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Journal of Hazardous Materials 158 (2008) 151–156
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Influence of the wastewater composition on denitrification and biological p-removal in the S-DN-P-process (b) Effect of acetate

Hee-Jeong Choi [a], Seung-Mok Lee [a,*], Chul-Ho Choi [a], Min-Chul Kwon [b], Hyeon-Yong Lee [b]

[a] *Department of Environmental Engineering, Kwandong University, Gangnung 210-701, Republic of Korea*
[b] *College of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Republic of Korea*

Received 2 March 2007; received in revised form 6 July 2007; accepted 18 January 2008
Available online 31 January 2008

Abstract

The effect of acetate was examined during the p-removal and denitrification of wastewater. The plant was operated based on the sequencing-batch-biofilm-reactor (SBBR) process. As microbial media, ca. 9 mm Bio-Flow granules made from polyethylene and polypropylene were used. Three preparations were made to compare the level of biological p-removal and denitrification. In comparison to the batch test, 42 mg/L (AC 30) and 84 mg/L (AC 60) of $NaCH_3COO$ were mixed with the 500 mL of raw wastewater and the effect of the acetate concentration on the level of p-removal was monitored. All samples were immediately filtered with 0.45 μm membrane filter, and PO_4-P, NO_3-N, NO_2-N and acetate were analyzed using Ion Chromatography, whereas P_{total} and chemical oxygen demand (COD) were measured by a spectrophotometer. The p-removals for the WW, WW + AC 30 and WW + AC 60 preparations were found to be 9.4, 9.1 and 13.1 mg/L, respectively. The WW + AC 30 preparation did not show any significant effect on the p-removal, while p-removal in WW + AC 60 preparation was higher than that in the other two preparations. A comparison of the data revealed the COD: NO_3-N:AC:P ratio of the WW, WW + AC 30 and WW + AC 60 preparations to be 18.07:2.90:6.87:1, 21.28:2.45:5.98:1 and 15.95:2.75:6.18:1, respectively. The experimental results showed that approximately 7 mg/L of acetate was consumed per 1 mg/L of p-removal.

Keywords: Acetate (AC); Biological phosphorus (bio-P) removal; Denitrification; Sorption denitrification P-removal (S-DN-P) process; Sequencing-batch-biofilm-reactor (SBBR); Wastewater (WW)

1. Introduction

Biological phosphorus removal is in the anaerobic zone, where acetate (and propionate) is taken up by phosphorus-storing bacteria and is converted to carbon storage products that provide energy for growth in the subsequent aerobic zones [1]. Schmolders et al. [2] reported that the transport of a negative loaded molecule such as acetates by the cell membrane exhibited different types of forces. A high concentration gradient of acetate into the cell favored the transportation process because of the high electro-chemical difference in potential (the charge difference between the cell interior and the external medium). Therefore, the energy required for the anaerobic bio-p metabolisms provides a suitable environment-dependent energy, facilitates the transport of the acetate and the conversion of acetate to PHA [3]. These reactions are interrelated, which means that the concentration of the materials involved can be evaluated along with the respective reaction rate for a set of equations. Since a total of thirteen different chemical species are involved in a set of these reactions, the reaction rates can be obtained by solving these equations [4]. Overall, the ions are represented as acids in order to simplify its form. Formally, the ratio of acetate uptake and soluble-P in the anaerobic phase can be described by the following equation:

$$Y_{P/AC} = \frac{\Delta c\,(PO_4\text{-}P)}{\Delta c\,(\text{acetate})} \left(\frac{\text{mol } PO_4\text{-}P}{\text{mol acetate}} \text{ or } \frac{\text{mg } PO_4\text{-}P}{\text{mg acetate}} \right) \quad (1)$$

* Corresponding author. Tel.: +82 33 649 7535; fax: +82 33 642 7635.
E-mail address: leesm@kwandong.ac.kr (S.-M. Lee).

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doi:10.1016/j.jhazmat.2008.01.066

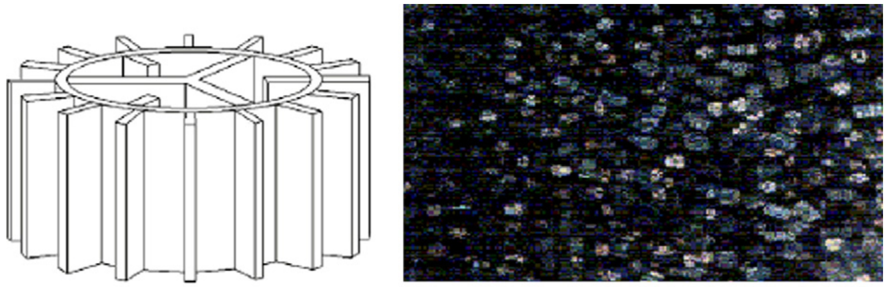

Fig. 1. (a) Bioflow-9-ring and (b) filling materials in the SBBR.

According to these reaction equations, the back solution of phosphate is necessary for both setting up the PHB (polyhydroxybutyrate) memory and for producing energy. On the other hand, acetate consumption occurs only in the soluble-P. The theoretical value of $Y_{P/AC}$ depends on the portion of the entire soluble-P, and thus on the metabolic pathway, which is based on the reported model [5]. Comeau et al. [6] and Wentzel and Ekama [7] reported the $Y_{P/AC}$ ratio to be 0.52 g P/g AC, whereas Mino et al. [8] reported 0.26 g P/g AC. The difference between these two groups highlights the stoichiometric connections. With Comeau et al. [6], it was associated with the $Y_{P/AC}$ overview of the stoichiometry connections for different substrates, whereas Mino et al. [8] considered decomposition. The uptake of phosphate and the regeneration of ATP by $NADH_2$ are described differently according to the environmental conditions. Generally, 7–10 mg/L of acetate is needed for approximately 1 mg/L p-removal. This suggests that the rate of cell growth increases with increasing acetate concentration, which means that more phosphorus is likely, removed [9]. In this study, the possible higher p-removal and denitrification were examined as a function of the acetate concentration.

2. Material and methods

2.1. Sequencing-batch-biofilm-reactor (SBBR)

This study was performed based on the wastewater of a pilot sewage plant. The plant was operated based on the SBBR process. The reactor possessed a total volume of filling materials of 1.5 and 0.6 m^3, respectively. The overall height of the reactor and the height of the filling material were 2.2 and 1.9 m, respectively. As microbial media, ca. 9 mm Bio-Flow granules made from polyethylene and polypropylene were used. Fig. 1(a) and (b) shows the filling materials in the reactor. As shown in the figure, the SBBR contained many rings lying in its interior portion, which may help increase the total quantity of biomass per ring. In contrast, they may also cause limited material conversions into the interior ring due to transportation, with a setting of microflora, which will compete with the denitrification of poly-P organisms.

2.2. Batch plant and sampling methods

Three different types of wastewater were prepared, two synthetic wastewaters and one raw wastewater. In comparison to the batch test, 42 mg/L (AC 30) and 84 mg/L (AC 60) of $NaCH_3COO$ was mixed with the 500 mL of raw wastewater and the effect of the acetate concentration on the level of p-removal was monitored. The raw wastewater was obtained from the preliminary sedimentation of the sewage plant at Waßmansdorf, Berlin.

A step-by-step batch test of S-DN-P-cycle was performed in the lab-scale plant with either real or synthetic wastewater. Fig. 2 shows a schematic of the plant. First, a 2-h anaerobic sorption phase (S-phase) was operated. After which the reactor was completely emptied, the following denitrification phase (DN-phase)

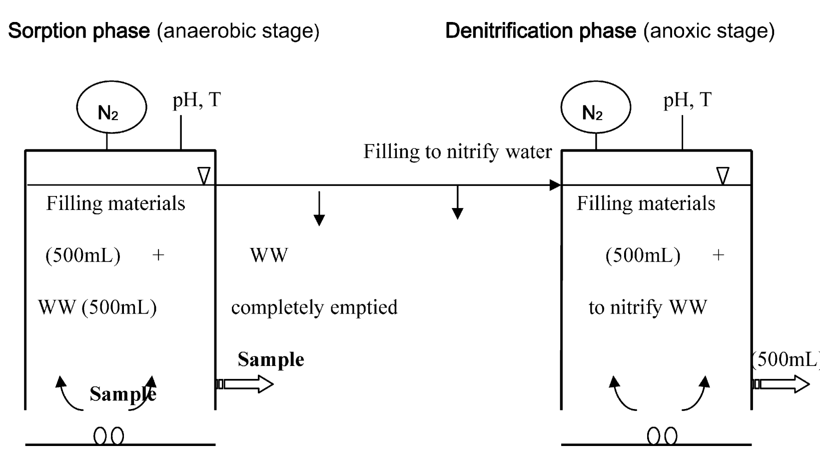

Fig. 2. A schematic of the SBBR plant.

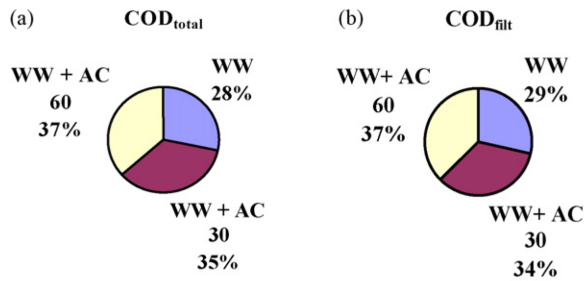

Fig. 3. (a) COD_{total} consumption and (b) COD_{filt} consumption in the S-phase for the WW, WW + AC 30 and WW + AC 60 preparations.

was established by filling with nitrifying water to the reactor. The anoxic phase, then, was performed for 4 h. Each test was examined with a liquid volume of 500 mL and about the same portions of thickened Bio-Flow media. The final batch volume in the plant was between 750 and 800 mL. The test of three preparations was performed in a parallel mode. The wastewater was mixed with a magnetic agitator.

The S-DN-P Bio-Flow media were rinsed several times with tap water to ensure the removal of the residual degradation products on the biofilm. The test was initiated with injecting nitrogen gas. The first sample was drawn immediately after nitrogen injection, and subsequent samplings were performed with defined time interval. All samples were immediately filtered with 0.45 μm membrane filter, and placed in a cold environment. The tests were performed under controlled environmental conditions, i.e., pH, time and temperature. The samples were subjected to analyze PO_4-P, NO_3-N, NO_2-N and acetate using Ion Chromatography according to DIN (German Institute for Standardization), whereas P_{total} and chemical oxygen demand (COD) were measured by a spectrophotometer.

3. Results and discussion

3.1. COD_{total} and COD_{filt} consumption

The COD indicates the quantity of oxygen, which is essential to the chemical oxidation of the wastewater constituents. It describes the quantity of substrates, which can be degraded chemically. The COD is a sum parameter that covers different groups of materials [10].

The COD_{total} obtained for these three preparations, the WW, WW + AC 30 and WW + AC 60 preparations, were found to be 164.4, 200.0 and 209.0 mg/L, respectively. This means that the absorbed COD_{total} was the highest for the WW + AC 60 preparation, i.e. 37%, followed in order by the WW + AC 30 preparation (35%) and the WW preparation (28%) (cf. Fig. 3(a): absorbed COD_{total}). These results clearly show that the level of COD_{total} consumption increased with increasing acetate concentration. The COD_{filt} for the WW, WW + AC 30 and WW + AC 60 preparations were 63.9, 75.7 and 84.0 mg/L, respectively. This suggests that the COD_{filt} consumption for the three preparations were 29%, 34% and 37%, respectively (cf. Fig. 3(b): absorbed COD_{filt}).

Table 1
AC consumption in the S-phase

Preparations	ΔAC (mg/L)	$\Delta AC/\Delta COD_{total}$ (g AC/g COD)	$\Delta AC/\Delta COD_{filt}$ (g AC/g COD)
WW	62.5	0.38	0.98
WW + AC 30	56.2	0.28	0.74
WW + AC 60	80.9	0.39	0.96

The COD_{filt}/COD_{total} ratio was pointed towards the easily degradable fraction group present in the wastewater. In these tests, the COD_{filt}/COD_{total} for the WW, WW + AC 30 and WW + AC 60 preparations were 0.39, 0.38 and 0.40, respectively. The COD_{filt}/COD_{total} ratio appeared to be about the same for all three preparations.

3.2. Acetate consumption

Table 1 shows the absorbed acetate concentration for three preparations. The WW + AC 60 preparation showed the highest level of acetate consumption. The level of acetate consumption in the WW + AC 30 preparation was lower than that in the WW preparation. Acetate in WW and WW + 60 preparations were almost removed, while 13.1 mg/L of acetate in WW + 30 was remained after 2 h of reaction. The cause for this is unclear. It was may be no good conditions for the RNA and poly-P in the WW + AC 30 preparation, because the relative cellular content of RNA and poly-P can vary significantly depending on the growth conditions. P is located in several types of compounds in bacterial cells: nucleic acids (RNA, DNA), nucleotides, phospholipids, sugar phosphates, orthophosphate and polyphosphates. The inorganic phosphates and nucleic acids, in particular RNA, are the dominant fractions. In polyphosphate (poly-P)-accumulating organisms, this compound is the main P fraction.

In rapidly growing cells, the RNA content was relatively high and the poly-P content was low (opposite at low growth rates). It was not likely that any of these mechanisms can explain enhanced P storage in cells in biological systems treating municipal wastewater. Sulfur, nitrogen and phosphorous reach such low concentrations were relevance, nor were inhibition from low pH values a problem in most practical cases. The lower level of acetate consumption results in a lower soluble-P. Not all the acetate in the WW + AC 30 preparation had decomposed in the anaerobic phase. The ratio of the absorbed AC and the absorbed COD_{total} in the S-phase for the WW, WW + AC 30 and WW + AC 60 preparations were 0.38, 0.28 and 0.39, respectively. Similarly, the ratio of the absorbed acetate and the absorbed COD_{filt} in the WW and the WW + AC 60 preparations were similar.

3.3. Nitrate- and nitrite-consumption

Ideally, the various decomposition phases are recognized as an anoxic phase, where the decomposition of easily degradable carbon compounds can take place. At the beginning of the batch test, the easily degradable carbon compounds are likely to be degraded, and the rapid denitrification process adjusts accord-

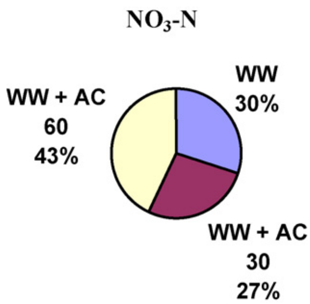

Fig. 4. NO_3-N consumption in the DN-phase for the WW, WW + AC 30 and WW + AC 60 preparations.

ingly. As soon as the easily degradable carbon compounds are removed, the rate of change decreases markedly [11]. The next-phase is the denitrification of the easily degradable substrates. Similar to denitrification, the level of biological p-uptake can be increased, and the high nitrate content in the biomass inhibits the soluble-P by increasing the level of the acetate consumption in the S-phase [12].

Fig. 4 shows the NO_3-N consumption in the DN-phase during the batch tests. The WW + AC 60 preparation showed 13% more NO_3-N consumption than the WW preparation. This might be because the WW + AC 60 contain more substrates (10% of AC, 8% of COD_{filt} and 9% of COD_{total}) than the WW preparation. It should be noted that the substrate quantity affects the denitrification process.

3.4. NO_3-N and substrate consumption in the S-phase

Table 2 shows specific substrate consumption during the batch experimentation. It should be noted that the extent of the substrate consumption in the S-phase greatly influenced the consumption of NO_3-N. Hence, the AC/nitrate ratio plays significant role towards the biological p-removal. With a high AC/nitrate ratio (>2), the level of acetate decreases to a larger extent than the denitrification process. In this study, the COD_{total}/NO_3 consumption ratios for the WW, WW + AC 30 and WW + AC 60 preparations were 5.66, 7.78 and 5.06, respectively. The WW and WW + AC 60 preparations have similar values, but the WW + AC 30 preparation showed a much higher value. The ratio of absorbed AC/NO_3 consumption for the WW, WW + AC 30 and WW + AC 60 preparations were 2.2, 2.2 and 2.0 g AC/g NO_3-N, respectively. Almost equal values were obtained for all preparations. There is only a small difference between the ratio of COD_{filt}/NO_3-N and the ratio of AC/NO_3-N because the level of COD_{filt} and NO_3-N consumptions are identical for most acetate. The C/NO_x-N ratio was considered that the COD used can be accounted by cell synthesis and COD oxidation by NO_x-N reduction to produce energy for the cell [13].

Fig. 5 shows the level of absorbed AC and NO_3-N consumption in the batch test. The NO_3-N consumption for the WW, WW + AC 30 and WW + AC 60 preparations were 28.7,

Table 2
Specific substrate consumption in the batch test

Preparations	$\Delta COD_{total}/\Delta NO_3$-N (g COD/g NO_3-N)	$\Delta COD_{filt}/\Delta NO_3$-N (g COD/g NO_3-N)	$\Delta AC/\Delta NO_3$-N (g AC/g NO_3-N)
WW	5.66	2.20	2.20
WW + AC 30	7.78	2.90	2.20
WW + AC 60	5.06	2.00	2.00

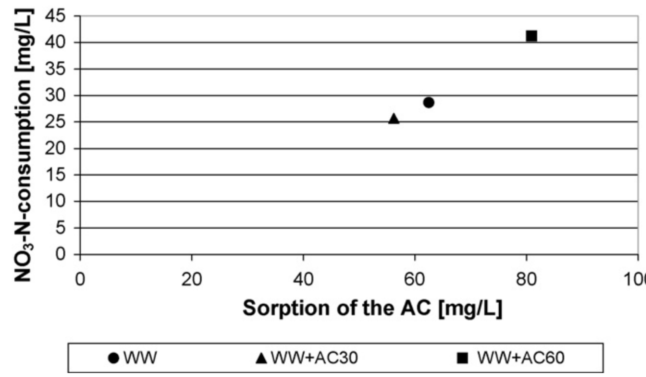

Fig. 5. NO_3-N consumption with AC consumption for the WW, WW + AC 30 and WW + AC 60 preparations.

Table 3
Substrate consumption and soluble-P in the S-phase

Preparations	Soluble-P/ΔCOD_{total} (g P/g COD_{total})	Soluble-P/ΔCOD_{filt} (g P/g COD_{filt})	Soluble-P/Δ AC (g P/g AC)
WW	0.03	0.08	0.08
WW + AC 30	0.02	0.06	0.07
WW + AC60	0.06	0.14	0.15

Table 4
NO_3-N consumption and P-uptake in the DN-phase

Preparations	NO_3-N consumption w/o NO_2-N (mg NO_3-N/L)	NO_3-N consumption w/ NO_2-N (mg O_2/L)	p-Uptake/ΔNO_3-N (g P/g NO_3-N)	p-Uptake /ΔNO_3-N (g P/g O_2)
WW	26.4	28.7	0.56	0.51
WW + AC 30	23.0	25.7	0.58	0.52
WW + AC60	36.0	41.3	0.70	0.60

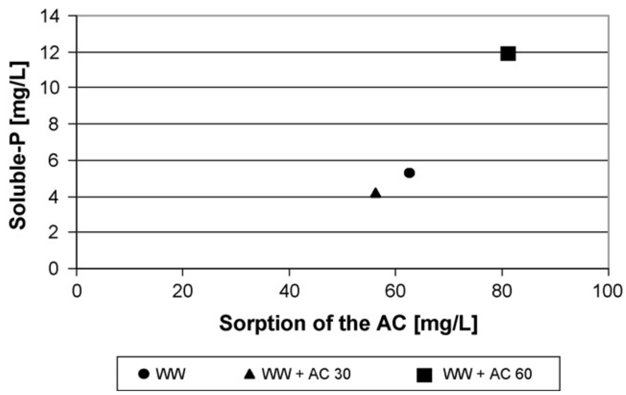

Fig. 6. Soluble-P with AC consumption in the S-phase for the WW, WW + AC 30 and WW + AC 60 preparations.

25.7 and 41.3 mg/L, respectively. This suggests that the level of NO_3-N consumption increases with increasing amount of acetate uptake.

3.5. Soluble-P and substrate consumption in the S-phase

Basically, the p-removal depends upon the presence of various substrates. Therefore, the soluble-P, the kind of substrate and the initial substrate concentration play an important role [14]. In biological p-removal plants, the poly-P organisms have the function of both accumulating poly-P, and also removing organic matter from the sewage [15]. When comparing the soluble-P and the substrate consumption in the S-phase, it was noted that the soluble-P was dependent on the substrate consumption. Table 3 shows the substrate consumption and soluble-P in the S-phase. The ratios of soluble-P/COD_{total} for the WW, WW + AC 30 and WW + AC 60 preparations were found to be 0.03, 0.02 and 0.06, respectively. The COD: soluble-P ratio in the three preparations was roughly 17:1 to 50:1. The soluble-P/COD_{filt} and soluble-P/AC ratios were similar in all these three preparations. The WW + AC 60 preparation showed a relatively higher soluble-P/COD_{filt} ratio. It was 0.14 g P/g COD_{filt} for the WW + AC 60 preparation, which was almost twice higher than the other two preparations. The soluble-P/AC ratios for the WW, WW + AC 30 and WW + AC 60 preparations were found to be 0.08, 0.07 and 0.15, respectively. The WW + AC 60 preparation was also higher than the other preparations. This means that the level of cell growth increases with increasing acetate concentration, which results in more phosphorus removal.

Fig. 6 shows the soluble-P and level of AC sorption in the S-phase. The soluble-P was obtained for these preparations; 5.3, 4.2 and 12.0 mg/L for the WW, WW + AC 30 and WW + AC 60 preparations, respectively. Fig. 7 gives p-uptake and p-removal in the DN-phase. For the p-uptake, 14.7, 13.3 and 25.1 mg/L were observed for the WW, WW + AC 30 and WW + AC 60 preparations, respectively. In addition, the level of p-removal was found to be 9.4, 9.1 and 13.1 mg/L for the WW, WW + AC 30 and WW + AC 60 preparations, respectively.

3.6. Relation of the PO_4-P and NO_3-N in the DN-phase

The majority of the microbiological conversion was based on the mechanism of increased p-removal. However, the electron acceptor, i.e. nitrate, is used mainly for other purposes, i.e. growth [16]. While comparing the level of p-uptake with that of NO_3-N-consumption in the DN-phase, it was noted that the complete reduction of nitrate to molecular nitrogen could not occur with partial formation of nitrite. The ratio of the p-uptake/NO_3-N-consumption increased with increasing acetate content. Table 4 shows the p-uptake and NO_3-N-consumption in the DN-phase. From the results of the test preparations, p-uptake/NO_3-N-consumption for the WW, WW + AC 30 and WW + AC 60 preparations were 0.56, 0.58 and 0.70, respectively. The WW + AC 60 preparation showed higher p-uptake/NO_3-N-consumption ratio than the other two preparations. When NO_3-N concentration increased, the residual phosphorus concentration decreased, which meant that no phosphorus was released when high concentration of NO_3-N was present [17].

Fig. 7 gives a comparison of the substrate uptake in the S-phase, NO_3-N consumption and PO_4-P-removal in the DN-phase. The results clearly show that the phosphorus removal efficiency was affected more by acetate concentration than that by the COD and NO_3-N concentrations. The COD: NO_3-N:AC:P ratios were 18.07:2.90:6.87:1, 21.28:2.45:5.98:1 and

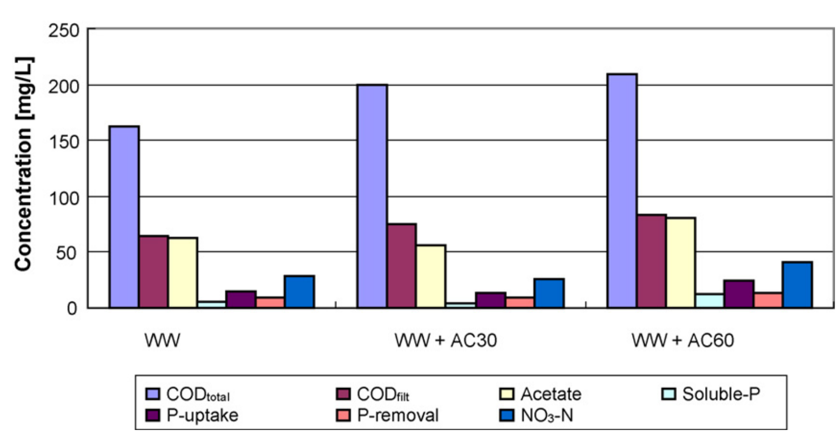

Fig. 7. Comparison of the substrate consumption, NO_3-N consumption and PO_4-P removal for the WW, WW + AC30 and WW + AC 60 preparations.

15.95:2.75:6.18:1 for the WW, WW + AC 30 and WW + AC 60 preparations, respectively.

4. Conclusions

The effect of acetate concentration on the denitrification and biological p-removal from the wastewater in the S-DN-P process was examined and the following results were obtained. The more substrate uptake and p-removal was obtained in the WW + AC60 preparation than those in the other two preparations. P-removal was found to be 9.4, 9.1 and 13.1 mg/L for the WW, WW + AC 30 and WW + AC 60 preparations, respectively. The ratio of absorbed AC/NO_3 consumption for the WW, WW + AC 30 and WW + AC 60 preparations were 2.2, 2.2 and 2.0, respectively. The COD: NO_3-N:AC:P ratio for the WW, WW + AC 30 and WW + AC 60 preparations were 17.49:2.81:6.69:1, 21.98:2.53:6.18:1 and 15.95:2.78:6.18:1, respectively. Generally, 7–10 mg/L of acetate was needed for 1 mg/L P of removal. From this experiment, it was found that approximately 6.18–6.69 mg/L of acetate can enable 1 mg/L of p-removal.

Acknowledgements

This study was carried out at the Institute of Environmental Technology, TU-Berlin, Germany. The valuable advice received from Prof. Dr. W. Hegemann is greatly acknowledged. This work was supported by a Grant (No. KRF-2007-314-C00271) from Korea Research Foundation, Republic of Korea).

References

[1] N.O. Yaqci, R. Tasli, N. Artan, D. Orhon, The effect of nitrate and different substrates on enhanced biological phosphorus removal in sequencing batch reactors, J. Environ. Sci. Health (Part A) 38 (8) (2003) 1489–1497.
[2] G.J.F. Schmolders, J. van der Meij, M.C.M. van Loosdrecht, J.J. Heijnen, Model of anaerobic metabolism of the biological phosphorus removal process: stoichiometry and pH influence, Biotechnol. Bioeng. 43 (1994) 461–470.
[3] N.H. Callado, E. Foresti, Removal of organic carbon, nitrogen and phosphorus in sequential batch reactors integrating the aerobic/anaerobic process, Water Sci. Technol. 44 (4) (2001) 263–270.
[4] C. Sieker, Kombination der Denitrifikation und der vermehrten biologischen Phosphorremoval in einer alternierend betriebenen Biofiltrationsanlage, Berichte zur Siedlungswasserwirtschaft TU Berlin 12 (1999).
[5] M. Pijuan, A. Guisasola, J.A. Baeza, J. Carrera, C. Casas, J. Lafuente, Aerobic phosphorus release linked to acetate uptake: influence of PAO intracellular storage compounds, Biochem. Eng. J. 26 (2005) 184–190.
[6] Y. Comeau, K.J. Hall, R.E.W. Hancock, W.K. Oldham, Biochemical model for enhanced biological phosphorus removal, Water Res. 20 (12) (1986) 1511–1521.
[7] M.C. Wentzel, G.A. Ekama, Denitrification kinetics in biological N and P removal activated sludge systems treating municipal wastewaters, Water Sci. Technol. 39 (6) (1999) 69–77.
[8] T. Mino, V. Arun, Y. Tsuzuki, T. Matsuo, Metabolism of different bacterial populations in enhanced biological phosphate removal process, Water Sci. Technol. 29 (7) (1994) 67–70.
[9] Z.-R. Hu, M.C. Wentzel, Anoxic growth of phosphate-accumulating organisms (PAOs) in biological nutrient removal activated sludge system, Water Res. 22 (2002) 565–570.
[10] I.K. Kapdan, R. Oztekin, The effect of hydraulic residence time and initial COD concentration on color and COD removal performance of the anaerobic–aerobic SBR system, J. Hazard. Mater. 136 (3) (2006) 896–901.
[11] W.J. Nq, S.L. Onq, J.Y. Hu, Denitrifying phosphorus removal by anaerobic/anoxic sequencing batch reactor, Water Sci. Technol. 43 (3) (2001) 139–146.
[12] R.J. Zeng, A.M. Saunders, Z. Yuan, L.L. Blackall, J. Keller, Identification and comparison of aerobic and denitrifying polyphosphate-accumulating organisms, Biotechnol. Bioeng. 83 (2003) 140–148.
[13] J.H. Kim, Effect on removal of nitrogen and phosphorus using exogenous carbon source in biofilm process, RIST 17 (3) (2003) 312–316.
[14] B. Pertersen, H. Temmink, M. Henze, S. Isaacs, Phosphate uptake kinetics in relation to PHB under aerobic conditions, Water Res. 32 (1) (1998) 91–100.
[15] M. Merzouki, N. Bernet, J.P. Delqenes, R. Moletta, M. Benlemlih, Biological denitrifying phosphorus removal in SBR: effect of added nitrate concentration and sludge retention time, Water Sci. Technol. 43 (3) (2001) 191–194.
[16] P.A. Castillo, I. Tejero, Biological phosphorus removal using a biofilm membrane reactor, in: IAWQ 19th Biannual International Conference, 22–26 Juni Vancouver Canada, V 2, 1998, pp. 270–277.
[17] P. Hung, S. Qin, Q. Zhao, X. Guo, Quick start-up of mudanjiang wastewater treatment plant and factors influencing phosphorus removal, Global NEST J. 8 (1) (2006) 1–8.