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ORIGINAL PAPER

The role of biodegradable particulate and colloidal organic compounds in biological nutrient removal activated sludge systems

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Abstract The efficiency of denitrification and enhanced biological phosphorus removal in biological nutrient removal activated sludge systems is strongly dependent on the availability of appropriate carbon sources. Due to high costs of commercial compounds (such as methanol, ethanol, acetic acid, etc.) and acclimation periods (usually) required, the effective use of internal substrates is preferred. The aim of this study was to determine the effects of slowly biodegradable compounds (particulate and colloidal), as internal carbon sources, on denitrification, phosphate release/uptake and oxygen utilization for a full-scale process mixed liquor from two large wastewater treatment plants located in northern Poland. Since it is difficult to distinguish the effect of slowly biodegradable substrate in a direct way, a novel procedure was developed and implemented. Four types of one- and two-phase laboratory batch experiments were carried out in two parallel reactors with the settled wastewater without pre-treatment (reactor 1) and pre-treated with coagulation–flocculation (reactor 2). The removal of colloidal and particulate fractions resulted in the reduced process rates (except for phosphate release). The average reductions ranged from 13 % for the oxygen utilization rate during the second phase of a two-phase experiment (anaerobic/aerobic), up to 35 % for the nitrate utilization rate (NUR) during the second phase of a conventional NUR measurement.

Keywords Activated sludge · Denitrification · Biological phosphorus removal · Internal carbon source · Nitrogen removal · pH dynamics · Slowly biodegradable substrate · Wastewater fractionation

Introduction

In modern biological nutrient removal (BNR) activated sludge systems for combined N/P removal, sufficient amounts of appropriate biodegradable organic compounds (substrates) should be ensured to achieve simultaneously high efficiency of both denitrification and enhanced biological phosphorus removal (EBPR). The reported data on wastewater fractionation in various countries, summarized by Makinia (2010), indicated that a content of the biodegradable organic fraction in total COD was highly variable and ranged from 38 % (Zele, Belgium) to 91 % (Istambul, Turkey).

The biodegradable organic fraction is composed of readily (S_S) and slowly (X_S) biodegradable compounds, but only the former are immediately metabolized by heterotrophic microorganisms. In contrast to the S_S fraction, the actual role of slowly biodegradable compounds has not been ultimately recognized in terms of both theoretical aspects (mechanism of utilization) and practical aspects (influence on process performance). In the traditional opinion, these compounds become available for utilization by microorganisms after rapid enmeshment into sludge flocs and follow-up hydrolysis to the readily biodegradable substrate (Henze et al. 1987; Melcer et al. 2003; Uan et al. 2013). The latter process is accomplished by heterotrophic microorganisms, which release extracellular enzymes that break down and solubilize polymers, such as proteins, lipids and polysugars. Recent experimental observations indicate, however, that the removal of particles is not instantaneous and better described by flocculation–

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hydrolysis rather than a one-step hydrolysis (Jimenez et al. 2005). The latest reported full-scale data on the effects of particulate and colloidal substrate on process performance are ambiguous and suggest that different behaviours may result from complex interactions occurring in the activated sludge systems (van Veldhuizen et al. 1999; Tas et al. 2009; Tuncal et al. 2009; Puig et al. 2010).

A better understanding of the effect of biodegradable particulate and colloidal organic compounds, which are major components of the slowly biodegradable fraction, is important for optimal design and operation of BNR activated sludge systems. Conventional activated sludge systems have primarily been designed for removal of soluble organic compounds, and most of them have been equipped with quite big primary clarifiers in order to reduce the organic (BOD) load to the biological stage. This approach changed in the 1980s with the introduction of denitrification and EBPR, and higher requirements of organic compounds for those processes. The hydraulic retention time (HRT) of primary clarifiers was shortened resulting in lower efficiency of suspended solids removal (Siegrist et al. 2008). Recently, however, an opposite trend has been observed in WWTPs due to an interest in enhancing biogas production in anaerobic digesters. The effects of colloidal and particulate organic compounds can thus be important in terms of optimization of sedimentation and chemical precipitation in primary clarifiers, i.e. balancing between the efficiency of nutrient removal in the bioreactor and biogas production in the digester. Gori et al. (2011) concluded that an increase in the particulate COD removed in primary clarifiers would result in reduction in the energy demand for aeration in the bioreactor, and the associated CO_2 emissions from respiration (direct emission) and power generation (indirect emission). The authors noted, however, that "care must be used during process analysis since a fraction of the COD necessary for proper nutrient removal downstream may be incidentally removed".

There are no straightforward methods for measuring a slowly biodegradable fraction in wastewater. Melcer et al. (2003) proposed to estimate this fraction by subtracting other fractions (readily biodegradable and inert) from total COD:

$$X_S = \mathrm{COD} - S_S - S_I - X_I \qquad (1)$$

The deficiency of that approach is that no reliable method exists for estimating the inert particulate fraction (X_I).

Alternatively, in the Dutch STOWA guidelines for wastewater characterization (Roeleveld and van Loosdrecht 2002), the biodegradable fraction of the influent COD ($S_S + X_S$) is determined based on a BOD analysis (measuring the BOD as a function of time). Assuming that the S_S fraction is known, the X_S fraction can be determined from the following equation:

$$X_S = \frac{\frac{\mathrm{BOD}_5}{1-e^{-5k_{\mathrm{BOD}}}}}{1 - Y_{\mathrm{BOD}}} - S_S, \qquad (2)$$

where BOD_5—5-day biochemical oxygen demand, g BOD/m^3; k_{BOD}—BOD decay coefficient, d^{-1}; Y_{BOD}—yield coefficient for BOD.

However, due to the uncertainty of the BOD analysis, the X_S fraction should be further calibrated along with the X_I fraction. Meijer et al. (2001) demonstrated a proper way to evaluate the estimated X_S/X_I ratio using mass balances over the activated sludge system. Furthermore, the authors emphasized that a variation in the X_S/X_I ratio is very sensitive to almost every modelled process, especially the sludge production.

Since the concentration of the slowly biodegradable fraction is not directly available, it is consequently difficult to directly distinguish the effect of this fraction on major biochemical processes in the activated sludge process. For this purpose, Drewnowski and Makinia (2011) developed and implemented a novel procedure based on the standard batch experiments and pre-treatment of settled wastewater sample. In this follow-up study, these effects were comprehensively evaluated with respect to denitrification, phosphate release/uptake and oxygen utilization for a full-scale process mixed liquor originating from two large BNR WWTPs in northern Poland. Moreover, the pH behaviour during those experiments was investigated in more detail. The effect of pH on biochemical processes is normally related the specific enzymatic activity in a bacterial cell, energy required for the active transport of substrate across the cell and the concentration of substrate (and inhibitor).

Materials and methods

Origin of the process biomass and wastewater for laboratory experiments

The process mixed liquor, i.e. fresh returned activated sludge (RAS) as recommended by Brdjanovic et al. (2000), and settled wastewater originated from two large WWTPs located in the cities of Gdansk and Gdynia (northern Poland). The operating parameters and average composition of the primary and secondary effluents at both studied plants are summarized in Table 1.

The Wschod WWTP in Gdansk is one of the largest facilities located upon the Baltic Sea. During the time of the study (2007–2009), the average influent flow rate to the plant was approximately 82,000 m^3/d and the pollutant load corresponded to approximately 570,000 population

Table 1 Operating parameters and average composition of the primary and secondary effluents during three study sessions at the Wschod WWTP and two study sessions at the Debogorze WWTP (average values ± SD)

Parameter	Unit	"Wschod" WWTP (MUCT process)			"Debogorze" WWTP (JHB process)		
		Influent	Effluent	Bioreactor	Influent	Effluent	Bioreactor
Q	m^3/d	82,000			53,500		
PE	–	574,000			422,000		
SRT	d			21 (±2.9)			28 (±6.3)
MLSS	kg/m^3			5.45 (±0.56)			4.95 (±0.66)
Temperature	°C			11.8–20.5			15.5–17.9
COD	g COD/m^3	626 (±82)	48 (±4.2)		901 (±52)	25.8 (±5.3)	
Soluble COD	g COD/m^3	194 (±38)					
BOD$_5$	g BOD/m^3	298 (±35)	6.7 (±2.1)		301 (±42)	4.2 (±0.63)	
Total P	g P/m^3	14.9 (±2.6)	0.60 (±0.10)		12.5 (±1.6)	0.50 (±0.15)	
Total N	g N/m^3	81.2 (±5.0)	11.1 (±1.1)		90.2 (±7.0)	8.77 (±0.7)	
NH$_4$–N	g N/m^3	58.9 (±3.4)	1.20 (±0.75)		67.5 (±4.6)	0.98 (±0.27)	
NO$_3$–N	g N/m^3		7.4 (±0.64)			5.9 (±0.53)	
TSS	g/m^3	288 (±31)	12.6 (±3.5)		260 (±28)	12.4 (±1.2)	
VSS	g/m^3	180 (±21)	5.8 (±2.8)		198 (±16)	5.3 (±0.4)	

equivalents (PE). The biological step consisted of six parallel bioreactors, designed according to the Modified University of Cape Town (MUCT) process configuration, and twelve circular secondary clarifiers.

The average influent flow rate to the Debogorze WWTP in Gdynia was approximately 53,500 m^3/d in 2009–2010 (study period), and the pollutant load corresponded to approximately 420,000 PE. The biological step, upgraded in 2009 (3 months before starting the experiments), was designed according to the Johannesburg (JHB) process configuration with a carrousel system in the last compartment for simultaneous nitrification/denitrification. The new biological step consists of 10 bioreactors and 8 secondary clarifiers.

For the laboratory experiments, samples of the process mixed liquor and settled wastewater were collected from the Wschod WWTP during three study periods (termed winter, summer and spring) between December 2007 and May 2009. The samples from the Debogorze WWTP were collected during two (fall and spring) study periods between September 2009 and May 2010. The average daily (24-h) time-proportional samples of the settled wastewater (after mechanical treatment) were collected at both studied plants.

Preparation of wastewater samples

Since it is difficult to determine the effects of slowly biodegradable substrate in a direct way, a novel laboratory procedure was developed based on the measurements conducted in two parallel batch reactors (Fig. 1). In reactor 1, the process biomass was mixed with the settled wastewater without further pre-treatment (containing soluble, colloidal and particulate organic fractions), whereas the supernatant of the settled wastewater after coagulation–flocculation was used in reactor 2. A similar concept was earlier proposed by Goel et al. (1999) to separate hydrolysis from storage by performing and analysing two parallel OUR measurements: one with filtered wastewater (including only soluble COD) and the other with non-filtered wastewater (including total COD).

The wastewater sample in reactor 2, containing only a soluble organic fraction, was prepared according to the rapid physical–chemical method of Mamais et al. (1993). That method is based on precipitation with ZnSO$_4$ at pH 10.5, which removes the colloidal particles leaving only truly soluble organic matter in the supernatant. After removing colloids and particulates, the pH was adjusted to its original value by adding 6 M HCl. This kind of pre-treatment also resulted in precipitation of phosphate so the initial PO$_4$–P concentrations in reactor 2 always decreased in comparison with reactor 1.

It should be noted that Mamais et al. (1993) justified the rationale for their coagulation–flocculation method by the fact that readily biodegradable organic matter consists of simple molecules such as VFAs and low molecular weight carbohydrates that pass through the cell membrane and are metabolized immediately. Hu et al. (2002b) indeed confirmed that the COD quantified with this method corresponded closely with the low (<1 kDa) molecular weight

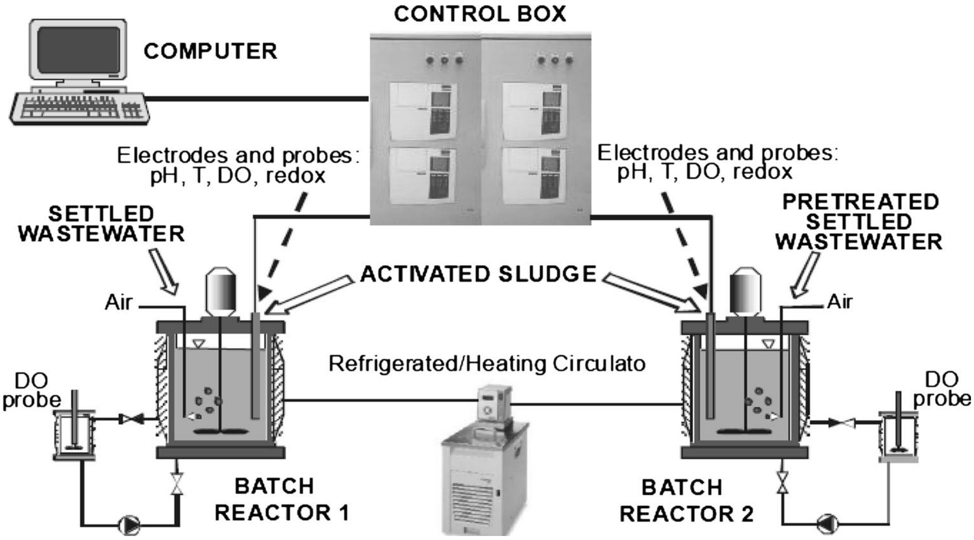

Fig. 1 Diagram illustrating the experimental apparatus and procedure

fraction, but that finding remains in contradiction to the observations from a few other studies with municipal wastewater (Makinia 2010).

Laboratory apparatus

The laboratory experiments were carried out in an experimental apparatus consisting of two parallel, plexiglass batch reactors (max. volume of 4.0 dm^3) and automated control system for temperature and dissolved oxygen (DO) concentration (Drewnowski and Makinia, 2011). The reactors were equipped with probes/electrodes (WTW GmbH, Germany) for continuous monitoring of pH (SenTix 21), oxidation–reduction potential (ORP) (SenTix ORP), temperature and DO concentration (CellOx 325). The reactor content was mixed with mechanical stirrers at the velocity of 180 rpm.

In addition, a small chamber for cyclic measurements of oxygen utilization rates (OURs) was connected with the main reactor. The OUR measurement was automatically controlled and one cycle (3.5 min) consisted of the following phases: emptying, rinsing, filling and measuring (3 min). The oxygen depletion in the OUR chamber was measured using a DO probe with a stirrer (WTW Stirrox G).

Experimental procedures

The laboratory experiments were carried out at the current process temperatures (12–21 °C) in the full-scale bioreactors at the studied WWTPs. The aim was to determine the kinetics of some major biochemical processes in BNR activated sludge systems, including nitrate utilization rates (NURs), phosphate release/uptake rates (PRR/PURs), OURs and ammonia utilization rates (AURs). In each study period, batch tests were carried out in duplicate for four different types of measurements described below.

Conventional NUR measurement

The fresh RAS was diluted with the settled wastewater without pre-treatment (reactor 1) and pre-treated with coagulation–flocculation (reactor 2) to obtain 4.0 dm^3 of mixed liquor in each reactor. The dilution rate was adjusted to obtain the mixed liquor suspended solids (MLSS) concentration at approximately 2–3 kg/m^3, but the actual MLSS and mixed liquor volatile suspended solids (MLVSS) concentrations in both reactors were measured at the beginning of each test. After adding the mixture to each reactor, the mixer, cooling system for the water coat and on-line monitoring system were turned on. Nitrate (KNO_3) was injected to increase the initial NO_3–N concentration to approximately 20 g N/m^3. The measurement was conducted for 4 h, and samples of the mixed liquor ($V = 50$ cm^3) were withdrawn with the frequency of 10–30 min, filtered under vacuum pressure on the Whatman GF/C filter and analysed for NO_3–N, PO_4–P and COD.

Anaerobic PRR and anoxic/aerobic PUR measurement

The measurement was conducted for 6.5 h and comprised two phases: anaerobic (2.5 h) followed by anoxic or aerobic (4 h). Such a sequence better reflects the actual conditions occurring in the activated sludge systems performing EBPR. The mixed liquor in the two parallel reactors was prepared in a similar way to the conventional NUR measurement but, in order to maintain anaerobic conditions, no nitrate was added. Nitrate (KNO_3) was injected at the beginning of the anoxic phase (in the anoxic PUR test) to increase the NO_3–N concentration by approximately 20 g N/m^3. In the aerobic PUR test, the aeration system was turned on at the beginning of the aerobic phase and the OUR measurement was initiated. The DO set point was kept at 6 g O_2/m^3 to avoid the effect of oxygen limitation. In each experiment, samples of the mixed liquor ($V = 50$ cm^3) were withdrawn with the frequency of 10–30 min, filtered under vacuum pressure on the Whatman GF/C filter and analysed for PO_4–P and COD (anaerobic phase), and PO_4–P, COD and NO_3–N/NH_4–N (anoxic/aerobic phase).

Conventional OUR measurement

This additional experiment under aerobic conditions was carried out to verify the yield coefficient, Y_H, in comparison with its theoretical value. An hour before starting the measurement, nitrification inhibitor [allylthiourea (ATU)] was added to the RAS in the amount of 10 mg/dm^3. The mixed liquor in the two parallel reactors was prepared in a similar way to the conventional NUR measurement but without adding nitrate. After placing the mixed liquor in the reactors, the automated OUR measurement was initiated and the DO set point was kept at 6 g O_2/m^3. The measurement was conducted for 6 h. Samples of the mixed liquor ($V = 50$ cm^3) were withdrawn with the frequency of 10–60 min, filtered under vacuum pressure on the Whatman GF/C filter and analysed for COD. Based on the measurements of total OUR ($OUR_{tot}(t)$), endogenous OUR ($OUR_{end}(t)$) and degraded COD ($COD_{degr.}$), the Y_H coefficient was determined from the following equation (Ekama et al. 1986; Kappeler and Gujer 1992; Orhon et al. 1994):

$$Y_H = \frac{COD_{degr.} - \int (OUR_{tot}(t) - OUR_{end}(t))dt}{COD_{degr.}} \quad (3)$$

Analytical methods

Total and volatile suspended solids were measured by the gravimetric method according to the Polish Standards (PN-72/C-04559). The total and soluble COD, PO_4–P, NO_3–N and NH_4–N were analysed by Hach–Lange "test-in-tube" using a Xion 500 spectrophotometer (Hach–Lange GmbH, Germany). The analytical procedures, which were adapted by Hach–Lange GmbH (Germany), followed the Standard Methods (APHA 1992).

Results and discussion

Characteristics of the wastewater samples

At the Wschod WWTP, the soluble fraction accounted for 19–39 % of total COD in 24 wastewater samples used in the experiments in reactor 2 (Fig. 2a). The average value of total COD was 627 (±81) g COD/m^3 including soluble COD = 194 (±38) g COD/m^3 versus non-soluble (colloidal and particulate) COD = 433 (±73) g COD/m^3. For comparison, the average values of total and soluble COD determined using the same method of Mamais et al. (1993) from the annual routine operating data were slightly deviating from the above concentrations, i.e. 594 and 172 g COD/m^3 (2007), and 715 and 192 g COD/m^3 (2008). At the Debogorze WWTP, the soluble fraction accounted for 23–46 % of total COD in 16 samples of the settled wastewater (Fig. 2b). The average value of total COD was 533 (±86) g COD/m^3 including soluble COD = 211 (±32) g COD/m^3 versus non-soluble COD = 322 (±95) g COD/m^3.

In the earlier modelling study at these two plants (Makinia, 2006), similar measurements were carried out at both plants and those data are also shown in Fig. 2a–b. The estimated ratio of biodegradable to non-biodegradable particulate (and colloidal) organic fractions varied in the range of 1.8–2.5 (Wschod) and 1.4–1.5 (Debogorze) to fit the waste activated sludge (WAS) production. As a consequence, the ratios of [$S_S/(S_S + X_S)$] at the Wschod WWTP (0.32–0.40) fitted well into a typical range of 0.3–0.5 (Sahlstedt et al. 2003), whereas the corresponding values at the Debogorze WWTP (0.50–0.54) slightly exceeded that range. Furthermore, Pagilla et al. (2008) reported results of the COD distribution in the primary effluents of both plants based on the filtration on different pore size filters. The dominant fraction originated from particulate organic compounds (>1.2 μm), which constituted 67 and 75 % of COD, respectively, at the Wschod and Debogorze WWTPs (the latter plant was before the upgrade).

Batch test results

The ranges of all the observed specific rates, including NURs, PRRs, anoxic/aerobic PURs and OURs, in the batch experiments with the settled wastewater without pre-treatment and after coagulation–flocculation are listed in Table 2 (Wschod) and Table 3 (Debogorze). The obtained kinetic results are discussed in the following sections. Results of similar experiments with the settled wastewater, carried out earlier by Makinia (2006) at the same plants, were used for comparison.

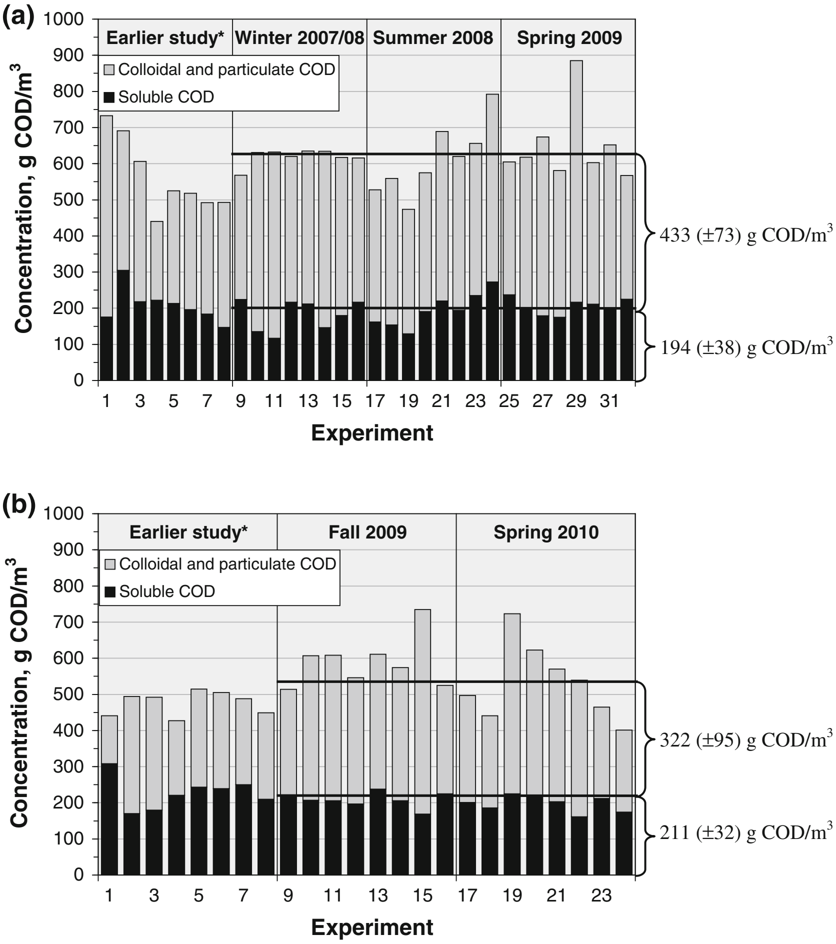

Fig. 2 Measured fractions of COD (soluble vs. colloidal and particulate) in the 24-h settled wastewater samples from **a** the Wschod WWTP and **b** the Debogorze WWTP [earlier study*—adapted from Makinia (2006)]

Conventional NUR tests

Figure 3a–d illustrates sample results of the conventional NUR experiments carried out at the two studied plants. In the experiments with the settled wastewater without the pre-treatment, the observed double rates (NUR1 and NUR2) were associated with utilization of soluble, readily biodegradable organic compounds (only NUR1), and slowly biodegradable (soluble, colloidal and particulate) organic compounds (both NUR1 and NUR2). During all the study periods, the average NUR1 values varied in the range of 3.2–5.3 and 3.2–3.6 mg N/(g VSS h), respectively, at the Wschod and Debogorze WWTPs. The observed higher rates at lower temperatures can be attributed to a different composition of the activated sludge biomass (e.g. a higher content of denitrifying heterotrophs) and/or influent wastewater characteristics (e.g. a higher content of readily biodegradable compounds). The calculated ΔCOD:ΔN ratios associated with the NUR1 ranged from 5.1 to 10.4 (Wschod) and 6.4 to 8.1 (Debogorze). The average NUR2 values in the corresponding experiments varied in similar ranges, i.e. 1.5–1.8 mg N/(g VSS h) (Wschod) and 1.7–2.0 mg N/(g VSS h) (Debogorze). The NUR1 and NUR2 values observed at both studied plants fit into the ranges reported by Naidoo et al. (1998) for similar experiments at eight municipal WWTPs in Europe. In that study, the NURs associated with utilization of the readily biodegradable and slowly biodegradable substrates remained in the range of 3.3–5.7 and 1.6–3.6 mg N/(g VSS h), respectively.

When the pre-treated samples of wastewater were used in the experiments, the observed lower NURs were associated with utilization of the remaining soluble fraction. The NUR1 values for the pre-treated wastewater varied in the range 2.4–3.4 g N/(kg VSS h) (Wschod) and 2.7–2.9 g N/(kg VSS h) (Debogorze), whereas the NUR2 values in the corresponding experiments were 1.0–1.2 g N/(kg VSS h) (Wschod) and 1.1–1.2 g N/(kg VSS h) (Debogorze). The calculated ΔCOD:ΔN ratios associated with the NUR1 ranged from 5.5 to 8.5 (Wschod) and 7.3 to 8.6 (Debogorze). Furthermore, the

Table 2 Average values (±standard deviations) of the specific process rates observed during the batch experiments with the settled wastewater without pre-treatment and pre-treated with coagulation–flocculation at the Wschod WWTP

Process	Unit	Winter session	(11.8–14.2 °C)	Spring session	(14.8–16.7 °C)	Summer session	(18.9–20.5 °C)
		Settled wastewater	Pre-treated settled wastewater	Settled wastewater	Pre-treated settled wastewater	Settled wastewater	Pre-treated settled wastewater
NUR1	mg N/gVSS h	4.4 (±0.92)	3.4 (±0.92)	5.3 (±0.14)	3.3 (±0.21)	3.2 (±0.35)	2.4 (±0.49)
NUR2	mg N/gVSS h	1.5 (±0.07)	1.1 (±0.07)	1.8 (±1.13)	1.2 (±0.28)	1.7 (±0.07)	1.0 (±0.01)
PRR	mg P/gVSS h	10.5 (±1.07)	8.1 (±2.28)	11.2 (±0.67)	13.1 (±0.91)	11.0 (±1.56)	10.2 (±2.56)
PUR_{Anoxic}	mg P/gVSS h	4.7 (±0.35)	1.2 (±0.07)	6.8 (±0.78)	6.7 (±1.20)	5.7 (±1.48)	3.4 (±1.27)
NUR	mg N/gVSS h	1.8 (±0.21)	1.0 (±0.35)	3.0 (±0.71)	2.2 (±0.35)	2.3 (±0.07)	1.8 (±0.01)
$PUR_{Aerobic}$	mg P/gVSS h	8.6 (±1.41)	4.0 (±2.33)	11.4 (±0.07)	11.0 (±0.57)	6.1 (±0.42)	3.8 (±1.41)
OUR_{Max}	mg O_2/gVSS h	22.1 (±0.07)	18.3 (±0.07)	26.8 (±1.41)	25.9 (±0.42)	32.6 (±1.13)	29.7 (±1.89)
AUR	mg N/gVSS h	2.5 (±0.57)	2.3 (±0.71)	3.5 (±0.21)	3.5 (±0.01)	3.9 (±0.35)	3.8 (±0.78)
OUR_{Max}	mg O_2/gVSS h	22.8 (±7.35)	16.9 (±7.99)	28.0 (±4.95)	20.8 (±6.51)	39.5 (±10.32)	27.8 (±7.99)
OUR_{Endog}	mg O_2/gVSS h	7.1 (±4.25)	4.95 (±2.76)	8.1 (±0.85)	5.7 (±0.14)	10.6 (±0.71)	6.6 (±1.27)
Y_H	g COD/g COD	0.65 (±0.06)	0.66 (±0.04)	0.66 (±0.01)	0.66 (±0.05)	0.67 (±0.05)	0.62 (±0.06)

Table 3 Average values (±standard deviations) of the specific process rates observed during the batch experiments with the settled wastewater without pre-treatment and after coagulation–flocculation at the Debogorze WWTP

Process	Unit	Fall session	(16.0–17.8 °C)	Spring session	(15.4–16.2 °C)
		Settled wastewater	Pre-treated settled wastewater	Settled wastewater	Pre-treated settled wastewater
NUR1	mg N/gVSS h	3.6 (±0.64)	2.7 (±0.07)	3.2 (±0.57)	2.9 (±0.35)
NUR2	mg N/gVSS h	1.7 (±0.07)	1.1 (±0.21)	2.0 (±0.35)	1.2 (±0.07)
PRR	mg P/gVSS h	9.6 (±0.44)	11.2 (±0.76)	7.9 (±0.85)	8.7 (±1.52)
PUR_{Anoxic}	mg P/gVSS h	2.0 (±0.07)	1.2 (±0.49)	1.8 (±0.14)	1.7 (±0.01)
NUR	mg N/gVSS h	1.8 (±0.42)	1.3 (±0.14)	2.3 (±0.49)	1.6 (±0.28)
$PUR_{Aerobic}$	mg P/gVSS h	5.9 (±1.06)	4.9 (±0.71)	5.6 (±0.35)	5.0 (±1.06)
OUR_{Max}	mg O_2/gVSS h	24.9 (±0.99)	21.8 (±1.34)	24.5 (±3.75)	19.1 (±1.70)
AUR	mg N/gVSS h	2.9 (±0.21)	2.9 (±0.14)	2.8 (±0.07)	3.1 (±0.07)
OUR_{Max}	mg O_2/gVSS h	20.4 (±1.98)	16.9 (±1.30)	21.8 (±3.46)	17.1 (±0.99)
OUR_{Endog}	mg O_2/gVSS h	7.6 (±2.67)	5.2 (±2.73)	8.1 (±1.38)	6.1 (±1.09)
Y_H	g COD/g COD	0.69 (±0.01)	0.71 (±0.05)	0.68 (±0.02)	0.69 (±0.07)

removal of colloidal and particulate organic compounds resulted in decreasing the overall efficiency of NO_3–N removal during the 4-h experiments by 21–37 and 24–28 %, respectively, at the Wschod and Debogorze WWTPs.

In addition to nitrate and COD, the behaviour of phosphate was also investigated during the experiments. At the beginning of the experiments, phosphate was released despite high concentrations of nitrate (in the range of approximately 10–20 g N/m^3) which, according the traditional opinion, inhibit that process (P release). The release continued until the readily biodegradable substrate was present in the solution (approximately 0.5–2.5 h) depending on the study period (temperature), biomass and initial COD concentrations in the batch reactors. A very similar behaviour of phosphate was observed by Brdjanovic et al. (2000) in the conventional NUR test with a full-scale plant mixed liquor and acetate as a carbon source. In that study, a significant amount of phosphate was released (from approximately 2 g P/m^3 to almost 10 g P/m^3) during the first hour of the test until a complete consumption of acetate. Simultaneously, the NO_3–N concentration decreased from approximately 25 g to 10 g N/m^3. These observations are in accordance with the conclusion of Yuan and Oleszkiwicz (2008) that phosphate release continues as long as the substrate is

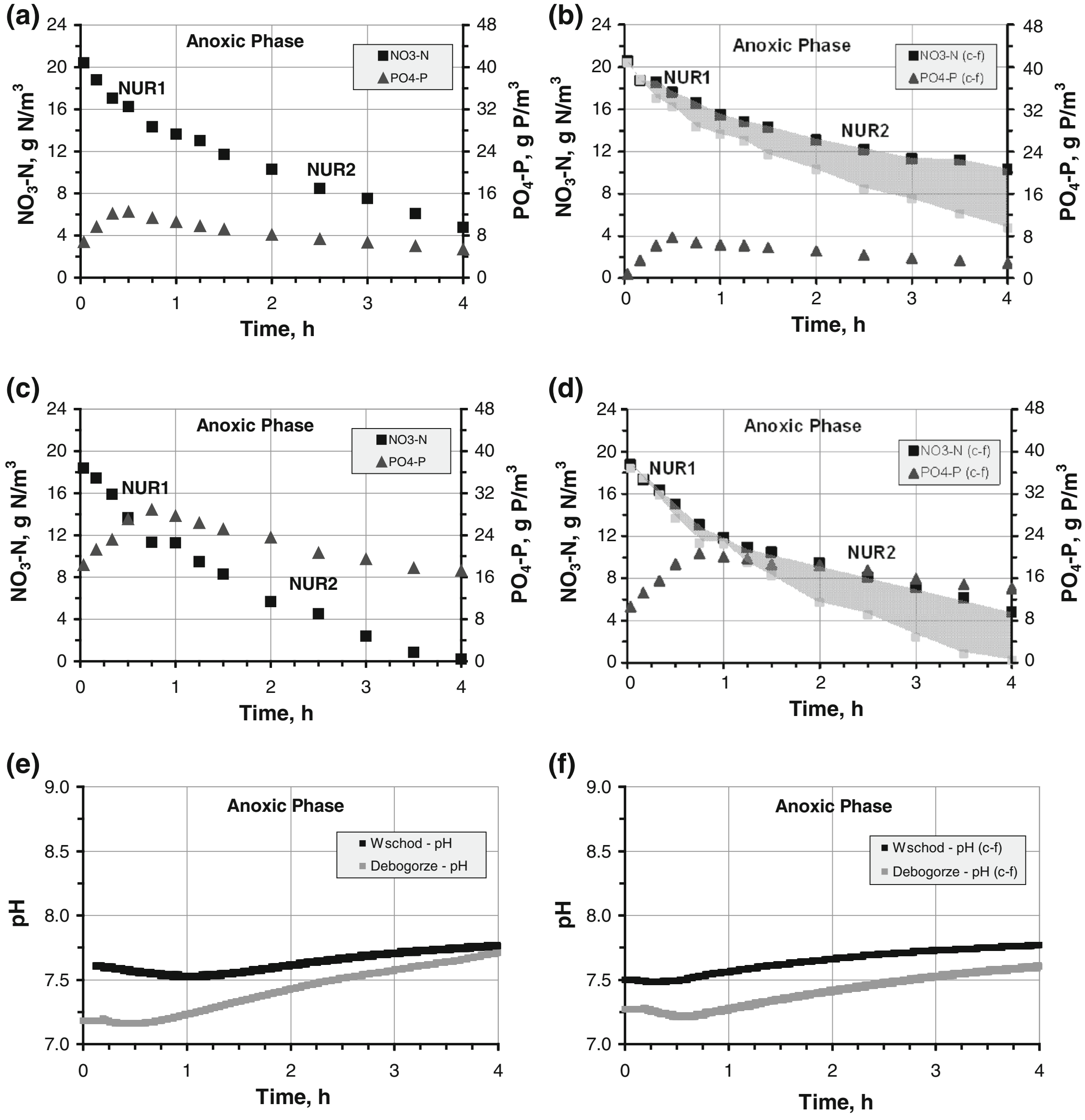

Fig. 3 Sample results of the conventional NUR measurement in two parallel reactors during the summer study period at the Wschod WWTP ($T = 20.1$ °C): **a** with the settled wastewater without pre-treatment (MLVSS = 1.8 kg/m^3), **b** with the settled wastewater after coagulation–flocculation (MLVSS = 1.7 kg/m^3), and during the fall study period at the Debogorze WWTP ($T = 17.4$ °C): **c** with the settled wastewater without pre-treatment (MLVSS = 2.5 kg/m^3), **d** with the settled wastewater after coagulation–flocculation (MLVSS = 2.6 kg/m^3), and pH variations at both studied plants: **e** with the settled wastewater without pre-treatment, **f** with the settled wastewater after coagulation–flocculation

present regardless of NO_3–N concentrations in the "anaerobic" phase.

PRR and anoxic/aerobic PUR tests

Sample results of the combined anoxic and aerobic PUR tests are shown in Figs. 4a–d and 5a–d, respectively. Both figures also illustrate sample results of the preceding PRR experiments carried out at the two studied plants. In the experiments with the settled wastewater, the maximum PRR values ranged from 10.5 to 11.2 mg P/(g VSS h) at the Wschod WWTP and 7.9 to 9.6 mg P/(g VSS h) at the Debogorze WWTP. At the former plant, the lowest PRRs were obtained in the winter study session ($T = 11.8$–14.2 °C), while the rates in the spring and summer study periods ($T = 14.8$–16.7 °C vs. $T = 18.9$–20.5 °C) were comparable. These results are thus ambiguous with respect to the effect of temperature on the kinetics of phosphate

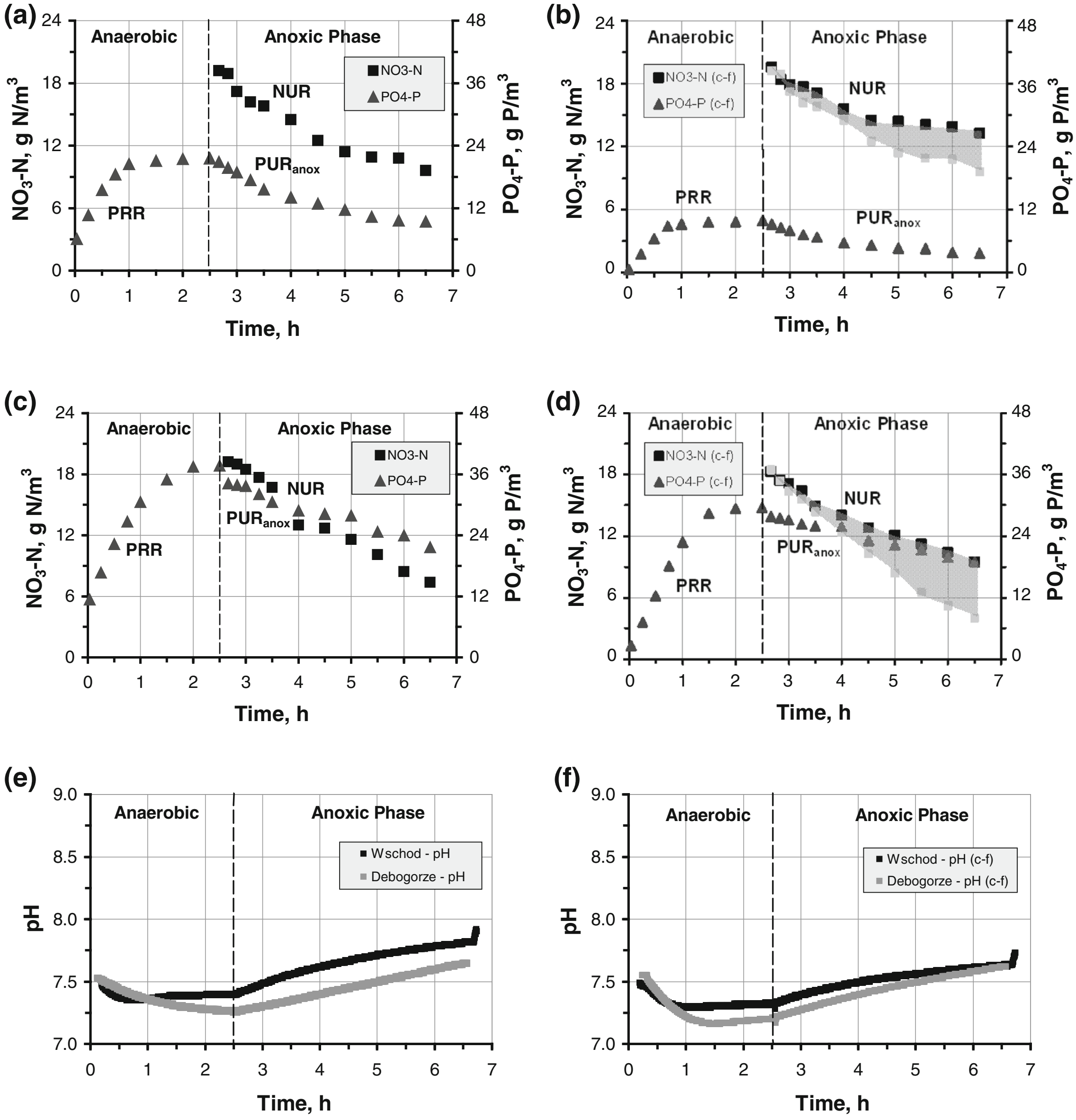

Fig. 4 Sample results of the PRR and anoxic PUR measurement in two parallel reactors during the summer study period at the Wschod WWTP ($T = 20.0$ °C): **a** with the settled wastewater without pre-treatment (MLVSS = 1.5 kg/m^3), **b** with the settled wastewater after coagulation–flocculation (MLVSS = 1.4 kg/m^3), and during the fall study period at the Debogorze WWTP ($T = 16.2$ °C): **c** with the settled wastewater without pre-treatment (MLVSS = 2.4 kg/m^3), **d** with the settled wastewater after coagulation–flocculation (MLVSS = 2.1 kg/m^3), and pH variations at both studied plants: **e** with the settled wastewater without pre-treatment, **f** with the settled wastewater after coagulation–flocculation

release. Also coagulation–flocculation had no explicit effect on the PRRs as the observed rates in the parallel reactors varied in the range of approximately ±20 %. Furthermore, the average amounts of P released per MLVSS were similar in both reactors, i.e. 13.7 versus 14.0 g P/g MLVSS (Wschod) and 10.8 versus 11.3 g P/g MLVSS (Debogorze). The stored P was not completely released at the end of the anaerobic phase as in the earlier study (Makinia 2006), and the amounts of P released in the presence of acetate were higher by 40–70 % compared to the experiments with the settled wastewater. It thus appears that anaerobic hydrolysis of slowly biodegradable organic compounds generates relatively insignificant amounts of the substrate for maintenance of PAOs under anaerobic conditions. Indeed, the estimated correction factor for anaerobic hydrolysis (η_f) was only 0.1 (Makinia 2006).

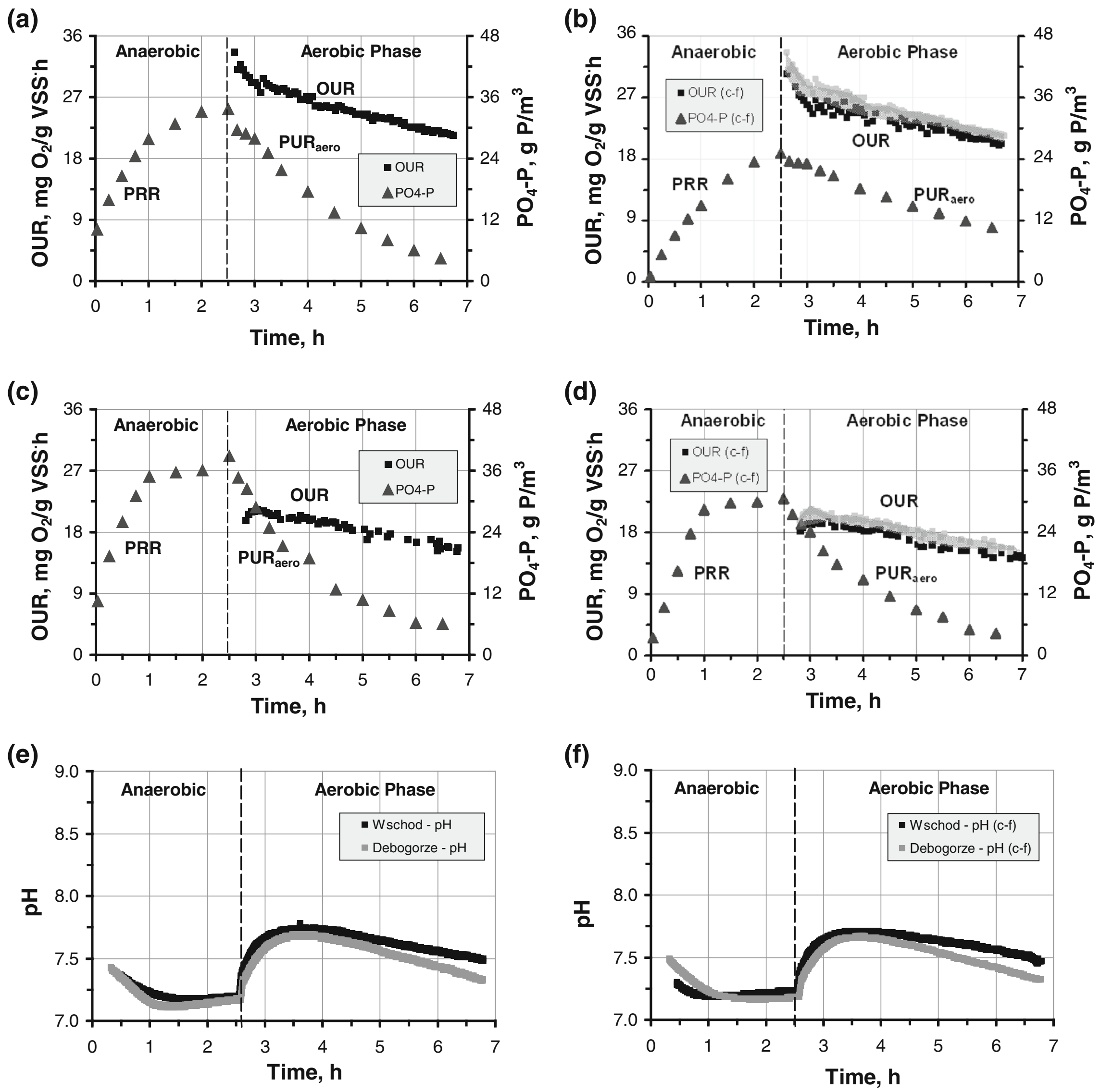

Fig. 5 Sample results of the PRR and aerobic PUR measurement in two parallel reactors during the summer study period at the Wschod WWTP ($T = 18.9$ °C): **a** with the settled wastewater without pre-treatment (MLVSS = 2.2 kg/m^3), **b** with the settled wastewater after coagulation–flocculation (MLVSS = 1.9 kg/m^3), and during the fall study period at the Debogorze WWTP ($T = 15.9$ °C): **c** with the settled wastewater without pre-treatment (MLVSS = 2.7 kg/m^3), **d** with the settled wastewater after coagulation–flocculation (MLVSS = 2.6 kg/m^3), and pH variations at both studied plants, **e** with the settled wastewater without pre-treatment, **f** with the settled wastewater after coagulation–flocculation

The PRRs observed at both plants remain in the range of literature data [4.4–18.8 mg P/(g VSS h)] reported in similar experiments carried out with the mixed liquor from full-scale BNR systems (Kuba et al. 1997; Sorm et al. 1998; Tuncal et al. 2009; Puig et al. 2010) and full-scale pilot plants performing EBPR (Petersen et al. 1998; Tykesson et al. 2002). In the study of Tuncal et al. (2009), the PRRs were 10.8 and 16.2 mg P/(g VSS h), respectively, in the line with and without primary clarification. Puig et al. (2010) found the opposite effect of by-passing primary clarifiers. The observed PRR decreased from 18.8 mg P/(g VSS h) during the standard operation to 13.6 mg P/(g VSS h) during by-passing the primary clarifiers.

The average ratio of PO$_4$–P released to COD utilized (Y_{PO4}), measured during the initial 60 min of the anaerobic phase, was similar in both reactors and varied in the range of 0.29–0.35 g P/g COD (Wschod) and 0.34–0.59 g P/g COD (Debogorze). These values are only slightly different from a typical range (0.35–0.5) reported for activated sludge systems. Lower Y_{PO4} can be justified by either long

SRTs or the presence of glycogen-accumulating organisms (GAOs) (Brdjanovic et al. 2000). Furthermore, the type of substrate is another factor influencing Y_{PO4}. In the earlier study (Makinia 2006), the ratios for acetate were significantly higher (by approximately 35–40 %) in comparison with the settled wastewater. Also, Ubukata (2005) found that the organic compounds present in real wastewater contribute to a different phosphate release than acetate.

The maximum PURs observed at both plants under aerobic conditions (during the initial 60 min of the test) varied in the ranges of 6.1–11.4 and 5.6–5.9 mg P/(g VSS h) in the settled wastewater without pre-treatment versus 3.8–11.0 and 4.9–5.0 mg P/(g VSS h) in the wastewater after coagulation–flocculation, respectively, at the Wschod WWTP (Table 2) and Debogorze WWTP (Table 3). The anoxic PURs were significantly lower, i.e. 4.7–6.8 and 1.8–2.0 mg P/(g VSS h) in the settled wastewater without pre-treatment versus 1.2–6.7 and 1.2–1.7 mg P/(g VSS h) in the wastewater after coagulation–flocculation. The anoxic PURs correlated strongly ($r^2 = 0.96$–0.99) with the NURs measured during the parallel experiments with the settled wastewater (Tables 2, 3). The calculated ratios of utilized nitrate and phosphate varied in the range of 0.51–0.78 and 0.74–1.41 g N/g P in the settled wastewater without pre-treatment versus 0.47–1.74 and 0.83–1.01 g N/g P in the wastewater after coagulation–flocculation, respectively, at the Wschod WWTP and the Debogorze WWTP. For comparison, Tuncal et al. (2009) found lower N/P ratios which were 0.4 and 0.5 g N/g P, respectively, in the line without and with primary clarification.

The capabilities of denitrifying PAOs cannot be significant at both studied plants in terms of contribution to the overall utilization of nitrate. Makinia (2006) found at both plants that the denitrification rates associated with the anoxic storage of polyphosphate and the anoxic growth of PAOs constituted 16–21 % of the denitrification rates associated with the anoxic activity of "ordinary" heterotrophs. This range was very close to the earlier findings of Hu et al. (2002a) that the specific denitrification rate of PAOs on internally stored PHB was only about 20 % of the rate of the "ordinary" heterotrophs on slowly biodegradable COD.

For comparison, the anoxic and aerobic PURs reported for full-scale BNR activated sludge systems varied in the range of 1.9–13 and 3.6–30 mg P/(g VSS h), respectively (Kuba et al. 1997; Sorm et al. 1998; Tuncal et al. 2009; Puig et al. 2010). In the studies of Tuncal et al. (2009) and Puig et al. (2010), the observed anoxic and aerobic PURs were approximately equal; however, the effects of primary clarification were opposite in those studies. In the former study, a significant increase in the PURs (by 70 %) was observed in the line without primary clarification compared to the line with primary clarification, whereas by-passing raw wastewater to the biological stage in the latter study resulted in a small decrease in the PURs (by 15 %), likely due to the shorter SRT and higher inert fraction in the sludge.

In the two-phase experiments with the pre-treated wastewater, the NURs decreased by approximately 22–44 % (anoxic phase), while only 3–22 % reductions were observed with respect to the OUR measurements (aerobic phase). The lower reductions in the aerobic phase resulted from an equal effect of nitrification in both reactors (that process was not affected by the removal of particulates and colloids) (Tables 2, 3). For comparison, Tuncal et al. (2009) reported a similar (25 %) reduction for the NUR measured in the line with primary clarification compared to the line without primary clarification [1.8 vs. 2.4 mg N/(g VSS h)].

Conventional OUR measurements

In the OUR curves (Fig. 6), the first phase reflects the primary metabolism of the added substrate (readily and slowly biodegradable), whereas the second phase implicitly originates from the utilization of the remaining slowly biodegradable substrate and/or stored polymers. The maximum OURs in the first phase varied in the range of 22.8–39.5 and 20.4–21.8 g O_2/(kg VSS h), respectively, at the Wschod and Debogorze WWTP. The differences between the parallel reactors were reached 20–30 % (Tables 2, 3). For comparison, Choi and Daehwan (2001) found that particulate COD contributed to the increase in total OUR (including nitrification) by approximately 5.5 % when the particulate COD constituted 65 % of total COD (50 % of the particulate fraction was estimated to be biodegradable). The soluble solution was made of filtered nightsoil, while the particulate solution was made of settled wastewater and nightsoil. In another study, Kristensen et al. (1992) demonstrated that hydrolysis of primary sludge can produce a broad range of readily biodegradable carbon sources. The authors concluded this based on the observation that OURs with hydrolysate (a liquid phase of the anaerobically hydrolysed primary sludge) as a carbon source were typically 10–20 % higher compared to the OURs with acetate.

The heterotrophic yield coefficients, Y_H, determined from Eq. 1 based on simultaneous measurements of COD and oxygen consumptions, are listed in Table 2 (Wschod) and Table 3 (Debogorze). The values of Y_H remained in a relatively narrow range at each plant, i.e. between 0.62–0.67 and 0.68–0.71 g cell COD/g COD, respectively, at the Wschod and Debogorze WWTP. This

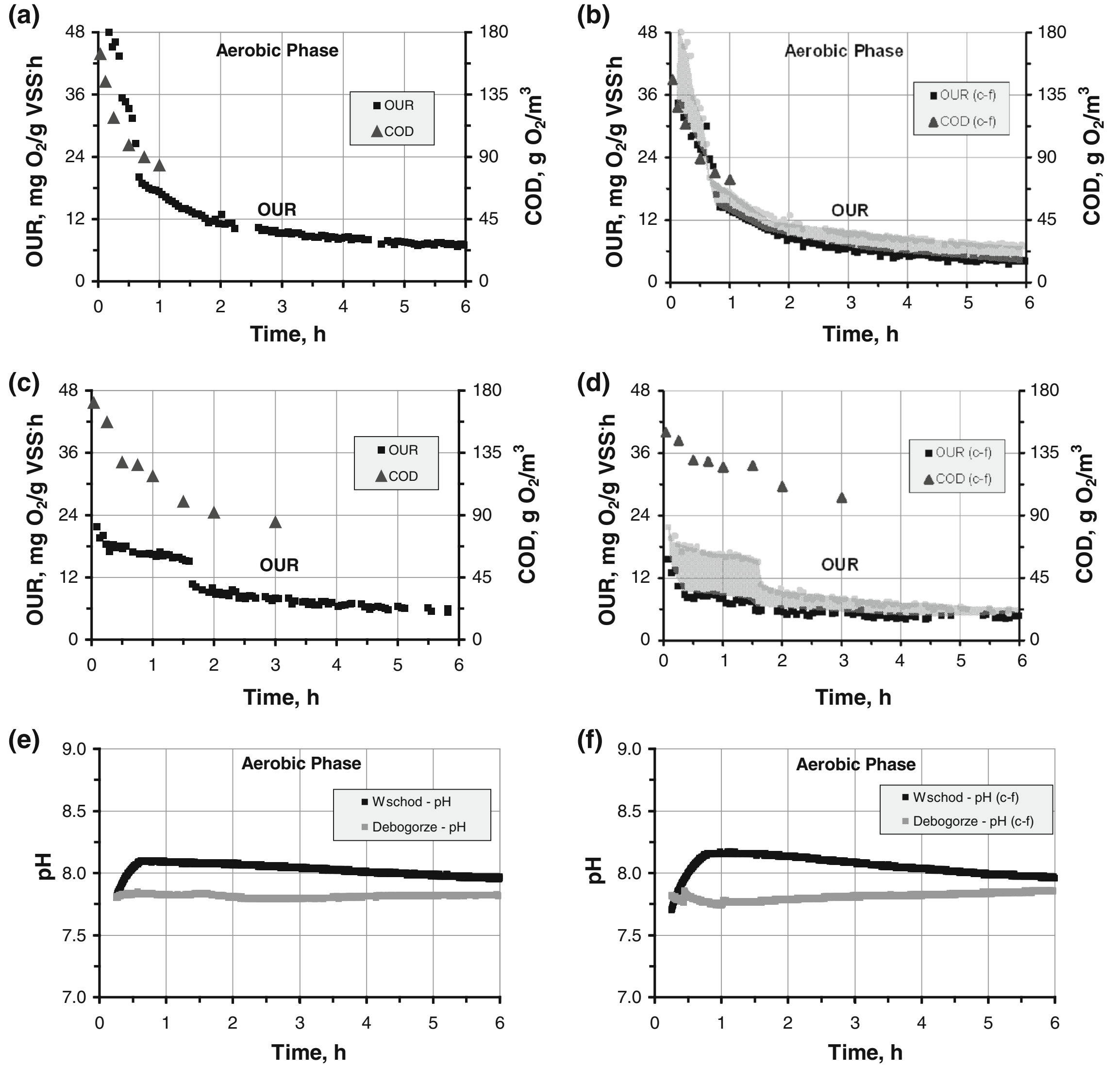

Fig. 6 Sample results of the conventional OUR measurement in two parallel reactors during the summer study period at the Wschod WWTP ($T = 20.2$ °C): **a** with the settled wastewater without pre-treatment (MLVSS = 1.4 kg/m^3), **b** with the settled wastewater after coagulation–flocculation (MLVSS = 1.2 kg/m^3), and during the fall study period at the Debogorze WWTP ($T = 16.0$ °C): **c** with the settled wastewater without pre-treatment (MLVSS = 2.0 kg/m^3), **d** with the settled wastewater after coagulation–flocculation (MLVSS = 1.8 kg/m^3), and pH variations at both studied plants: **e** with the settled wastewater without pre-treatment, **f** with the settled wastewater after coagulation–flocculation

may thus suggest that the composition of the settled wastewater is slightly different at the two studied plants. The typical values of Y_H for municipal wastewater, reported by Henze et al. (1987), were within the range of 0.46–0.69 g cell COD synthetized/g COD removed with the default value equal to 0.67 g cell COD/g COD used in the Activated Sludge Model No. 1 (ASM1). A higher range of Y_H values (0.72–0.78 g COD/g COD) was found at the Wschod WWTP with a few external carbon sources (acetate, ethanol, fusel oil and distilled raw alcohol). Dircks et al. (1999) proved that the difference in the Y_H coefficient could indeed result from the type of substrate. The reported Y_H values for the activated sludge from two studied Danish WWTPs were 0.71–0.72 and 0.66–0.67 g COD/g COD for acetate and ethanol, respectively.

pH as a control parameter of biochemical processes in BNR systems

On-line measurements of nutrient concentrations (NH_4–N, NO_3–N, PO_4–P) are often expensive and difficult in use for different full-scale BNR activated sludge systems. However, according to the literature data (Yu et al. 1997; Lee et al. 2001, Li et al. 2008), the dynamics of nutrient concentrations can be related with on-line monitoring of pH. For example, the on-line profile of pH can accurately detect the ends of PRR, PUR or NH_4–N conversions, and be used as a real-time control parameter (Lee et al. 2001).

In this study, the pH values observed as a control parameter at both studied plants remained in the range 7–8 during all the laboratory tests. The pH trends in the reactors with the settled wastewater without pre-treatment and after coagulation–flocculation were similar. The pH continuously increased during the conventional NUR experiments, however, at slower rate at the beginning of the experiments (during 1 h) when the PO_4–P release was observed (Fig. 3). The calculated ratios of $\Delta pH/\Delta NO_3$–N varied in the range of 0.91–1.19 and 0.73–1.01 in the settled wastewater without pre-treatment versus 0.54–1.02 and 0.53–0.76 in the wastewater after coagulation–flocculation, respectively, at the Wschod WWTP and the Debogorze WWTP. The pH variations at both reactors with the settled wastewater without pre-treatment and after coagulation–flocculation were similar during the two-phase PRR and anoxic/aerobic PUR tests. Sharp decrease in pH was observed at the beginning of anaerobic phase mainly due to the PO_4–P release. The pH continuously decreases but at slower rate until the end of phosphate release. Second phase of PUR tests showed different pH variations between anoxic and aerobic phase. The calculated ratios of $\Delta pH/\Delta PO_4$–P varied in the range of 0.23–0.49 and 0.23–0.28 (anoxic), 0.25–0.43 and 0.27–0.48 (aerobic) in the settled wastewater without pre-treatment versus 0.40–0.50 and 0.44–0.47 (anoxic), 0.33–0.62 and 0.35–0.69 (aerobic) in the wastewater after coagulation–flocculation, respectively, at the Wschod WWTP and the Debogorze WWTP. A rapid increase in pH occurred at the beginning of aerobic phase, primarily due to the stripping of CO_2 out of the system. Subsequent decrease in pH in the aerobic phase was mainly caused by the release of H^+ from nitrification. The pH profile reached a typical short plateau, which might coincide with the end point of NH_4–N conversion to NO_3–N and NO_2–N (Fig. 5). In contrast during the anoxic PUR tests, slowly increase in pH was observed due to significant concentrations of nitrate (15–20 g N/m^3) at the beginning of anoxic phase. After that occurred rapid increase in pH mainly caused by anoxic PUR consumed H^+ in the reactor (Fig. 4). The calculated ratios of $\Delta pH/\Delta NO_3$–N varied in the range of 0.48–1.09 and 0.55–1.05 in the settled wastewater without pre-treatment versus 0.49–0.88 and 0.55–0.81 in the wastewater after coagulation–flocculation, respectively, at the Wschod WWTP and the Debogorze WWTP. No significant dependence on pH was observed during conventional OUR tests, mainly caused by ATU addition at the beginning of aerobic phase. The pH value continuously increased but at very slowly rate mostly up to pH 8 until the end of experiment (Fig. 6).

According to the different studies (Yu et al. 1997; Lee et al. 2001; Li et al. 2008), it has been reported that nutrient concentrations were related to dynamic characteristics of on-line pH sensor during different type of experiments (e.g. SBR, batch tests) carried out with the mixed liquor from pilot or full-scale BNR systems. In the activated sludge system examined, the pH in bulk wastewater should be always in narrow range 7 and 8. However, the strong pH dependency is typical for an enzymatic reaction with a maximum activity around pH 8 and with little activity at pH values below 6 and above 9 (Li et al. 2008). In this study, based on pH dynamics, the nutrient removal processes were estimated during different type of batch tests carried out with the mixed liquor from full-scale BNR systems. The pH continuously increased during the one-phase conventional NUR experiments, however, at slower rate at the beginning of the experiments (during 1 h) when the PO_4–P release was observed. During the two-phase PRR and anoxic/aerobic PUR tests, a significant drop of pH was observed due to the lack of dissolved oxygen at the early part of anaerobic phase. In the subsequent anoxic phase, increase in pH was observed as anoxic phosphate uptake consumed H^+. Subsequently in aerobic phase, pH increased continuously and reached a relatively constant value when maximum PUR was ended. The accumulated CO_2 from the respiration activity using the residual dissolved oxygen might cause the pH decrease at the later part of aerobic phase. Therefore, the on-line profile of pH can accurately detect the ends of phosphate release/uptake activity as well as nitrogen/ammonia conversion, and be used as a real-time control parameter.

Reported full-scale results on the role of organic particulates

Tuncal et al. (2009) investigated performances of two parallel treatment lines operated with and without primary sedimentation at the Izmir Wastewater Treatment Plant (WWTP) (Turkey). During the study period (more than 1 year), the average effluent concentrations of NO_3–N and PO_4–P were lower in the line without primary sedimentation (0.82 g N/m^3 and 1.7 g P/m^3) compared to the second line (1.6 g N/m^3 and 3.8 g P/m^3). The authors attributed these results to two mechanisms. Firstly, increased organic loading rates, even in the particulate form, supported survival and proliferation of denitrifying PAOs. Secondly, sedimentation of particulate solids in the anaerobic compartments resulted in fermentation of the settled organic compounds and increased EBPR performance. Significant effects of primary settling on denitrification were also observed in the study of Tas et al. (2009). It was estimated that the particulate COD fraction removed by primary settling could represent up to approximately 40 % of the total denitrification potential of activated sludge systems. It was concluded that the settleable COD fraction could provide an additional carbon source in systems where the denitrification potential associated with the influent becomes rate-limiting for the denitrification efficiency (Tas et al. 2009).

The contrary findings have been reported in Dutch studies. Puig et al. (2010) found that by-passing raw wastewater (27 % of the influent flow) directly to the biological reactor did not improve effluent quality and nutrient removal efficiency of the studied plant. The observed increase in the influent C/N and C/P ratios was attributed to a high non-biodegradable COD fraction and particulate compounds with low COD/VSS ratios. van Veldhuizen et al. (1999) also found that hydrolysis and fermentation processes did not play a major role in the overall treatment performance, especially for P removal. A primary effluent in the studied plant was primarily composed of soluble COD (46 % of total COD), including VFAs (30 % of soluble COD). The authors emphasized, however, that hydrolysis and fermentation processes would be much more important in the plants fed with wastewater from aerobic sewers in which the ratio between soluble and particulate organic material (S_S/X_S) is much lower.

Conclusion

In a present work, a novel procedure, based on the standard batch experiments and pre-treatment of wastewater sample with coagulation–flocculation, was proposed to evaluate the effects of colloidal and particulate organic compounds (slowly biodegradable substrate) on denitrification and EBPR in BNR activated sludge systems. The colloidal and particulate organic fractions play important role in enhancing denitrification and EBPR at the studied WWTPs receiving strong municipal wastewater. Except for one process (phosphate release), the removal of these fractions resulted in reduced process rates. At both studied WWTPs, the observed average reductions were as follows: 24 and 35 %, respectively, for the NUR1 and NUR2 during the conventional denitrification tests, and 30 % for the NUR during the anoxic PUR test; 32 % and 25 %, respectively, for the anoxic and aerobic PURs; 13 % for the average OUR during the aerobic PUR tests, and 24 % during the conventional OUR test (maximum measured values). Anaerobic hydrolysis of slowly biodegradable organic compounds is slow and generates small amounts of the substrate for maintenance of PAOs under anaerobic conditions. In addition to the experimental observations, the results of such a comparative study can provide a comprehensive database for calibration and evaluation of dynamic models of the hydrolysis process. Based on pH dynamics, the nutrient removal processes were estimated during different types of batch tests carried out with the mixed liquor from full-scale BNR systems. Therefore, the on-line profile of pH can accurately detect the ends of phosphate release/uptake activity as well as ammonia conversion, and be used as a real-time control parameter. The observed effects of colloidal and particulate organic compounds are important in the overall energy balance of WWTPs. Sedimentation and chemical precipitation in primary clarifiers for the removal of particulate and colloidal organic matter should be optimized in terms of balancing between the efficiency of nutrient removal in the bioreactor and biogas production in the digester. Moreover the experimental observations, the results of such a comparative study can provide a comprehensive database for calibration and evaluation of dynamic models of the hydrolysis process.

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