAOs group was most probably limited by the presence of both aerobic (aeration of the reactor) and anaerobic (mixing) conditions. This was most likely the cause of the lack of a clear correlation between simultaneous removal of TP and TN. Furthermore, at the ratio C:N:P of 140:7:7 and 140:35:7, the absence of nitrates in the treated wastewater after 0.5 hr and 3 hr, respectively, prevented their use by bacteria of the DNPAOs group, and thus limited their activity. It could explain the different

profiles of phosphate release in series 1 and 2 compared to series 3 and 4 (Figs. 2–5). Higher concentrations of nitrates and thus a transport to deeper layers, where there were anoxic and anaerobic and never aerobic conditions, have most probably enabled the activation of DNPAOs, and thus a simultaneous removal of TP and TN. Furthermore, longer nitrate presence in the treated wastewater allowed for their use by bacteria of the DNPAO group. Thanks to this they could win a competition for an organic substrate against other microorganisms in series 3 and 4.

The profiles of phosphate release and phosphate uptake indicated the highest activity of DNPAOs in the series with higher ratio of nitrate to carbon and phosphorus.

The C:N:P ratios of 140:70:7 and 140:140:7 enabled complete consumption of the organic substrate within the 6-hr anaerobic phase, and the rate of its consumption reached, respectively, 263 and 212 mg C/(L·hr) (Figs. 4 and 5).

Both in series 3 and 4, the highest quantities of released phosphates were recorded in the first hour. After a short period when phosphate concentration remained at a stable level, it decreased significantly despite anaerobic conditions (Figs. 4 and 5).

The denitrifying activity of PAOs could explain the increase of denitrification rate in series 3, observed during anaerobic uptake of phosphates. The rate of denitrification increased from 9.66 to 14.02 mg N/(L·hr) (Fig. 4). In series 4 a decrease was noted in the denitrification rate from 10.78 to 6.18 mg C/(L·hr). The quantity of organic carbon supplied in this series was, however, insufficient to meet the total demand in denitrification, which is confirmed by the concentration of total nitrogen at the effluent reaching 84.6 ± 4.2 mg N/L. In order to complete the denitrification process, with acetic acid, the minimum quantity of organic carbon supplied would need to be 2.5 mg per 1 mg of nitrate nitrogen.

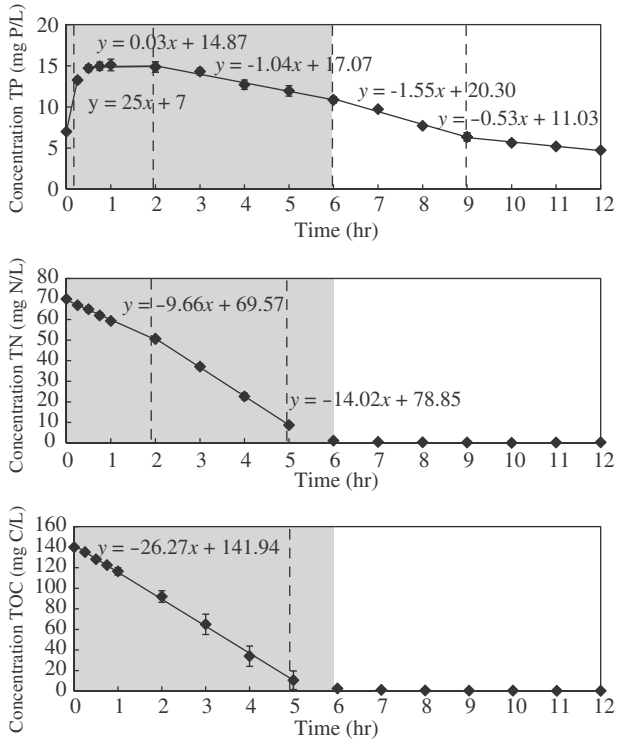

Fig. 4 – Changes of TOC(total organic carbon), TN (total nitrogen) and TP (total phosphorus) in series 3 (the ratio of C:N:P 140:70:7).

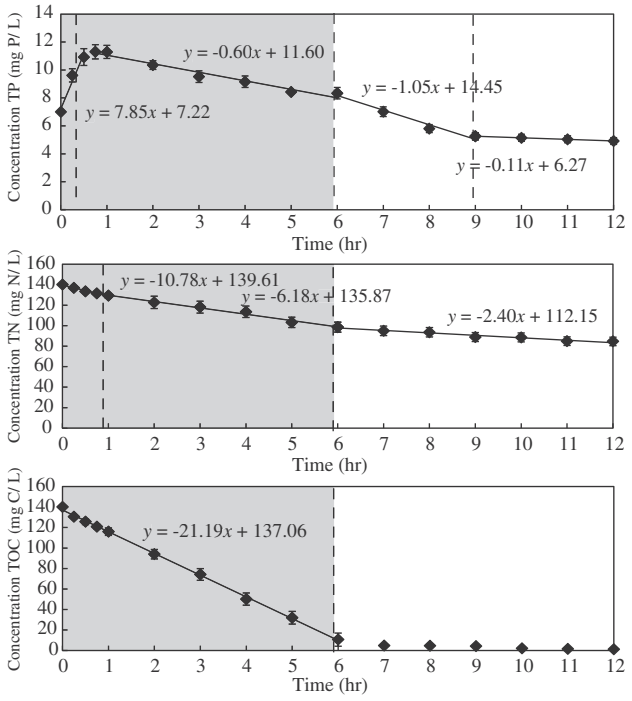

Fig. 5 – Changes of TOC (total organic carbon), TN (total nitrogen) and TP (total phosphorus) in series 4 (the ratio of C:N:P 140:140:7).

Nitrogen removal in series 4 occurred also under aerobic conditions, but with a lower rate of 2.4 mg N/(L·hr) (Fig. 5). This is because the organic substrate was insufficient, it might be speculated that at this stage the removal of nitrogen resulted from both biomass synthesis and denitrifying dephosphatation. A significant effect upon PAO and DNPAO bacteria could also be ascribed to a high concentration of nitrite, the maximum value of which reached 32.8 ± 2.1 mg N-NO_2/L at the end of anaerobic stage and 28.3 ± 3.4 mg N-NO_2/L at the end of aeration stage. This was also the most likely reason of the lower rate of organic substrate consumption in this series and of the collapse of the treatment processes. It was indicated by a lower load of removed contaminants compared to series 3 (Table 1). In the other series, the maximum concentration of nitrite did not exceed 2 mg N-NO_2/L. In a study conducted with activated sludge, Meinhold et al. (1999) observed inhibition of the anoxic treatment of phosphates already at nitrite concentration above 8 mg N-NO_2/L. During the aeration stages of series 3 and 4, a significant decrease was noted in phosphate concentration in the wastewater. The highest rate of phosphate uptake was determined in the first 3 hr of the cycle, *i.e.*, 1.55 mg P/(L·hr) in series 3 and 1.05 mg P/(L·hr) in series 4. These rates were higher compared to the phosphorus uptake rate in the mixing stage. This would confirm literature studies that show the rate of anoxic uptake of phosphates is lower than that of phosphates accumulation under aerobic conditions (Kuba and van Loosdrecht, 1996).

Table 2 – pH changes in SBBR (sequencing batch biofilm reactor) cycle.

Series	pH					
	1 hr	2 hr	3 hr	6 hr	9 hr	12 hr
1	6.21 ± 0.12	6.32 ± 0.10	6.47 ± 0.06	6.73 ± 0.17	8.21 ± 0.09	8.26 ± 0.03
2	6.30 ± 0.07	6.77 ± 0.10	6.99 ± 0.11	7.20 ± 0,08	8.08 ± 0.09	8.12 ± 0.11
3	6.38 ± 0.08	7.22 ± 0.12	7.63 ± 0.17	7.77 ± 0.12	8.39 ± 0.11	8.45 ± 0.16
4	6.36 ± 0.06	7.21 ± 0.12	7.61 ± 12	7.68 ± 0.22	8.43 ± 0.09	8.55 ± 0.09

The progress of denitrification and dephosphatation in activated sludge systems is not only impacted by the occurrence of the appropriate aerobic–anoxic–anaerobic conditions and the presence of oxidized forms of nitrogen in the wastewater but is also dependent on the pH value of wastewater (Serralta et al., 2006).

In this study, the introduction of acetic acid caused a decrease of wastewater pH to the value of 6.1. A low pH value at the beginning of the cycle could inhibit the activity of PAOs (Liu et al., 2007; Table 2). The population of glycogen accumulating organisms (GAOs) may also develop in activated sludge. Unlike PAOs, the GAOs derive energy for PHA accumulation from glycolysis and not from the hydrolysis of polyphosphates, whereas in the aerobic stage they compete for organic substrate with PAOs. As a result, the development of GAOs contributes to the reduced effectiveness of biological dephosphatation. Filipe et al. (2001a, 2001b) conducted a study on the effect of pH on anaerobic metabolism of PAOs and GAOs in activated sludge. They observed a linear increase in the load of phosphates released by PAOs in the pH range of 6.0–8.0, whereas PHA synthesis and glycogen degradation by PAOs did not depend on the pH value. In the case of GAOs, the synthesis of PHA and degradation of glycogen increased along with an increase in pH. The authors suggest that, in the anaerobic stage, the pH value should be kept above 7.25, which ensures faster uptake of organic substrate by PAOs than by GAOs and, consequently, increases the effectiveness of biological dephosphatation. In the aerobic stage, the pH value should be kept below 7.0 because the PAOs are more susceptible to pH values in the aerobic stage than GAOs. In this study, in the mixing stage, the pH value of wastewater was increased along with the successive consumption of acetic acid. Depending on the C:N:P ratio, at the end of the anaerobic stage the pH value of wastewater reached 6.73 ± 0.17, 7.20 ± 0.08, 7.77 ± 0.12, and 7.68 ± 0.22 in series 1, 2, 3 and 4, respectively (Table 2). Maintaining the lower pH of the wastewater for a longer period in series 1 and 3 could affect the occurrence and activity of PAOs, GAOs as well as DNPAOs, which consequently influenced the profiles of phosphate release and phosphate uptake in these series compared to series 3 and 4. The emission of CO_2 and additional wastewater treatment at the aeration stage caused an increase in the pH value which at the end of the cycle reached 8.26 ± 0.03, 8.12 ± 0.11, 8.45 ± 0.16, and 8.55 ± 0.09 in series 1, 2, 3 and 4, respectively (Table 2).

3. Conclusions

A series of investigations were conducted using a SBBR type reactor under different ratios of C:N:P. Several major conclusions were drawn: Bacteria of the biofilm exposed to alternate conditions of mixing and aeration exhibit enhanced intracellular accumulation of polyphosphates. Also the structure of the biofilm, encourages anaerobic–aerobic as well as anoxic–anaerobic and absolutely anaerobic conditions in the one reactor. These heterogeneous conditions in the presence of nitrates may be the significant factor determining the promotion of DNPAO development. These conditions are not easily created in the activated sludge system which means that in such cases this method is less applicable.

The promotion of DNPAOs and the occurrence of dephosphatation denitrification contribute to the reduced demand for organic carbon which is indispensable for nitrogen and phosphorus removal. Reduced organic carbon requirement may affect operating costs of SBBRs.

C:N:P ratios had a significant effect on the profiles of phosphate release and phosphate uptake, of organic substrate consumption and nitrogen removal. The highest rate of both release and uptake of phosphates was recorded at the C:N:P ratio of 140:70:7, *i.e.*, 25 and 1.55 mg P/(L·hr), respectively. In turn, the lowest rate of phosphates release (0.10 mg P/(L·hr)) was noted at C:N:P of 140:35:7, whereas the lowest rate of phosphates uptake (0.18 mg P/(L·hr)) was stated at C:N:P of 140:7:7.

The C:N ratio of 2.5:1 ensured complete denitrification. The highest rate of denitrification (14.59 mg N/(L·hr)) was achieved at the C:N:P ratio of 140:35:7. In contrast, the lowest rate of denitrification, ranging from 6.18 to 10.78 mg N/(L·hr), was noted at the ratio of 140:140:7 (series 4). Also in this series, analyses showed a high nitrogen removal rate at 2.40 mg N/(L·hr) at the aeration stage and with the lack of organic substrate. Irrespective of the C:N:P ratio, organic substrate was almost completely consumed.

The increase of nitrogen load fed to the reactor caused an increase in the quantity of removed phosphates until a ratio C:N:P of 140:140:7, at which point a decrease in the effectiveness of wastewater treatment was noted.

Acknowledgments

The project was funded by the National Science Centre, Poland (the decision nr DEC-2012/07/N/ST8/03201).

REFERENCES

APHA-AWWA-WEF, 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed. Washington, USA.

Elefsiniotis, P., Wareham, D.G., Smith, M.O., 2004. Use of volatile fatty acids from an acid-phase digester for denitrification. J. Biotechnol. 114 (3), 289–297.

Filipe, C.D.M., Daigger, G.T., Grady Jr., C.P.L., 2001a. pH as a key factor between glycogen accumulating organisms and phosphorus accumulating organisms. Water Environ. Res. 73 (2), 223–232.

Filipe, C.D.M., Daigger, G.T., Grady Jr., C.P.L., 2001b. Effects of pH on the rates of aerobic metabolism of phosphate-accumulating and glycogen-accumulating organisms. Water Environ. Res. 73 (2), 213–222.

Helness, H., Ødegaard, H., 2000. Biological phosphorus and nitrogen removal in a sequencing batch moving bed biofilm reactor. Water Sci. Technol. 43, 233–240.

Hu, J.Y., Ong, S.L., Ng, W.J., Lu, F., Fan, X.J., 2003. A new method for characterizing denitrifying phosphorus removal bacteria by using three different types of electron acceptors. Water Res. 37 (14), 3463–3471.

Janczukowicz, W., Rodziewicz, J., Filipkowska, U., 2011. Evaluation of process of biological nitrate and phosphate removal in SBR type reactor with external carbon source. Ann. Environ. Prot. 13, 453–470 (in Polish).

Janczukowicz, W., Rodziewicz, J., Czaplicka, K., Kłodowska, I., Mielcarek, A., 2013. The effect of volatile fatty acids (VFAs) on nutrient removal in SBR with biomass adapted to dairy wastewater. J. Environ. Sci. Health A. 48 (7), 809–816.

Kerrn-Jespersen, J.P., Henze, M., 1993. Biological phosphorus uptake under anoxic and aerobic conditions. Water Res. 27 (4), 617–624.

Kuba, T., van Loosdrechtt, M.C.M., 1996. Effect of cyclic oxygen exposure on activity of denitrifying phosphorus removing bacteria. Water Sci. Technol. 34 (1-2), 33–44.

Liu, Y., Chen, Y., Zhou, Q., 2007. Effect of initial pH control on enhanced biological phosphorus removal from wastewater containing acetic and propionic acids. Chemosphere 66 (1), 123–129.

Meinhold, J., Filipe, C., Daigger, G., Isaacs, S., 1999. Characterization of the denitrifying fraction of phosphate accumulating organisms in biological phosphate removal. Water Sci. Technol. 39 (1), 31–42.

Meyer, R.L., Zeng, R.J., Giugliano, V., Blackall, L.L., 2005. Challenges for simultaneous nitrification, denitrification, and phosphorus removal in microbial aggregates: mass transfer limitation and nitrous oxide production. FEMS Microbiol. Ecol. 52 (3), 329–338.

Mielcarek, A., Rodziewicz, J., Janczukowicz, W., Thornton, A., 2015. The feasibility of citric acid as external carbon source for biological phosphorus removal in a sequencing batch biofilm reactor (SBBR). Biochem. Eng. J. 93, 102–107.

Patel, J., Nakhla, G., 2006. Interaction of denitrification on P removal in anoxic P removal systems. Desalination 201, 82–99.

Serralta, J., Ferrer, J., Borrás, L., Seco, A., 2006. Effect of pH on biological phosphorus uptake. Biotechnol. Bioeng. 95 (5), 875–882.

Wachtmeister, A., Kuba, T., Loosdrecht, M., Heijnen, J.J., 1997. A sludge characterization assay for aerobic and denitrifying phosphorus removing sludge. Water Res. 31 (3), 471–478.

Zhang, S.-H., Huang, Y., Hua, Y.-M., 2010. Denitrifying dephosphatation over nitrite: effects of nitrite concentration, organic carbon, and pH. Bioresour. Technol. 101 (11), 3870–3875.