The role of colloidal and particulate organic compounds in denitrification and EBPR occurring in a full-scale activated sludge system

J. Drewnowski and J. Makinia

ABSTRACT

The efficiencies of denitrification and enhanced biological phosphorus removal (EBPR) in biological nutrient removal (BNR) activated sludge systems are 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 carbon sources for denitrification is preferred. The aim of this study was to determine the immediate effects of slowly biodegradable substrates on the denitrification capability and phosphate release/uptake interactions for a full-scale biomass process from the “Wschod” wastewater treatment plant (WWTP) in Gdansk (Poland). Since it is hard to distinguish the slowly biodegradable substrate in a direct way, a novel procedure based on batch experiments was developed and implemented. The laboratory experiments were carried out in two parallel, fully automatic batch reactors with the settled wastewater without pretreatment and after coagulation-flocculation. The removal of colloidal and particulate fractions resulted in the reduced observed process rates, such as denitrification, phosphate release and phosphate uptake (under aerobic and anoxic conditions). The reduction ranged from approximately 14% for the anaerobic P release to approximately 46% for the anoxic P uptake.

Key words | activated sludge, denitrification, EBPR, nitrogen removal, wastewater fractionation

INTRODUCTION

The efficiencies of denitrification and enhanced biological phosphorus removal (EBPR) in biological nutrient removal (BNR) activated sludge systems are strongly dependent on the availability of appropriate carbon sources. Denitrification is accomplished by a variety of facultative heterotrophic microorganisms, which can utilize nitrate (or nitrite) instead of oxygen as the final electron acceptor. Sufficient amounts of organic carbon (electron donor) must also be ensured to provide energy for the conversion. The energy sources can be categorized as internal (present in the influent wastewater), endogenous (self-generated within the system as a result of organism decay) and external (not present in wastewater). Many of the operating systems to enhance the denitrification process and improve the overall efficiency of N removal within the existing capacities of activated sludge systems use of latter (external) sources. Their addition in pre-denitrification anoxic zones increases the denitrification rates and nitrogen removal efficiencies, while addition of carbon to the anaerobic zone of enhanced biological phosphorus removal (EBPR) systems may help improve the process performance and stability. There is a number of effective, commercially available organic compounds (such as methanol, ethanol, acetic acid, sodium acetate and glucose) which can be categorized as the “conventional” carbon sources. Among them, methanol has been most commonly used and best documented. However, due to high costs of commercial compounds and acclimation periods (usually) required, the effective use of internal carbon sources for denitrification is preferred.

The EBPR process is accomplished by heterotrophic microorganisms collectively referred to as phosphate accumulating organisms (PAOs). The principal mechanism for
attaining the EBPR process is a continual circulation of the activated sludge biomass through an alternating anaerobic/ aerobic (or anoxic) phases or zones, and provision of specific carbon sources during the anaerobic phase. These sources primarily include acetate and propionate which are most common volatile fatty acids (VFAs) in domestic wastewater (Pijuan et al. 2004). The contribution of other carbon sources than VFAs for proliferation of PAOs in the anaerobic phase is currently not clear, although the EBPR process also occurred successfully with other organic substrates, such a mixture of peptone and glucose, or even only glucose (Carucci et al. 1999) as well as carboxylic acids and amino acids (Mino et al. 1998). In “normal” municipal wastewater, the VFAs concentrations are usually insignificant. Therefore, in practice, fermentation of “complex” readily biodegradable substrates, accomplished by “ordinary” heterotrophs, plays an important role in EBPR allowing to sustain PAOs in the system.

In order to improve complex wastewater characteristics, different organic fractions have to be described separately. Ekama & Marais (1979) were the first, who divided the wastewater into distinct biodegradable fractions, that are degraded at two different rates. The readily biodegradable (S$_b$) fraction consists mainly of soluble organic compounds and the slowly biodegradable (X$_b$) fraction consists of large molecules, colloids and particles. According to the literature data (e.g. Orhon et al. 1997; Lagarde et al. 2005), municipal wastewater after primary treatment contains readily and slowly biodegradable compounds in the amounts of approximately 10–30% and 40–60% of total COD, respectively. An investigation in four municipal WWTPs in northern Poland revealed that particulate and colloidal fractions constituted more than 60% of total COD (Czerwionka et al. 2008). These two physical fractions mostly contain the slowly biodegradable substrate, which can be taken up and degraded by microorganisms after hydrolysis to the readily biodegradable substrate (Levine et al. 1985).

The influence of the readily biodegradable COD fraction in biological wastewater treatment systems has extensively been investigated and reported, but it is still very little knowledge about the effects of slowly biodegradable substrate on denitrification and EBPR. The aim of this study was to determine the immediate effects of slowly biodegradable (colloidal and particulate) substrates, as a internal carbon sources, on the denitrification capability and phosphate release/uptake interactions for a full-scale biomass process from the “Wschod” wastewater treatment plant (WWTP) in Gdansk (Poland). For this purpose, a novel procedure based on batch experiments was developed and implemented.

**MATERIALS AND METHODS**

**Study site**

The “Wschod” WWTP in Gdansk is one of the largest facilities located upon the Baltic Sea. The plant treats wastewater originating from the city of Gdansk and adjusting communities. The influent flow rate is approx. 81,000 m$^3$/d, and the pollutant load corresponds to approx. 570,000 population equivalents (PE). Industrial sources contribute to about 10% of the total pollutant load. The biological step, completed in 1998–1999, consists of twelve circular secondary clarifiers and six parallel bioreactors, which run in the Modified University of Cape Town (MUTC) system. A minor modification to the original configuration is a deoxic zone in the internal recirculation line from the aerobic to the anoxic zone. The treated wastewater is directly discharged to the Bay of Gdansk which is part of the Baltic Sea.

**Laboratory set-up**

Lab experiments were carried out in a specially designed and constructed experimental set-up consisting of two parallel, plexiglass batch reactors (max. volume of 4.0 dm$^3$), control system and computer (Figure 1). The reactors were equipped with electrodes for a continuous monitoring of pH, ORP, temperature and dissolved oxygen (DO). The automated control system maintained a desired DO concentration and temperature in the reactors. The temperature set points were set close to the actual process temperature in the full-scale bioreactors during each study session, i.e. 12–14°C (winter), 15–17°C (spring) and around 20°C (summer). The experimental data (pH, DO, T, ORP) were collected on-line by computer from the control box and probes installed in each batch reactor.

**Sample preparation**

It is hard to distinguish the slowly biodegradable substrate in a direct way from wastewater. Therefore, the laboratory experiments were conducted in two parallel batch reactors (Figure 2). In the first reactor, the process biomass (fresh returned activated sludge) was mixed with the settled wastewater without pretreatment (containing soluble, colloidal and particulate organic fractions), whereas the settled wastewater after coagulation-flocculation was used in the second reactor. The latter sample of wastewater, only containing a soluble organic fraction, was prepared according to the rapid physical-chemical method of Mamais et al. (1993). That...
The method is based on the rationale that membrane filtration of the sample that has been flocculated (by precipitating with ZnSO$_4$ at pH 10.5) will remove the colloidal particles from a filtrate leaving only truly soluble organic matter in the filtrate. After removing colloids and particulates, the pH was adjusted to its original value by adding 6 M HCl.

For comparison, Goel et al. (1999) proposed a similar experimental procedure to separate hydrolysis from storage by performing and analyzing two parallel oxygen uptake rate (OUR) measurements: one with filtered wastewater (including soluble COD) and the other with non-filtered wastewater (including total COD).

**Batch tests**

Batch tests were conducted in three experimental series, termed winter, spring and summer, during the period from December, 2007 to May, 2009. Three different kinds of batch tests were carried out to determine nitrate utilization rates (NURs) and specific phosphate release/uptake rates (PRR/PURs). The measurement procedures were based on similar tests described in more detail by Makinia et al. (2004, 2006).

In the so-called “conventional” NUR measurements, a source of nitrate (KNO$_3$) was injected at the beginning of the test (20 gN/m$^3$) and the test was run for 4 hours. The samples were withdrawn with the frequency of 10–30 min., filtered under vacuum pressure on the Whatman GF/C filter and analyzed for NO$_3$-N, PO$_4$-P and COD. The actual MLSS and MLVSS concentrations in both reactors were determined at the beginning and at the end of the each batch test.

In the PRR/PUR tests, a 2.5-hour anaerobic phase was followed by the anoxic or aerobic phase, both lasting 4 hours. Such a sequence better reflects the actual conditions occurring in activated sludge systems with EBPR. In the anoxic PUR test, a source of nitrate (KNO$_3$) was injected at the beginning of the anoxic phase (20 gN/m$^3$). In the aerobic PUR test, the air supply system was turned on at the beginning of the aerobic phase (DO set point = 6 gO$_2$/m$^3$) and the OUR measurement was initiated. The samples were withdrawn with the frequency of 10–30 min., filtered under vacuum pressure on the Whatman GF/C filter and analyzed for PO$_4$-P, COD (anaerobic phase) and PO$_4$-P, NO$_3$-N/NH$_4$-N, COD (anoxic/aerobic phase). The actual MLSS and MLVSS concentrations in both reactors were measured at the beginning and at the end of the each batch test.

**Analytical methods**

Total and volatile suspended solids were measured by the gravimetric method according to the Polish Standards (PN-72/C-04559). The total or soluble COD, PO$_4$-P, NO$_3$-N and NH$_4$-N were analyzed using Xion 500 spectrophotometer (Dr Lange GmbH, Germany). The analytical procedures,
which were adapted by Dr Lange GmbH (Germany), followed the Standard Methods (APHA, 1992).

RESULTS AND DISCUSSION

The soluble fraction in the 24 wastewater samples used in the experiments accounted for 19 to 39% of total COD (Figure 3). The average value of total COD was 627 gCOD/m$^3$ including soluble COD = 194 (±38) gCOD/m$^3$ vs. colloidal and particulate COD = 433 (73) gCOD/m$^3$. For comparison, the average values of total and soluble COD determined from the routine operating data at the “Wschod” WWTP were 594 and 172 gCOD/m$^3$ (2007), and 715 and 192 gCOD/m$^3$ (2008). In the earlier modelling study (Makinia 2006), the estimated ratio of biodegradable to non-biodegradable particulate (and colloidal) organic fractions ranged from 1.8 to 2.5 to fit the waste activated sludge production in three study periods.

Table 1 contains a list of the ranges of all the specific rates, i.e. NURs, PRRs, anoxic/aerobic PURs and OURs, observed in the three kinds of batch experiments with the settled wastewater without pre-treatment and after coagulation-flocculation. The obtained results in all study sessions (winter, spring and summer) are discussed in the following sections.

“Conventional” NUR tests

In the experiments with the settled wastewater, the observed NURs (NUR1 and NUR2) were associated with the utilization of readily biodegradable organic compounds ($S_R$) and colloidal and particulate organic compounds ($X_S$). The NUR1 values varied within the range 3.7–5.0, 4.4–5.5 and 4.6–5.1 g N/(kg VSS·h), respectively, during the winter, spring and summer sessions. The NUR2 values in the corresponding experiments varied within the range 1.3–1.4 (winter), 1.0–2.0 (spring) and 1.6–1.7 g N/(kg VSS·h) (summer). These ranges are comparable with the values reported by Naidoo et al. (1998) for the experiments at the WWTPs located in

| Table 1 | Specific process rates observed in the three kinds of batch experiments with the settled wastewater without pre-treatment and after coagulation-flocculation |
|-----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Process rates   | Winter session                  | Spring session                  | Summer session                  |
| Type            | Unit                            | (Temp. range 11.8–14.2 C)      | (Temp. range 14.8–16.7 C)      | (Temp. range 20–20.5 C)        |
|                 | Settled wastewater              | Pretreated settled wastewater  | Settled wastewater              | Pretreated settled wastewater  | Settled wastewater              | Pretreated settled wastewater  |
| NUR1            | mg N/gVSS·h                     | 3.7–5.0                         | 2.6–4.2                         | 4.4–5.5                         | 3.4–5.7                         | 4.6–5.1                         | 3.5–4.1                         |
| NUR2            | 1.3–1.4                         | 1.0–1.1                         | 1.0–2.0                         | 1.0–1.4                         | 1.6–1.7                         | 1.5–1.6                         |
| PRR             | mg P/gVSS·h                     | 7.8–9.3                         | 3.9–8.8                         | 10.9–13.0                       | 9.9–11.6                       | 8.4–10.8                       | 7.7–10.5                       |
| PUR$_{Anoxic}$  | mg P/gVSS·h                     | 3.4–4.2                         | 1.2–1.4                         | 5.9–6.6                         | 5.3–6.0                         | 4.1–4.4                         | 1.2–1.9                         |
| NUR             | mg N/gVSS·h                     | 1.6–1.7                         | 0.7–1.1                         | 2.4–2.7                         | 2.2–2.7                         | 2.0–2.1                         | 1.3–1.6                         |
| PUR$_{Aerobic}$ | mg P/gVSS·h                     | 5.3–12.5                        | 2.1–6.2                         | 11.4–13.8                       | 10.1–12.6                       | 6.1–6.8                         | 2.8–5.7                         |
| OUR$_{Max}$     | mg O$_2$/gVSS·h                 | 22.0–22.1                       | 18.2–18.3                       | 25.8–27.8                       | 25.6–26.2                       | 31.8–33.4                       | 26.2–31.0                       |

Figure 3 | Measured fractions of COD (soluble vs. colloidal and particulate) in the samples used for three series of the laboratory experiments.

"Conventional" denitrification test
eight European cities. In that study, the NURs associated with utilization of the readily biodegradable and slowly biodegradable substrates varied within the range of 3.3–5.7 g N/(kg VSS·h) and 1.6–3.6 g N/(kg VSS·h), respectively. Slightly higher rates, i.e. NUR1 = 4.9–6.8 g N/(kg VSS·h) and NUR2 = 2.2–3.0 g N/(kg VSS·h), were observed during earlier studies conducted at the “Wschod” WWTP (Makinia et al. 2004).

When the pre-treated samples of wastewater were used in the experiments, the observed NURs were associated with the utilization of SS and the remaining colloidal organic fraction (part of XS). Consequently, the values of NUR1 and NUR2 were lower in comparison with the parallel tests with the settled wastewater. The NUR1 values varied within the range 2.6–4.2 (winter), 3.4–3.7 (spring) and 3.5–4.1 g N/(kg VSS·h) (summer), whereas the NUR2 values in the corresponding experiments were 1.0–1.1 (winter), 1.0–1.4 (spring) and 1.5–1.6 g N/(kg VSS·h) (summer).

Figure 4 illustrates an example of the “conventional” denitrification test carried out during the winter and summer sessions. An interesting behaviour of phosphate was observed in both reactors. At the beginning of the experiments, phosphate was released despite high concentrations of nitrate which, in the common opinion, inhibit that process (P release). The release continued until the readily biodegradable substrate was present in the solution.

**PRR & anoxic/aerobic PUR tests**

In the experiments with the settled wastewater, the maximum PRR values ranged from 7.8 to 9.3 g P/(kg VSS·h) in the winter, 10.9 to 13 g P/(kg VSS·h) in the spring and 8.4 to 10.8 g P/(kg VSS·h) in the summer study session. Coagulation-flocculation had only a minor effect on the kinetics of phosphate release as the observed rates in the parallel reactor with the pre-treated wastewater were lower by approximately 10% (Figures 5 and 6). All these values are comparable with the literature data (6–12.3 P/(kg VSS·h)) reported by Makinia (2006) for a number of different full-scale, pilot-scale and lab-scale BNR systems, but significantly lower compared to the results obtained during the previous study (Makinia et al. 2004), i.e. 15.1–21.4 g P/(kg VSS·h).
The results from PRR & anoxic/aerobic PUR (Test 2 and Test 3), carried out with the settled wastewater without pre-treatment and after coagulation-flocculation, also showed different processes rates between two kinds of internal carbon sources used in parallel batch reactors (Figures 5 and 6). The anoxic PUR varied within the range 3.4–4.2, 5.9–6.6 and 4.1–4.4 g P/(kg VSS·h) vs. 1.2–1.4, 5.3–6 and 1.2–1.9 g P/(kg VSS·h), respectively, in the winter, spring and summer session. The PURs observed under aerobic conditions were significantly higher, i.e. 5.3–12.5, 11.4–13.8 and 6.1–6.8 g P/(kg VSS·h) vs. 2.1–6.2, 10.1–12.6 and 2.8–5.7 g P/(kg VSS·h) in the corresponding study sessions.

In the experiments with the pretreated wastewater, lower values of the NURs (on average 46%) were observed under the anoxic conditions (Figure 5). The additional OUR measurements under the aerobic condition were also lower for the pretreated wastewater (Figure 6). However, the average difference of OUR max was only 20% due to an equal effect of nitrification in both reactors.

CONCLUSIONS

From this study, the following conclusions can be derived:

- The knowledge of the wastewater characteristics is important in terms of optimization of denitrification and EBPR in the BNR activated sludge systems.
- A novel batch test procedure was proposed to evaluate the effects of colloidal and particulate substrate on these two biochemical processes.
- The removal of colloidal and particulate fractions by coagulation-flocculation resulted in the reduced process rates (to a variable extend). The average reductions were:
  - 24 and 14%, respectively, for the NUR1 and NUR2 during the “conventional” denitrification tests, and up to 26% for the NUR during the anoxic PUR test;
  - 14% PRR under anaerobic conditions (except for one particular case);
  - 46% for the anoxic PUR;
- 34% for the aerobic PUR;
- 11% for the maximum OUR during the aerobic PUR tests.

• The results of this study will be subject to a further evaluation using a dynamic model with a special attention to modeling the hydrolysis process.

ACKNOWLEDGEMENTS

This research has been financially supported by Polish Ministry of Science and Higher Education under the grants no. N-523-069-32/2871 and N-523-422-437. Jakub Drewnowski was supported by the European Union within the framework of the European Social Fund under the system project of the Pomorskie Voivodeship "InnoDoktorant - Scholarships for Ph.D. students, II edition".

REFERENCES


