A Semi-Batch (On-line) Method for Biokinetics Determination in an Enhanced Biological Phosphorus Removal System
Proceedings of European Congress of Chemical Engineering (ECCE-6)
Copenhagen, 16-20 September 2007

A Semi-Batch (On-line) Method for Biokinetics Determination in an Enhanced Biological Phosphorus Removal System

A. Eleni Vaiopoulou,[a] B. Alexander Aivasidis,[a]

[a]*Department of Environmental Engineering, Democritus University of Thrace ,67 100 Xanthi, Greece*

Abstract

Kinetics of nitrification, denitrification and dephosphatation were studied in the aerobic, anoxic and anaerobic stages of a pilot scale Biological Nutrient Removal plant treating municipal wastewater. The configuration of the plant's design is based on the combination of the UCT (University of Cape Town) process and the step feeding in a three-stage denitrification cascade. In order to study the process kinetics and to obtain actual values for the investigated kinetic parameters semi-batch experiments were performed. Continuous feeding of the treating system was interrupted and instantly the pilot plant was turned into a batch mode operation. Thereafter, substrate was added and its consumption was monitored. Aeration was implemented in the anaerobic tank to reach maximum phosphorus release. At this point and after transition to anaerobic conditions acetic acid was added to provide a readily biodegradable carbon source for phosphorus uptake. The results show that the nitrification process follows Monod type kinetics. In general, nitrification activity is enhanced along the treatment train, whereas the low values of the half saturation constant show that nitrifiers with high affinity to the influent substrate grow in the system. Denitrification experimental results indicated zero order kinetics. Two specific denitrification rates, an initial high one, $q_{DN,1}$ and a following low one, $q_{DN,2}$, until complete substrate consumption, were determined in the anoxic tanks. In any case, maximum denitrification activity is recorded in the first anoxic tank of the cascade. Specific denitrification rates with real wastewater supplementation were lower than them produced by synthetic wastewater addition, which is expected as real municipal wastewater contains a lower concentration of readily biodegradable organics. By testing the anaerobic sludge of the BNR unit, specific anaerobic phosphorus release rate and specific aerobic uptake rate was low. On-line biokinetics determination using the proposed semi-batch method reflects realistic characteristics of the microorganisms used for biodegradation and results in real data acquisition and thus, to actual unit monitoring under operating conditions.

Keywords: biokinetics, nitrification, denitrification, biological phosphorus removal

1. Introduction

Recently several wastewater treatment methods have been developed and modified to eliminate nutrient discharge, since nitrogen and phosphorus are responsible for the eutrophication of surface water. Among them, enhanced biological phosphorus removal (EBPR) processes have been regarded as the most efficient and economic treatment methods.

In particular, biological nitrogen removal is a two step process including ammonia oxidation by nitrification, followed by reduction of nitrogen oxides to nitrogen gaseous compounds by denitrification (Wagner *et al.*, 2002). As a result, nitrifiers and denitrifiers play the crucial role for nitrogen removal. Nitrifiers are categorized to nitrisifying (oxidizers of ammonia to nitrite) and nitrifying (oxidizers of nitrite to nitrate) bacteria, which come from the genus *Nitrosomonas* and *Nitrobacter* or *Nitrospira* respectively (Grady and Felipe, 2000; Wagner *et al.*, 2002). Mostly common denitrifiers belong to the genus *Pseudomonas*, *Micrococus*, *Achromobacter*, *Alcaligenes*, *Methylobacterium*, *Bacillus*, *Paracoccus* and *Hyphomicrobium* (Horan, 1996; Wagner et al., 2002). Biological phosphorus removal from wastewaters exploits the potential of some microorganisms, known as Phosphate Accumulating Organisms (PAOs), to accumulate phosphate (as intracellular polyphosphate) in excess of their normal metabolic requirements under aerobic conditions (Seviour *et al.*, 2003). In the anaerobic phase, sufficient readily biodegradable carbon sources, such as volatile fatty acids (VFAs), must be available to induce PAOs to take up the acids, store them as poly-β-hydroxyalkanoates (PHA) and release orthophosphates into the solution (Seviour *et al.*, 2003). Recent research has shown that PAOs do not consist of a certain dominating bacterium, but of many different microbial teams (Mino *et al.*, 1998; Mino *et al.*, 2000; Wong *et al.*, 2005). It is obvious that a large number of chemical and biological reactions take simultaneously place in an EBPR treatment plant and a broad frame of bacteria species is involved. As a result, bacteria characteristics are of crucial importance for a successful biological nutrient removal.

Kinetic characterization of nitrification, denitrification and phosphorous removal may provide useful information for process feasibility and efficiency, as well as operation optimisation and monitoring. Biokinetic coefficients are also regarded as design parameters for wastewater treatment plants. The most well known model applied to describe ammonia oxidation (nitrification) is the following one of Monod, under the condition that oxygen and carbon dioxide concentrations are in abundance (Monod, 1949):

$$q_N = q_{N,\max} \frac{S_{NH_4-N}}{K_N + S_{NH_4-N}}, \text{ where}$$

q_N: specific nitrification rate [$g_{NH4\text{-}N}/(g_{XAO}\cdot d)$]
$q_{N,max}$: maximum specific nitrification rate [$g_{NH4+\text{-}N}/(g_{XAO}\cdot d)$]
$S_{NH4\text{-}N}$: concentration of ammonia – nitrogen [$mg_{NH4\text{-}N}/L$]
K_N: half saturation constant for ammonia – nitrogen [$mg_{NH4\text{-}N}/L$]
X_{AO}: concentration of nitrifying biomass [mg/L]

The specific denitrification rate, q_{DN} ($g_{NO3\text{-}N}/(g_{XHET} \cdot d)$), is related to the denitrification rate:

$\frac{d(NO_3 - N)}{dt} = q_{DN} \cdot X_{HET}$, where X_{HET} is the heterotrophic biomass expressed in g/L.

Most investigators agree that denitrification by a bacteria consortium is a zero order reaction with respect to nitrate, since half saturation constant for nitrate-nitrogen has been estimated at K_{DN}<1 mg/L (Beccari *et al.*,1983; Elefsiniotis *et al.*, 2004; Foglar and Briski, 2003; Li *et al.*, 2001; Timmermans and van Haute, 1983). Denitrification rates vary depending on the process/system applied, e.g. nitrification-denitrification activated sludge systems (NDAS), biological nutrients removal activated sludge systems (BNRAS) and external nitrification BNRAS (ENBNRAS), denitrifiers' concentration and operational conditions (Kappeler and Gujer, 1992).

Tchobanoglous et al. claim that biological phosphorus kinetics are within the same order of magnitude of other heterotrophic bacteria (Tchobanoglous *et al.*, 2003). Other scientists support Michaelis-Menten kinetics for *Acinetobacter* cultures (Pauli and Kaitala, 1996). On the other hand, batch tests for sludge characterization, such as anaerobic P release test, aerobic P uptake test, anoxic P uptake test, endogenous P release test etc, show an initial high rate and a following low one (Pala and Bolukbas, 2005; Ostgaard et al., 1997).

Many studies of kinetic parameters calculations use separate bench-scale reactors, apart from the reactor in which the biodegradation actually takes place. As a result, the scale effect and parameters that affect the hydrodynamic structure of the system, such as turbulence, the way of aeration, size etc, are not all taken into consideration. In such experiments the activity of the treatment plant cannot reflect the real system thoroughly. Another important point is that, especially in long-term studies, influent characteristics and consequently sludge characteristics change. Therefore, in-situ experiments would allow actual (on-line) monitoring of the unit. Except for influent wastewater effect on sludge characteristics, plant configuration also exerts great influence on the microbial population synthesis and characteristics. Thus, biokinetic parameters estimation experiments should be conducted in wastewater treatment plant and especially in modified configurations for complete understanding of the unit's properties.

Objective of this study is to present a semi-batch (on-line) method for the determination of biokinetic parameters in a recently proposed EBPR plant configuration (Vaiopoulou and Aivasidis, 2007). Nitrification, denitrification, as well as phosphorus release and uptake specific rates are investigated.

2. Materials and Methods

The experimental unit includes an anaerobic selector and stepwise feeding in subsequent anoxic and oxic vessels for simultaneous removal of BOD_5/COD,

suspended solids, as well as nitrogen and phosphorus. A schematic layout of the treating system is presented in Figure 1. It consists of an anaerobic tank followed by a cascade of three identical double-vessels (each one includes an anoxic and an aerobic compartment) and finally of a sedimentation tank (clarifier). After sludge separation in the clarifier, part of the sludge is pumped back as return sludge to the first anoxic tank and the rest is removed as excess sludge. Air is pumped at the bottom of the aerobic tanks according to the diffused aeration method. All tanks are gently stirred. A more detailed description of the plant is given in Vaiopoulou and Aivasidis, 2007).

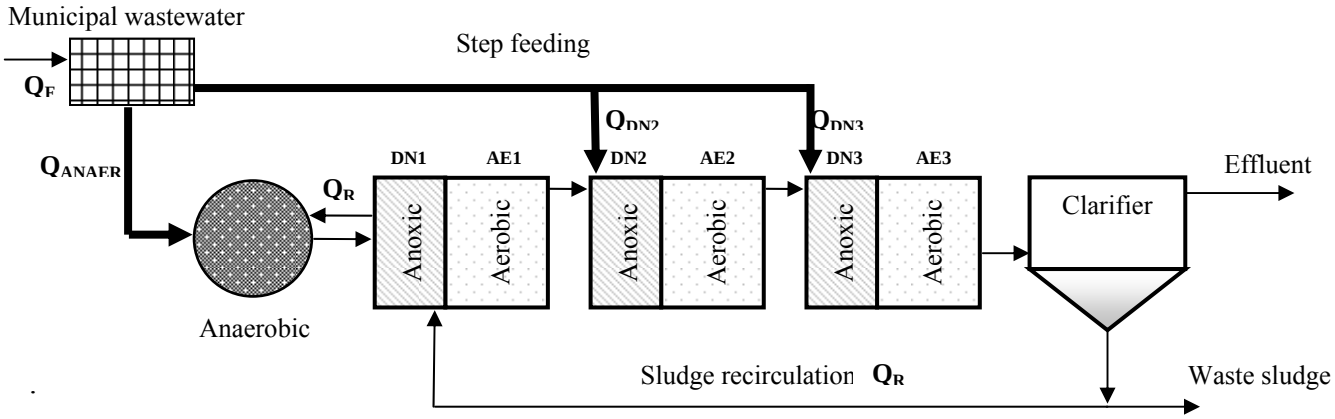

Figure 1. Experimental set-up of the EBPR system.

Due to the plant's configuration, nitrification takes place in the aerobic tanks (AE), denitrification in the anoxic tanks (DN), whereas biological phosphorus removal is achieved by phosphorus release in the anaerobic selector (AN), which then stored intracellularly under aerobic conditions, and thus removed by excess sludge.

In order to study the process kinetics and to obtain realistic values for the investigated kinetic parameters a series of semi-batch (or semi-continuous) experiments were performed. For this purpose, continuous feeding of the treating system under steady-state conditions was interrupted and instantly the pilot plant was turned into a batch mode operation by hydraulic isolation of each single tank. Thereafter, instant addition of substrate took place and substrate consumption was monitored. No sludge was extracted and experiments were conducted in the actual environment of bacteria life cycle under constant temperature (20˚C) and pH control.

In particular, for nitrification and denitrification tests organic substrate, in form of real or synthetic wastewater, and nitrate (KNO_3) were added into the anoxic tanks, whereas organic substrate and ammonium nitrogen (NH_4Cl) were added in the aerobic tanks of the treatment plant. In nitrification tests with real wastewater no addition of NH_4Cl took place. Synthetic wastewater consisted of 20 g/L peptone, 20 g/L saccharose and 5 ml/L acetic acid, which was diluted to reach a concentration of 500 mg/L COD in the tank. Real wastewater came from raw municipal wastewater of the combined sewer system of Xanthi city, Greece. Raw wastewater characteristics include a concentration of 330 mg/L COD and 50 mg/L TKN. Thereafter, sampling and analysing took place. Nitrite concentration was also monitored in case of

nitrification inhibition. Autotrophic and heterotrophic biomass was calculated according to the influent BOD_5/TKN ratio (Tchobanoglous, 1985).

Phosphorous release and uptake tests were conducted in the anaerobic selector. Initially, aeration was implemented in the anaerobic tank to reach maximum phosphorus uptake. At this point, anaerobic conditions were re-established by gaseous nitrogen (N_2) supply until dissolved oxygen concentration dropped to zero. There were no anoxic conditions, since initial ammonium nitrogen concentration before aeration was low. After transition to anaerobic conditions acetic acid was added to provide a readily biodegradable carbon source for phosphorus uptake. pH was adjusted to 7.2. Phosphorus was released in the mixed liquor, whereas sampling took place until phosphate concentration reached a baseline.

Samples of mixed liquor were taken by means of a syringe from each single tank at certain time intervals and were immediately filtered. The sampling volume does not affect the experimental results as it can be neglected in comparison with the total tank volume. Volatile suspended solids (VSS) and nitrate nitrogen were analysed for samples from the anoxic tanks, whereas VSS and ammonium nitrogen were analysed for samples withdrawn under aerobic conditions. Orthophosphates, nitrate and COD concentrations were monitored in phosphorus release and uptake tests. All analysis was conducted according to Standard Methods (APHA/AWWA/WPCF, 1998). A High Pressure Liquid Chromatography with a 550 conductivity detector and a 530 column heater of Alltech, USA was used for anion determination in isocratic mode after filtration of samples through 0.2 μm filters. A Marathon IV HPLC pump with flow rate of 2 ml/min was driving the filtered samples to pre-columns before entering the Hamilton PRP-X100 column. P-hydroxybenzoic acid was used as eluent. The system was running on EZ Chrom software. Wastewater temperature, pH and dissolved oxygen (DO) were constantly monitored.

3. Results

3.1. Nitrification tests

Six series of nitrification semi-batch tests were conducted to estimate maximum specific nitrification rate, $q_{N,max}$, and half saturation constant, $K_{m,N}$. The nitrification process was found to follow Monod type kinetics. The maximum specific nitrification rate, $q_{N,max}$, was determined to vary between 1.71 and 2.95 g NH_4^+-N/(g VSS_{aut}·d) for synthetic wastewater addition. The half saturation constant for the nitrification process, $K_{m,N}$, was estimated graphically at 2.2 – 6.4 mg NH_4^+-N/L with an average value of 4.76 mg NH_4^+-N/L. When the substrate was real wastewater, $q_{N,max}$ averaged at 1.14 g NH_4^+-Nr/(g VSS_{aut}·d) and $K_{m,N}$ at 1 mg NH_4^+-N/L. These values are low and show that the EBPR system configuration encourages growth of autotrophes with high affinity to the influent wastewater. In general, our results show that nitrification activity is enhanced along the treatment train, since half saturation constant values decrease from the AE1 tank to AE3 one. This finding implies that nitrifiers with high affinity to the influent substrate are formed into the system. An important point is that the real nitrification rates should be lower than the ones calculated, since both ammonia oxidation by nitrifiers and intracellular uptake of nitrogen by heterotrophes

take place at the same time. It is roughly estimated that VSS in activated sludge consist by 12% of nitrogen. Therefore, the substrate consumption should be attributed to both processes.

The values determined by the semi-batch experiments are considered to be in good agreement with other scientific results. In comparison with the results obtained by sludge characterization in a biological nutrients removal plant located also in the Mediterranean (Pala and Bolukbas, 2005), our values of $q_{N,max}$ are quite high. However, their values are below 0.096 g/ (g d), which, according to other researchers are not considered representative (Drtil *et al*., 1993). Moreover, our results of nitrification rates are in accordance with some other experimental works (Chandran and Smets, 2005; Grady *et al.,* 1996; Surmacz-Gorska *et al*., 1996), which show that the initially added substrate, measurements "noise", sampling intervals and sludge state are the main factors effecting the extent of $q_{N,max}$ and $K_{m,N}$.

By raw wastewater addition (as carbon and nitrogen substrate), experimental results of a typical nitrification batch test for the three aerobic tanks of the pilot plant are demonstrated in Figure 2. All diagrams indicate that high NH_4^+-N concentrations ensure the process' description by zero order kinetics with respect to nitrogen substrate. As ammonia becomes limiting, i.e. concentrations below 5 mg/L, the reaction is being described by a transition between zero and first order kinetics. According to Monod kinetics the substrate concentration for which the process rate becomes equal to the half of its maximum value, ($q_N = q_{N,max/2}$), represents the half saturation constant, K_N. Ammonia nitrogen consumption was accomplished in 2-3 hours after addition. As shown in Figure 2, the maximum specific nitrification rate increases from 0.8 g NH_4^+-N/(g VSS_{aut}·d) in AE1 to 1.2 g NH_4^+-N/(g VSS_{aut}·d) in AE2 and to 1.4 g NH_4^+-N/(g VSS_{aut}·d) in AE3, whereas the half saturation constant for the nitrification process was found almost constant in all tanks. Increase in maximum specific nitrification rate in the last aerated tanks is explained by the fact that the pre-denitrification, which takes place in the three anoxic tanks, eliminates organic substrate and thus, oxygen is available for ammonia oxidation without restriction of carbon oxidation by heterotrophes.

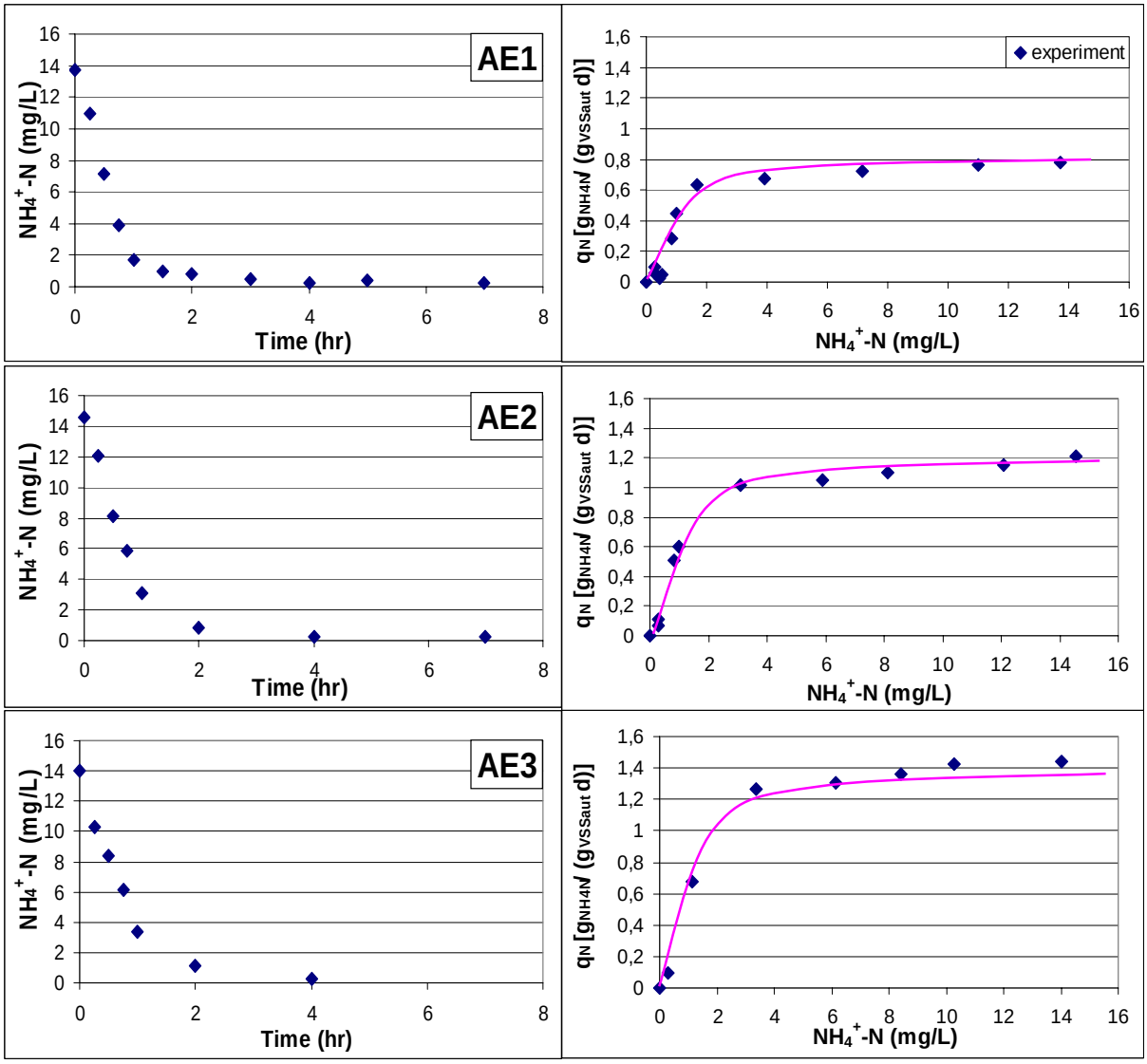

Figure 2. Real wastewater: Substrate consumption as well as specific nitrification rate in the 1st, 2nd and 3rd aerobic tank.

By synthetic wastewater addition (as carbon and nitrogen substrate), the maximum specific nitrification rates, as well as the half saturation constant for the nitrification process, are higher (Fig. 3). This finding can be attributed to several experimental aspects, such as the different influent flow distribution and the initially higher concentration of organic substrate. In particular, influent flow was distributed by 60% to the anaerobic selector, by 25% to 2nd anoxic tank and by 15% to the 3rd anoxic tank, whereas by real wastewater addition influent flow was distributed by 40% to the anaerobic selector, by 30% to 2nd anoxic tank and by 30% to the 3rd anoxic tank. It has been shown that this EBPR system operates optimally at the feeding ratio of 60/25/15 (Vaiopoulou and Aivasidis, 2007). The synthetic wastewater addition resulted in a concentration of 500 mg/L COD and 50 mg/L NH_4^+-N in each aerobic tank, which was primarily oxidized by heterotrophes restricting oxygen availability for ammonia oxidation by autotrophes. This finding complies with other results

showing that the extent of $q_{N,max}$ and $K_{m,N}$ is depending also on initial substrate addition (Chandran and Smets, 2005; Grady *et al.*, 1996; Surmacz-Gorska *et al.*, 1996).

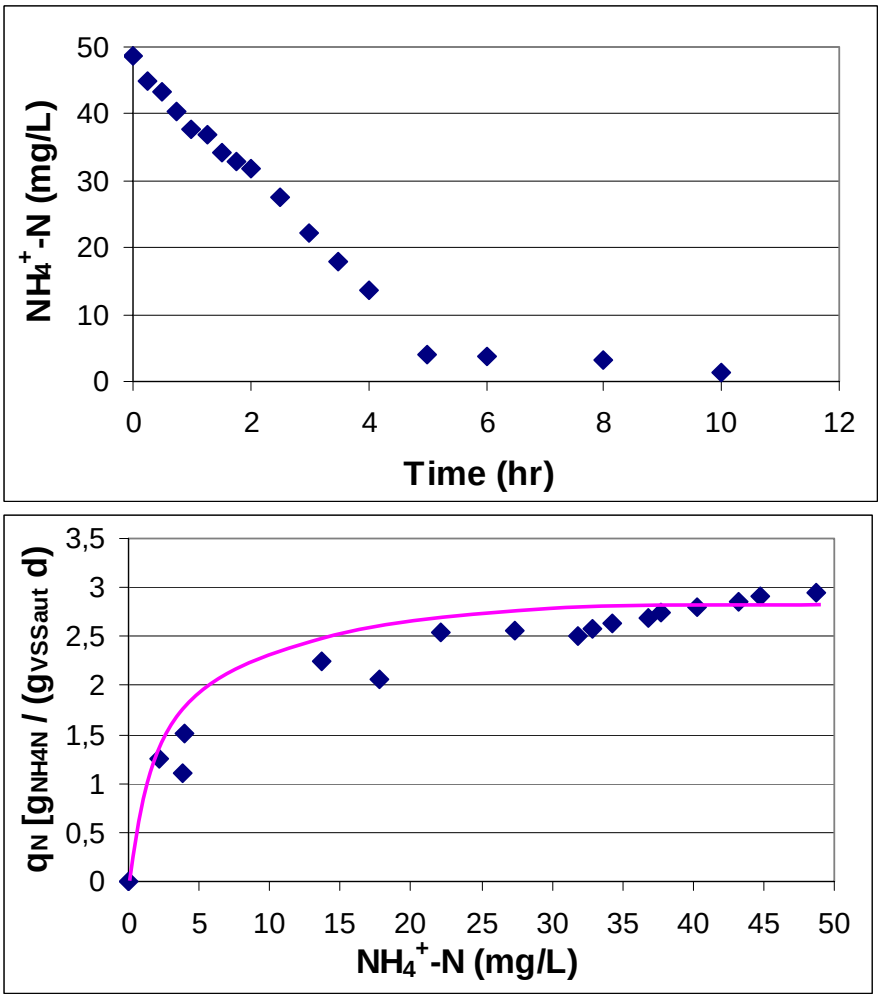

Figure 3. Synthetic wastewater: Substrate consumption as well as specific nitrification rate in the 2nd aerobic tank.

3.2. Denitrification tests

Five series of denitrification semi-batch tests were conducted to estimate maximum specific nitrification rate, $q_{DN,max}$, and half saturation constant, $K_{m,DN}$. Sufficiency and the type of organic substrate was well considered, as they are significant factors for the denitrification process. No nitrite accumulated during experimental process. Denitrification experimental results indicated zero order kinetics. Two specific denitrification rates were determined in the anoxic tanks; an initial high one, $q_{DN,1}$ and a following low one, $q_{DN,2}$, until complete substrate consumption. Using synthetic wastewater $q_{DN,1}$ and $q_{DN,2}$ varied between 0.078-0.186 g NO_3^--N/(g VSShet·d) and 0.057-0.0.77 g NO_3^--N/(g VSShet·d) with mean values of 0.12 and 0.06 g NO_3^--N/(g VSShet·d) respectively. When the added substrate was real wastewater, $q_{DN,1}$ and $q_{DN,2}$ were found to obtain mean values of 0.11 g NO_3^--N/(g VSShet·d) and 0.02 g NO_3^--N/(g VSShet·d) respectively. Since raw municipal wastewater contains a low

concentration of readily biodegradable organics, specific denitrification rates are lower. These values are in accordance with other experimental results (Barker and Dold, 1996; Chuang *et al.*, 1996; Ostgaard *et al.*, 1997; Yu *et al.*, 2000) and quite higher than the ones determined by Pala and Bolukbas, 2005. Some researchers used sludge full of PHA from the anaerobic tank of a modified UCT system without external addition of organics, and thus obtained lower specific rates (Ostgaard *et al.*, 1997), whereas others determined extremely low values (Pala and Bolukbas, 2005), which they attribute to low denitrification capacity of their sludge. In comparison with other reported denitrification rates, our experimental results are within reported limits.

Results show that maximum denitrification activity is recorded in the first anoxic tank of the cascade in all experiments, which complies with steady-state experimental results in the same unit (Vai and Aiv, 2007). It has also been observed that denitrification velocity decreases from DN1 to DN3. This finding distinguishes DN1 tank from the rest, which may be attributed to the fact that DN1 contains return sludge from the clarifier with polyphosphate storage, mixed liquor coming from the anaerobic selector, which is rich in phosphates, and biomass from the anaerobic selector with PHA storage. As a result, under anoxic conditions and phosphorus abundance the following are in process: 1) simultaneous denitrification and phosphate uptake from PAOs that come from the anaerobic selector, 2) simultaneous organic substrate transformation and phosphate release from PAOS in return sludge, and 3) definitely carbon consumption and nitrite from ordinary heterotrophic organisms (OHOs). Thus, organics consumption should be attributed not only to OHOs denitrification, but also to PHA storage from PAOs. That is to say that nitrite consumption is accomplished both by ordinary heterotrophes and PAOs with simultaneous phosphate release.

In general, the values of the initial higher specific denitrification rate ($q_{DN,1}$) are of the same order of magnitude either when the substrate is synthetic or real wastewater. However, values of the following specific denitrification rate ($q_{DN,2}$) are by four times lower in the case of raw wastewater addition. A probable explanation could be the low content of raw wastewater in readily biodegradable substrates, which are consumed very fast, having as a consequence the transition of the microbial population to the endogenous respiration phase. On the other hand, the synthetic wastewater, except for the readily biodegradable substrates, such as acetic acid and KNO_3, consists of more complicate ones, e.g. peptone, which are taken up after their break-up /hydrolysis. However, the issue is not that clear, since PAOs' ability for denitrification is a fact, which means that whatever distinction of rates ($q_{DN,1}$, $q_{DN,2}$) according just to the substrate type (readily or not readily biodegradable) and without taking into account the intracellularly stored PHA is otiose (Kujawa and Klapwijk, 1999). This is to say that denitrification rates do not stand just for nitrate consumption by the ordinary heterotrophes, but they also include denitrification capacity of denitrifying PAOs with use of intracellular PHA, hydrolysis /break-up of macroaggregated compounds, the concentration and biodegradability of organic wastewater constituents.

A crucial parameter for immediate uptake of nitrate during denitrification is the abundance of organic substrate, which should overcome the minimum theoretical ratio of 3.4-3.7 g COD/g NO_3-$^-$N. In case that carbon concentration is just enough for denitrification, it will take longer for complete nitrate removal. Hence, in case of raw wastewater addition as carbon substrate, the C/N ratio obtained values close to the theoretical ratio and thus, in a short interval (0.5-1 hr) the microbial population entered the endogenous respiration phase and denitrification rate turned slow.

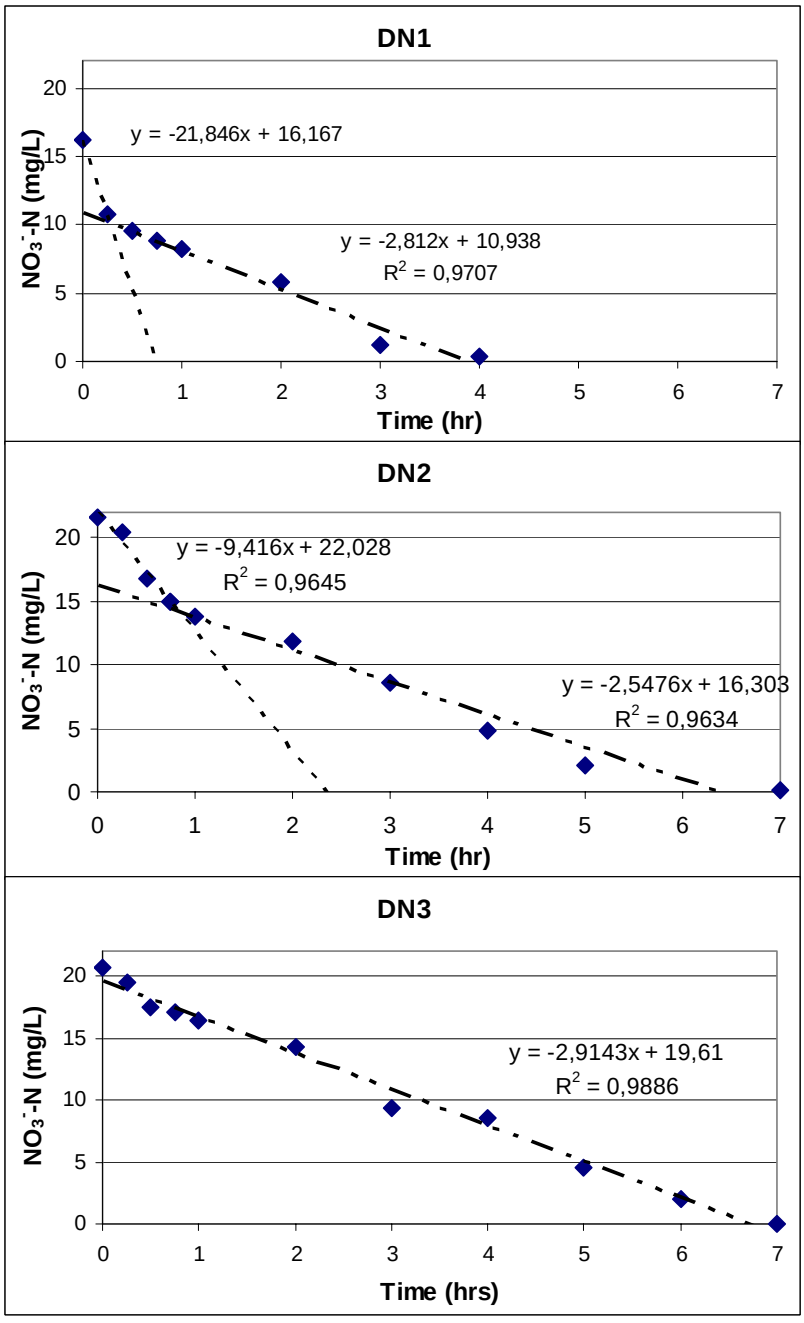

Figure 4. Real wastewater: Nitrate consumption in the three anoxic tanks.

The specific denitrification rate after synthetic wastewater addition is quite high and nitrate is quickly eliminated, which is attributed to the over-sufficiency of carbon substrate, e.g. C/N > 8 for experimental results shown in Figure 5. In this case of synthetic wastewater addition, the lower denitrification rate is absent.

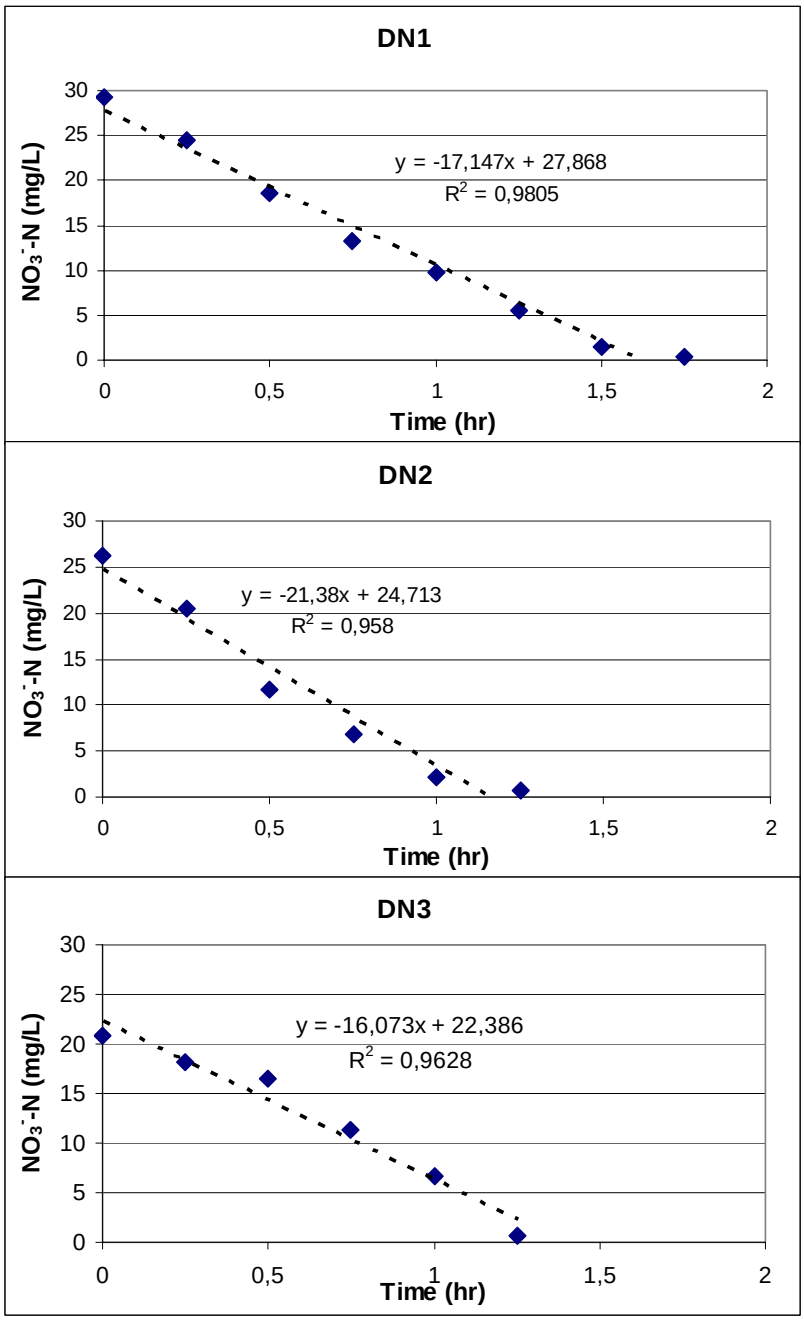

Figure 5. Synthetic wastewater: Nitrate consumption in the three anoxic tanks.

During denitrification phosphate concentration was fluctuating, whereas phosphorus was instantly released in nitrate absence. Specific phosphate release rates, phosphate profiles and maximum phosphate release were quite similar in all anoxic tanks.

Denitrifying phosphate accumulating organisms (DPAOs) was estimated at 25% (experiments not shown here).

3.3. Phosphorous release and uptake tests

Aeration was applied in the anaerobic selector of the pilot scale treatment plant having as a result phosphate uptake and polyphosphate intracellular storage into PAOs, whereas by transition to anaerobic conditions and acetate addition phosphate was released in the mixed liquor. Maintenance in anaerobic conditions lasted until phosphate was stabilized to a maximum as shown in Figure 6. The final phosphate concentration after anaerobic conditions establishment is higher than the initial one before aeration, which implies that longer residence times are possibly required in the anaerobic selector for complete phosphorus release. This value is also higher than the ones reported in studies conducting batch tests for sludge characterization from a biological nutrients removal plant (Pala and Bolukbas, 2005).

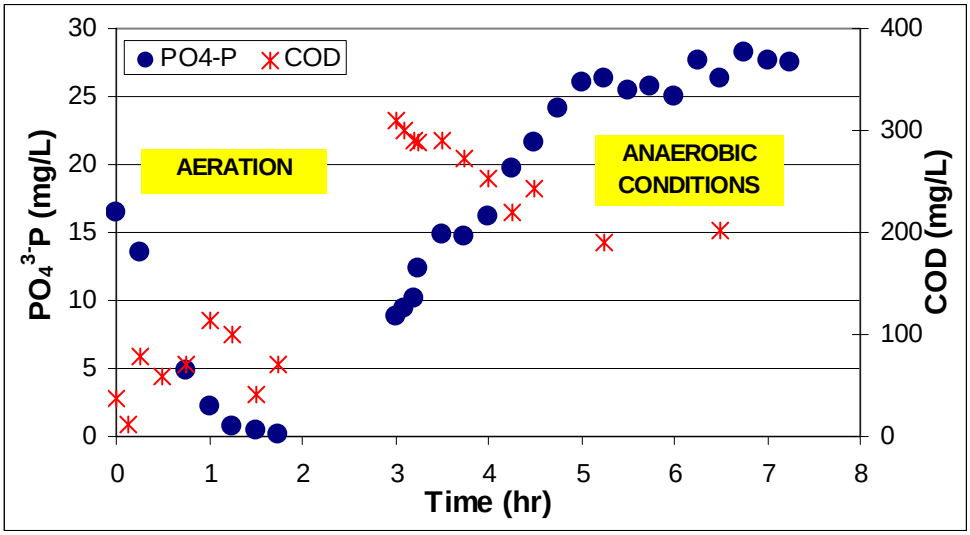

Figure 6. Aerobic uptake and anaerobic release of phosphate.

The specific anaerobic phosphorus release rate was determined averagely at 0.03 g PO_4^{3-}-P/(g VSS_{het}·d), which maybe higher than endogenous release rates of other research results (Ostggard *et al.*, 1997; Pala and Bolukbas, 2005), however, they are characterised as low. Sludge characterization results, which came from a SBR unit, are also on the same order of magnitude (Kargi *et al.*, 2005). The specific aerobic phosphorus uptake rate was estimated averagely at 0.05 g PO_4^{3-}-P/(g VSS_{het}·d). Although these values are also considered low, there are reports of low aerobic phosphorus uptake rates too (Brdjanovic *et al.*, 2000; Kargi *et al.*, 2005; Kerrn-Jespersen and Henze, 1993; Kuba *et al.*, 1997; Ostggard *et al.*, 1997; Pala and Bolukbas, 2005; Pauli and Kaitala, 1996).

4. Conclusions

The determination of kinetic parameters can be considered as a useful tool for the process design, operation and improvement of wastewater treatment plants. A semi-batch method for the determination of biokinetic parameters has been developed and applied in an EBPR system. Continuous feeding of the treating system was interrupted and instantly the pilot plant was turned into a batch mode operation. Thereafter, substrate was added and its consumption was monitored. All experiments were conducted in the actual environment of bacteria with no use of separate bench-scale reactors. Hence, the kinetic parameters determined by the proposed semi-batch method reflect the reality of the microbial activity in the treatment plant and allow actual (on-line) monitoring of the unit. Moreover, biokinetic parameters estimation experiments in modified UCT wastewater treatment plant provide information especially for complete understanding of the unit's properties.

The nitrification process was found to follow Monod type kinetics. The maximum specific nitrification rate, $q_{N,max}$, was determined to vary between 1.71 and 2.95 g NH_4^+-N/(g VSS_{aut}·d) for synthetic wastewater addition, whereas for real wastewater addition $q_{N,max}$ averaged at 1.14 g NH_4^+-Nr/(g VSS_{aut}·d). The half saturation constant for the nitrification process, $K_{m,N}$, was estimated graphically at an average value of 4.76 mg NH_4^+-N/L and $K_{m,N}$ at 1 mg NH_4^+-N/L for synthetic and real wastewater addition respectively. These values are low and show that the EBPR system configuration encourages growth of autotrophes with high affinity to the influent wastewater.

Denitrification experimental results indicated zero order kinetics. Two specific denitrification rates were determined in the anoxic tanks; an initial high one, $q_{DN,1}$ and a following low one, $q_{DN,2}$, until complete substrate consumption. Using synthetic wastewater $q_{DN,1}$ and $q_{DN,2}$ attained mean values of 0.12 and 0.06 g NO_3^--N/(g VSShet·d) respectively. When the added substrate was real wastewater, $q_{DN,1}$ and $q_{DN,2}$ were found to obtain mean values of 0.11 g NO_3^--N/(g VSShet·d) and 0.02 g NO_3^--N/(g VSShet·d) respectively. A crucial parameter for immediate uptake of nitrate during denitrification is the abundance of organic substrate. The specific denitrification rate after synthetic wastewater addition is quite high and nitrate is quickly eliminated, which is attributed to the over-sufficiency of carbon substrate. Another interesting point is that maximum denitrification activity is recorded in the first anoxic tank of the cascade in all experiments.

Aeration was implemented in the anaerobic tank to reach maximum phosphorus release. At this point and after transition to anaerobic conditions acetic acid was added to provide a readily biodegradable carbon source for phosphorus uptake. The specific anaerobic phosphorus release rate and specific aerobic phosphorus uptake rate was determined averagely at 0.03 g PO_4^{3-}-P/(g VSS_{het}·d) and 0.05 g PO_4^{3-}-P/(g VSS_{het}·d) respectively. Both are considered low, but comply with other reported values.

References

APHA/AWWA/WPCF. *Standard Methods for the Examination of Water and Wastewater*. 20th ed., American Public Health Association, Washington, USA (1998).

Barker, P.S. and Dold, P.L., (1996) *Wat. Res.*, 30(4), 769-780.

Beccari, M., Passino, R., Ramadori, R. and Tandoi, V., (1983) *J. Water Pollut. Control. Fed.*, 55, 58-64.

Brdjanovic, D., van Loosdrect, M.C.M., Versteeg, P., Hooijmans, C.M., Alaerts, G.J., Heijnen, J.J., (2000) *Wat. Res.*, 34(3), 846-858.

Chandran, K. and Smets, B.F., (2005) *Wat. Res.*, 35(13), 3153-3156.

Chuang, S.H., Ouyang, C.F., Wang, Y.B., (1996) *Wat. Res.*, 30(12), 2961- 2968.

Drtil, M., Nemeth, P., Bodik, I. (1993). *Wat. Res.*, 27(1), 35-39.

Elefsiniotis, P., Wareham, D.G., Smith, M.O., (2004) *J. of Biotech*.,114, 289-297.

Foglar, L. and Briski, F., (2003) *Proc. Biochem*., 39, 95-103.

Grady, L.C.P. Jr., Smets, B.F., Barbeau, D.S., (1996) *Wat. Res.*, 30(3), 742-748.

Grady, L.C.P. Jr. and Filipe, C.D.M., (2000) *Water, Air and Soil Pollution*, 123, 117-132.

Horan N.J. *Biological Wastewater Treatment Systems: Theory and Operation*. John Wiley & Sons, England (1996).

Kappeler, J. and Gujer, W., (1992) *Wat. Sc. Tech.*, 25(6), 125-139.

Kargi, F., Uygur, A., Baskaya, H.S., (2005) *J. of Env. Manag.*, 76, 71-75.

Kerrn-Jespersen J.P. and Henze M. (1993) *Wat. Res.*, 27(4), 617-624.

Kuba, T., van Loosdrect, M.C.M., Brandse, F.A., Heijnen, J.J., (1997) *Wat. Res.*, 31(4), 777-786.

Kujawa, K. and Klapwijk, B. (1999) *Wat. Res.*, 33(10), 2291-2300.

Li, Y., Gu, G., Zhao, J., Yu, H., (2001) *Process Biochemistry*, 37, 81-86.

Mino, T., van Loosdrecht, M.C.M., Heijnen, J.J., (1998) Wat. Res., 32(11), 3193-3207.

Mino, T. (2000) *Biochemistry (Moscow)*, 65(3), 405- 413.

Monod, J., (1949) *Ann. Rev. Microbiol.* 3, 371 -394.

Ostgaard, K., Christensson, M., Lie, E., Jonsson, K., Welander, T., (1997) *Wat. Res.*, 31(11), 2719-2726.

Pala, A., Bolukbas, O., (2005) *Process Biochemistr*y, 40, 629-635.

Pauli, A. S.-L. and Kaitala, S., (1996) *Biotechnol. Bioeng.*, 53, 304-309.

Seviour, R., Mino, T., Onuki, M. (2003) *FEMS Microbiology Reviews*, 27, 99-127.

Surmacz-Gorska, J., Gernaey, K., Demuynck, C., Vanrolleghem, P., Verstraete, W., (1996) *Wat. Res.*, 30(5), 1228-1236.

Tchobanoglous, G., *Wastewater Engineering: Treatment – Disposal – Reuse*, 3rd ed., Metcalf & Eddy, Inc.MacGraw – Hill, New York (1985).

Tchobanoglous, G., Burton, F.L., Stensel, D.H., *Wastewater Engineering: Treatment and Reuse*, 4th ed., Metcalf & Eddy, Inc., MacGraw – Hill, New York (2003).

Timmermans, P. and van Haute, A., (1983) *Wat. Res.*, 17(10), 1249-1255.

Vaiopoulou, E. and Aivasidis, A., (2007) *European Congress of Chemical Enginneering* (accepted)

Wagner, M., Loy, A., Nogueira, R., Purkhold, U., Lee, N., Daims, H. (2002) *Antonie van Leeuwenhoek*, 81, 665-680.

Wong, M.-T., Mino, T., Seviour, R.J., Onuki, M., Liu, W.-T. (2005) *Wat. Res.*, 39, 2901-2914.

Yu, R-F., Liaw, S-L., Cheng, W-Y., Chang, C-N., (2000) *J. of Envir. Eng.*, 126(10), 943-948.