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Enhanced post-denitrification without addition of an external carbon source in membrane bioreactors

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Abstract

In this study a post-denitrification process without addition of an external carbon source combined with enhanced biological phosphorus removal (EBPR) in a membrane bioreactor (MBR) was investigated. Three trial plants with two different process configurations were operated on two different sites, and a variety of accompanying batch tests were conducted. It was shown that even without dosing of external carbon source denitrification rates (DNR) much above endogenous rates could be obtained in post-denitrification systems. Furthermore, the anaerobic reactor located ahead of the process had a positive impact on the DNR. Given these surprising results, the project team decided to identify the carbon source used by the microorganisms in the post-denitrification process. Batch tests could demonstrate that lysis products do not play a major role as a C-source for post-denitrification. A hypothesis was proposed to explain the observations: the glycogen internally stored by the substrate accumulating bacteria if anaerobic conditions are followed by aerobic conditions could act as carbon source for denitrification in post-denitrification system. First exploratory batch tests where the glycogen evolution was monitored corroborate this assumption.

Key words post-denitrification, EBPR, glycogen, MBR, metabolism, internal substrate, denitrification rate

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Nomenclature

AC	acetate
AE	aerobic
AN	anaerobic
AX	anoxic
DNR	specific denitrification rate $\frac{\Delta\, NO_3 - N}{\Delta t \cdot MLVSS}$ ($mgNO_3$-N/h gMLVSS)
DNR_O	operational DNR ($mgNO_3$-N/h gMLVSS)
DO	dissolved oxygen (mg/L)
DOC	dissolved organic carbon (mg/L)
F/M	Feed-to-microorganism ratio (gCOD/gMLSS)
ML(V)SS	mixed liquor (volatile) suspended solids (g/L)
N_T	total nitrogen (mg/L)
RW	raw water
r_D	actual volumetric denitrification rate (mgN/L h)
r_S	actual volumetric substrate utilisation rate (mg COD/L h)
SRT	solids retention time (d)
TOC	total organic carbon (mg/L)
Y_H	biomass yield coefficient (mg/mg)
ϑ	temperature (°C)

Introduction

In conventional waste water treatment plants (WWTP) nitrogen removal is mostly achieved with pre-denitrification for two major reasons: the use of biodegradable organic matter available in the anoxic zone to improve denitrification rate, hence reduce the required volume of biological reactor, and the use of the oxidation capacity of nitrate to degrade part of the organic matter, hence to reduce oxygen demand and to achieve savings in aeration requirement. On the other hand, N-removal rate depends on the recirculation ratio which transfers the nitrate produced by nitrification in the aerated zone back to the anoxic zone and is therefore limited to 75 to 90%.

In post-denitrification, without carbon source, the denitrification rate is expected to be low and close to

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the endogenous denitrification rate, the minimum denitrification rate observed when no bacteria growth occurs (under maintenance and/or decay metabolism). However, as N-elimination occurs according to the natural nitrification / denitrification order, almost complete N-removal can be theoretically achieved, providing sufficient reactor volumes. In actual installed post-denitrification WWTP always a carbon source is added to the anoxic reactor in order to minimize reactor volume.

In membrane separation activated sludge processes, commonly referred to as membrane bioreactor (MBR), pre-denitrification has been traditionally implemented for nitrogen removal. To the authors knowledge, post-denitrification was never attempted with MBR. However, some characteristics of the MBR technologies could make post-denitrification processes attractive, even without addition of C-source (Gnirss et al. 2003). These specific features are summarized here:

(i) low denitrification rates reported in actual pre-denitrification MBR plants: due to the combination of several detrimental parameters (high operation sludge age; high oxygen carry-over to the anoxic zone from the membrane system, separated or submerged in a sequenced aeration reactor; one single totally mixed anoxic reactor instead of a multi-stage design, etc). In some cases, reported denitrification rates approached or even passed down the endogenous rate. MUNLV (2003) suggests a volume ratio $V_{denitrification}/V_{nitrification}$ of 50%/50% for pre-dentrification MBR plants while the ratio for conventional plats is 25%/75%. This can be theoretically accentuated by the presence of the up-front anaerobic zone in MBR processes featuring enhanced biological phosphorus removal (EBPR) as an important amount of the biodegradable substrate does not reach the denitrification zone.

(ii) insignificance of greater air requirement with post-denitrification mode: the savings due to nitrate recycling are minor in comparison with the important air requirement for membrane aeration in MBR.

(iii) less equipment and energy requirement: as the aerobic/anoxic sludge recirculation loop is not required.

(iv) better biomass repartition: due to the sludge recirculation pattern over the entire reactor volume (Figure 1), which leads to less sludge in contact with the membrane, but more sludge present in the anoxic zone. This advantage is not relevant with conventional processes as the sludge returns from the (anoxic) clarifier to the head of the biological lane (except University of Cap Town (UCT) and Virginia Initiative Plant (VIP) processes, Tchobanoglous and Burton, 1991).

(v) no bulking issue: This is often reported as a drawback of conventional plants working with EBPR and/or post-denitrification systems, but is obviously not an issue with a membrane separation system.

Post-denitrification was therefore identified as a promising configuration in MBR technology when enhanced nitrogen removal is required. In this research project, two MBR process configurations were compared to achieve enhanced biological phosphorus and nitrogen removal. The configurations included both an anaerobic zone, and resorted either to pre-denitrification or to post-denitrification without addition of external carbon source. The salient results obtained over more than 2 years on three different MBR trials plants are presented in Adam et al (2002), Lesjean et al. (2003) and Gnirss et al. (2003). Unexpected and surprising results were monitored for post-denitrification. This paper summarizes the observations related to the denitrification rates in sludge starving conditions, and attempts to provide some explanations of the possible mechanisms encountered.

Materials and Methods

Three trials plants were operated with different configurations on two different sites:

- one pilot plant (2m³, V_{AE}/V_{AX}=50/50%) with pre-denitrification and an anaerobic zone for EBPR (configuration 1, Figure 1), here called PP1;
- one pilot plant (2m³, V_{AE}/V_{AX}=45/55%) with post-denitrification without carbon addition and an

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anaerobic zone for EBPR (configuration 2, Figure 1), here called PP2;

- one bench scale plant (167-210L, V_{AE}/V_{AX}=42/58%) with post-denitrification operating for a period with anaerobic reactor and another period without anaerobic reactor; here called BSP.

PP1 and PP2 were fed with municipal raw sewage screened through 1 mm punch holes (rotative drum). Solid retention time (SRT) was varied stepwise between 8 and 26d and hydraulic retention time (HRT) was between 11 and 17h. The average sludge concentration in the aerobic reactor was 12.5 gMLSS/L at 26d SRT and around 8.5 gMLSS/L at 8d. MLVSS was around 64% of MLSS.

The BSP was fed with domestic waste water from a separated sewer after screening through 1mm slits. It was constantly operated at 15 d SRT. Depending on the configuration HRT was 17 or 20h. The sludge concentration was between 6 and 8 gMLSS/L in the aerobic reactor.

In all plants, the aerobic and anoxic zones were cascaded (3 to 4 stages). For the determination of operational denitrification rates (DNR_O) within the plants, filtered samples were taken from each reactor. Denitrification rates in each reactor were then calculated with the respective contact times. Temperature correction of DNR_O was calculated by:

$$DNR(20°C) = DNR(\vartheta) \cdot 1{,}15^{(20-\vartheta)}$$

Four kinds of batch tests were implemented with the sludge taken from each pilot plants. All tests were conducted at 20°C in stirred batch reactors.

Determination of DNR. These tests were done to clarify the denitrification potential of the starving sludge, taken just before the filtration stage, and to verify the DNR_O measured in the plants. Therefore sludge was spiked with sodium-nitrate to a concentration of app. 10 $mgNO_3$-N/L. Filtered samples were taken every 15 mn and analysed with an ion chromatograph.

Long-term DNR. The DNR was measured over 24h, in order to investigate if any change of DNR occurs. Sodium-nitrate was dosed to starving sludge to a start concentration of around 120 $mgNO_3$-N/L. Filtered samples were taken every 20 mn. If necessary sodium-nitrate was dosed again after 12h to avoid a nitrate limitation of denitrification.

Parallel tests. In order to have a closer look on the carbon source used for denitrification in starving sludge, three small (250ml) vessels were sampled in parallel. All were filled with starving sludge. To the first sodium-acetate and sodium-nitrate was dosed. In the second only sodium-nitrate was added. Nothing was dosed to the third vessel (blank). All were flushed with nitrogen and kept under anoxic resp. anaerobic (blank) conditions during the test. Nitrate and COD evolution were monitored over 1 hour. For COD determination the DOC was measured in a Dimatec TOC-Analyser; COD/DOC ratio was identified to be 2.9. Some tests were carried out with washed sludge. In such case, a buffer solution of following composition was used: 552 mg/L NaH_2PO_4 * H_2O, 760 mg/L Na_3PO_4 * 12 H_2O, 526 mg/L NaCl, 74,6 mg/L KCl.

Glycogen batch tests. These tests should simulate the evolution of Glycogen in a post-denitrification process featuring EBPR. Sodium-acetate was dosed to sludge from the last anoxic stage of PP2 and kept under anaerobic conditions for 1.5h. Afterwards the reactor was aerated and phosphate was added to avoid P-limitation. When P-uptake was almost complete, sodium-nitrate was dosed and anoxic conditions were established for 2h. PO_4-P and NO_3-N were measured with an ion chromatograph, glycogen was measured with a standard method presented for example by Brdjanovic et al. (1998).

Results

DNR_O in PP1 and PP2. The results from the trials with PP1 and PP2 are just shortly discussed. More information can be found in Gnirss et al. (2003). Depending on the SRT a DNR_O of 1.2 up to 3.0 $mgNO_3$-N/h gMLVSS was measured in PP1 (pre-denitrification). Given rates are without temperature correction. The temperature in the pilot plants was between 12°C and

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26°C with an average of 19°C. These rates were confirmed in batch tests. Increasing DNR_O was observed with decreasing SRT. It is assumed that this effect results from a more active biomass in systems with lower SRT and a greater presence of soluble substrate, i.e. greater F/M ratio. In the post-denitrification configuration without addition of C-source (PP2) the DNR_O was monitored between 0.47 and 1.17 $mgNO_3$-N/h gMLVSS (no temperature correction) when nitrate was not limited. The variation, lower than in PP1, could not be clearly related to any evolution of operation condition. However the DNR_O was most of the time clearly above the postulated endogenous rate. Endogenous denitrification is normally considered to be in the range of 0.2 to 0.6 $mgNO_3$-N/h gMLVSS (Kujawa and Kapwijk 1999). This brought up the question of the carbon source which is used in the post-denitrification system, and motivated other tests.

DNR_O in the BSP. The operation of the BSP was divided into two periods during which the BSP was operated under a constant sludge age of 15d. In the first period post-denitrification without addition of any external carbon source was implemented in combination with an anaerobic reactor for EBPR (Configuration 2 in Figure 1). During this period the effluent N_T concentration was between 2-5 mgN_T/L resulting from a N-elimination around 95% (Adam 2004). In profile measurements an average DNR_O of 2.2 $mgNO_3$-N/h gMLVSS (ϑ=20°C, MLVSS=4.5 g/L) was observed. The DNR batch tests confirmed this rate, which was much above the expected endogenous rate. For the second period, the anaerobic reactor was removed and the plant was seeded with sludge from a non-EBPR plant. The effluent concentration of N_T rose to 20-30 mgN_T/L. The average DNR_O measured in the plant dropped by nearly half down to 1.2 $mgNO_3$-N/h gMLVSS. This rate was also confirmed in DNR batch tests. The average nitrogen elimination dropped down to circa 80%. These results provided a first indication of the impact of the upfront anaerobic conditions on the post-denitrification performances. This second unexpected observation motivated the needs of further tests to improve our understanding of the biological mechanisms.

Long-term DNR. Two kinds of long-term DNR batch tests were undertaken: with EBPR sludge from PP2 and with non-EBPR sludge from BSP. The results from the latter test are shown in Figure 2. It is obvious that the DNR was constant at ca. 1.25 $mgNO_3$-N/h gMLVSS over the entire test length of 25h.

The EBPR-sludge showed a completely different behaviour (Figure 3). After app. six hours the DNR diminished suddenly by 36% from 1.07 to 0.68 $mgNO_3$-N/h gMLVSS. Since there was no other limitation for denitrification mechanisms, this must result from a change of C-source used for denitrification. The reduction of 36% is striking, this is almost the difference of DNR observed with the BSP during the periods with and without anaerobic reactor (-45%). We can expect that a similar evolution of C-source induced this reduction of DNR in the two systems. Small parallel batch tests were therefore initiated in order to find out if the evolution of C-source could be attributed to a change of sludge lysis pattern resulting from a different equilibrium of cell maintenance and decay conditions.

It could be argued that the denitrification rates in the BSP were higher than in PP2, even without anaerobic reactor, but ones has to take in account that the plants were operating with different types of sewage and pretreatment leading to a different biocenosis and different kinetics.

Parallel batch tests Parallel batch tests with unwashed and washed sludge were implemented. Typical results with unwashed sludge are presented in Figure 4 and Figure 5. The DNR in the vessel fed with acetate and nitrate was, as expected, much higher (5.82 $mgNO_3$-N/h gMLVSS) than in the vessel where only nitrate was added (1.46 $mgNO_3$-N/h gMLVSS). This was due to the abundant presence of easily degradable substrate (Figure 4). The results for the COD evolution were more surprising (Figure 5). The COD-concentration in the acetate fed vessel declined, because the acetate was used as carbon source for denitrification. This was

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anticipated, but in the vessel fed with nitrate only and in the anaerobic vessel the COD concentration remained constant. An increasing concentration was expected in the anaerobic vessel resulting from lysis and hydrolysis processes. According to van Loosdrecht and Henze (1999) it is unlikely that microorganisms are dieing in a big amount in natural and activated sludge systems even under starving conditions. This explains the constant COD concentration in the anaerobic vessel.

In the anoxic vessel the situation is different. Because of the on-going denitrification (Figure 4) there is a need of carbon source and a declining concentration was expected. The substrate needed can be calculated by (Kujawa and Klapwijk 1999):

$$r_S = \frac{2{,}86 \cdot r_D}{1 - Y_{H,NO_x}}$$

For the observed nitrate degradation a need of 35 mg/L COD is computed with a yield Y_{H,NO_x} = 0,66 for municipal waste water proposed by Kujawa and Klapwijk (1999). Since there was no COD production (as shown in the anaerobic vessel) and the dissolved COD concentration was constant, the carbon used for the denitrification in the anoxic vessel without acetate must originate from internal cell sources.

This result was substantiated by the trials with washed sludge. It is apparent in Figure 6 that washing the sludge, and herby washing out a potential external C-source, did not change significantly neither the DNR of the acetate fed vessel (6.6 $mgNO_3$-N/h gMLVSS) and, more important, nor this of the only-nitrate-dosed vessel (1.4 $mgNO_3$-N/h gMLVSS) . The DNR in the acetate fed vessel dropped to 1.1 $mgNO_3$-N/h gMLVSS after one hour when acetate was completely depleted (Figure 7).

The COD evolution with washed sludge differs to the behavior with unwashed sludge. The declining concentration in the acetate fed batch matches with the unwashed sludge but for the two other reactors a rising concentration was observed. The augmentation for both reactors was similar. Hence again, an external C-source for denitrification was implausible. The end value of all three reactors was around 50 mg COD/L which matched with the constant value measured in unwashed sludge. Other experiments showed, that the COD increase stopped at this level and the concentration remained afterwards constant. It is therefore unlikely, that lysis caused this increase of COD in the sludge filtrate. A possible reason could be the new build-up of extra cellular polymeric substances (EPS) which were washed out. Once the EPS-layer is fully renewed, the COD concentration remains constant.

The parallel batch tests showed, that no external C-source was used for denitrification in starving sludge. Since the presence of an upfront anaerobic reactor impacted significantly on the post-denitrification rates, as shown with the long-term DNR batch tests and in the BSP trials, it was necessary to have a closer look on the storage compounds in the EBPR process.

The widely accepted EBPR metabolism theory is presented by Mino et al. (1998). Under anaerobic conditions phosphate is released, polyhydroxyalcanolates (PHA) are stored and stored glycogen is used as reduction power source and energy source. In the following aerobic reactor phosphate is taken up going along with PHA degradation and glycogen storage (Figure 8). This means that in the following anoxic zone glycogen can possibly act as carbon source for denitrification.

Glycogen batch tests In these tests a post-denitrification process combined with EBPR was simulated. Of special interest was the evolution of the glycogen storage.

The orthophosphate concentration showed the postulated increase under anaerobic conditions and decreases under aerobic conditions. The leap in concentration at the beginning of the aerobic zone resulted from P-spiking. When entering anoxic conditions, the P-uptake was almost completed.

After nitrate addition in the anoxic zone, a DNR of 1.26 $mgNO_3$-N/h gMLVSS was measured. There was no nitrate formed under aerobic conditions since the sludge originated from the last anoxic zone of PP2 where no ammonia was present.

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As postulated, the glycogen storage was degraded under anaerobic conditions and rebuilt in the aerobic zone. With the onset of denitrification the glycogen concentration started to decline and was therefore possibly used as C-source.

We can estimate the amount of glycogen which is theoretically needed for denitrification according to Kujawa and Klapwijk (1999). In our case, the anoxic substrate utilisation rate r_S is calculated by:

$$r_S = \frac{2{,}86 \cdot (r_{D1} - r_{D2})}{1 - Y_H}.$$

For this case r_{D1} is the overall DNR and r_{D2} is the DNR not based on glycogen. From Figure 3 it is apparent that r_{D2} was 64% of r_{D1}. The glycogen consuming DNR was therefore 36% of the overall DNR. Goel et al. (1998) stated a yield Y_H of 0.45 mg biomass COD/ mg carbohydrate COD for glycogen consumption. With these figures and an overall DNR of 1.26 mgNO_3-N/h gMLVSS a glycogen-DNR of 0.45 mgNO_3-N/h gMLVSS was calculated, corresponding to a theoretical glycogen consumption of 2.36 mg COD/h gMLVSS. This matches well with the measured 2.2 mg COD/h gMLVSS.

Discussion

Based on the different results, we can propose a hypothesis to explain the observed metabolism of post-denitrification. Indeed, it could well be that glycogen, which is clearly stored in EBPR configuration by substrate accumulating bacteria species under aerobic conditions, is later consumed for denitrification. This would explain the lower DNR observed in the BSP when operated without anaerobic reactor, as no glycogen was internally stored by cells and the microorganisms had to use a less favourable C-source. This would also explain the results of the long-term DNR batch tests (Figure 2 and 3). With EBPR sludge glycogen was used as C-source over the first hours. When the internal glycogen pool was depleted, the organisms switched to another C-source to carry on the denitrification and the DNR slowed down. This effect could not be observed with non-EBPR sludge, as the biomass did not possess this ability to store glycogen.

While it is clear that the upfront anaerobic reactor entails substrate storage it is unknown which microorganisms participate at post-denitrification process. The anaerobic-aerobic process management can enrich two kinds of organisms: phosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO) (Mino et al 1998, Sudiana et al 1999). It is assumed that GAOs use the same metabolism as PAOs at the exception of poly-P granules accumulation. Meinhold et al (1999) and Hu et al. (2002) could show that there is definitely a group of denitrifying organisms within the PAOs (DNPAO). These authors always observed denitrification with simultaneous P-uptake. A contemporary glycogen storage is therefore assumed. From Figure 9 it is apparent that this is not the case here: no significant P-uptake occurs and glycogen is depleted. Figure 10 shows that the enhanced post-denitrification took also place when no solved phosphate could be detected anymore in the anoxic stages. The denitrification can therefore not be linked directly to DNPAO, according to the metabolism as described in the literature. The tests indicate that another species of bacteria is responsible for the observed phenomena. This species could possibly be part of the PAO-group.

The second known group of bacteria capable of internal carbon storage is the GAO-group. Little is found in literature about the denitrification potential of GAOs. Manga et al. (2001) had hints, that GAO might not have ability of denitrification but pre-denitrification was implemeted in this study. Saunders et al. (2003) showed that GAOs are present in most EBPR plants. Their fraction of the biomass depends on influent characteristics and can reach the same percentage by weight as PAOs. Provided that DNPAOs are unable to denitrify without P-uptake, the existence of DNGAOs is postulated, even if no evidence of their existence was published to the authors knowledge. The present results provides the first indication of their presence and DNGAOs could possibly play an important role in post-denitrification.

However, further trials are necessary to validate the proposed theory, such as long term batch tests with glycogen measurement, to investigate if the evolution of DNR mimics this of glycogen storage. Furthermore it is important to identify the involved bacteria species and whether this mechanism is also valid in conventional process or just in MBR.

The identification of this new biological mechanism provides a potential of MBR process development and optimisation. Indeed, other solutions of MBR configurations based on the combination "upstream anaerobic zone and downstream post-denitrification", can be envisaged with other intermediate processes such as the use of one or several successions of one anoxic and one aerobic reactor, or a carousel reactor, or the inclusion of a sequenced aeration zone for simultaneous nitrification and denitrification, or the development of novel separated sludge systems adapted to MBR technology and inspired from treatment schemes such as the Dephanox process.

Conclusions

The nitrogen removal ability of two enhanced biological phosphorus removal (EBPR) configurations adapted to MBR, one with pre-denitrification and the other one with post-denitrification without addition of carbon, were assessed and compared during long-term trials with pilot plants. The mechanisms of post-denitrification, and especially the question of the carbon source, were investigated. The salient results were:

- The denitrification rates observed with post-denitrification without dosing of external carbon source were clearly, and surprisingly, much above endogenous rates.
- Removing the anaerobic reactor reduced the DNR in the downstream anoxic zone by 45% and effluent values for nitrate were significantly higher. Long-term DNR batch tests showed that the DNR dropped suddenly after six hours by 36% in EBPR sludge and remained constant afterwards. In contrast, the DNR of non-EBPR sludge was steady over more than 24h. Hence, EBPR sludge offers a C-source for post-denitrification which is not present in non-EBPR sludge. Moreover this C-source evolved in time, as a reduction of DNR was noticed after few hours.
- Results from parallel batch tests comparing anoxic and anaerobic conditions with wash or unwashed sludge could show that lysis phenomena and dissolved substrate cannot explain the difference of C-source. The organic matter used for post-denitrification in starving sludge must therefore originate from internal storage mechanism.
- Finally, it was shown that glycogen, stored by the cells during the aerobic phase of the EBPR process, was degraded under anoxic conditions, when P-uptake was almost completed.
- We can therefore express the hypothesis that the stored glycogen was used as internal carbon source for denitrification in post-denitrification system when combined with EBPR.
- This biological mechanism, responsible for improved nitrogen removal performances, and which opens new avenues of wastewater treatment processes, could not be explained by the action of denitrifying phosphate accumulating bacteria (DNPAO) only, considering the so far observed DNPAO metabolisms. The tests indicated that some glycogen accumulating bacteria (GAO) are probably responsible for these enhanced denitrification.

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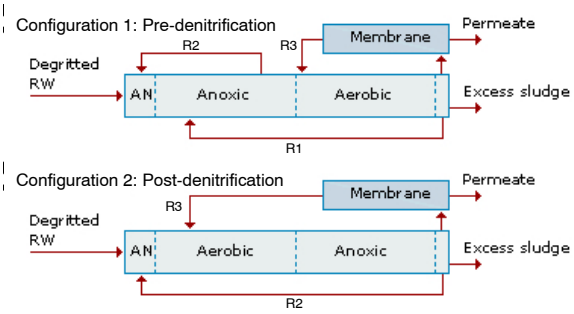

Figure 1: Flow sheet of pilot plants PP1 and PP2.

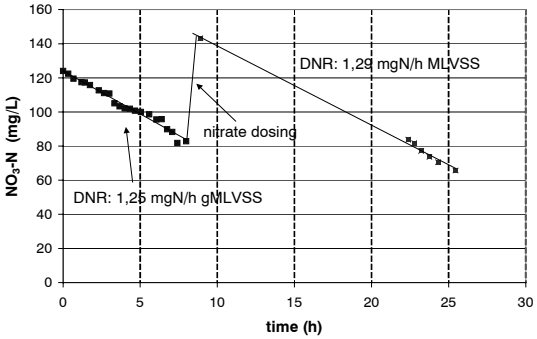

Figure 2: Long-term DNR of non-EBPR sludge from BSP.

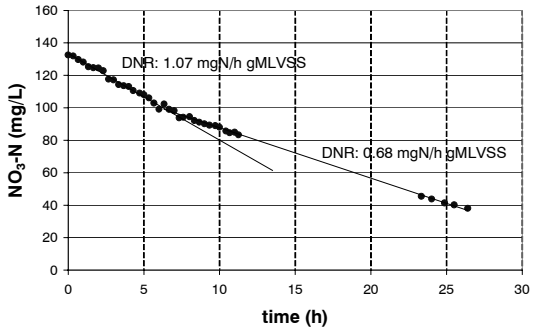

Figure 3: Long-term DNR of EBPR sludge from PP2.

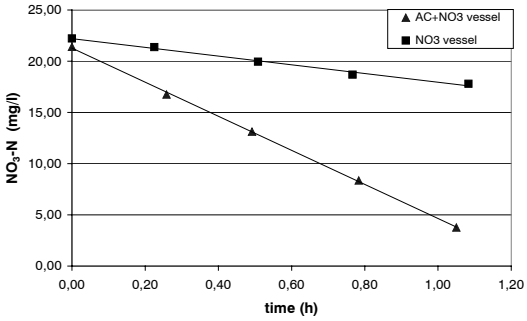

Figure 4 Nitrate evolution in the acetate+nitrate and the only nitrate vessel (unwashed sludge).

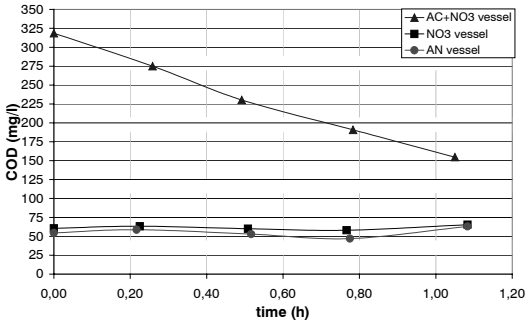

Figure 5 COD evolution for in three vessels (unwashed sludge).

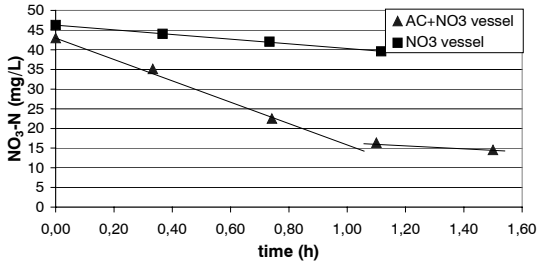

Figure 6: Nitrate evolution in acetate+nitrate and only-nitrate vessel (washed sludge).

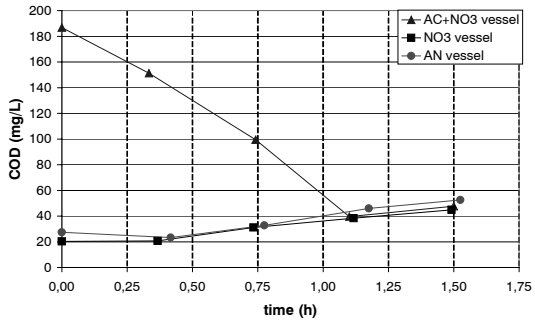

Figure 7: COD evolution in all three vessels (washed sludge).

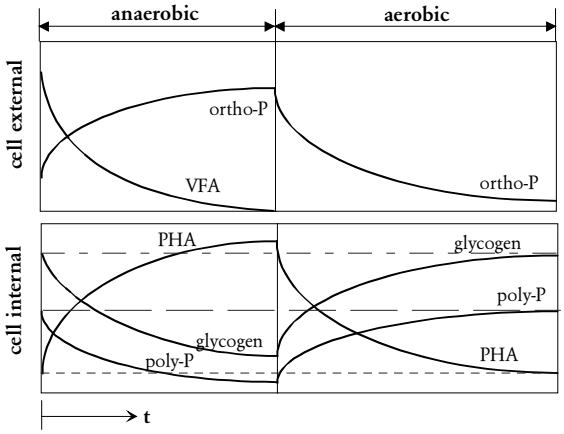

Figure 8: Proposed behavior of cell internal and external compounds under anaerobic and aerobic conditions within a EBPR process by Mino et al. (1998).

Preprint of an article published in:
Water Res. 39 (14) 3360-3368 (2005).

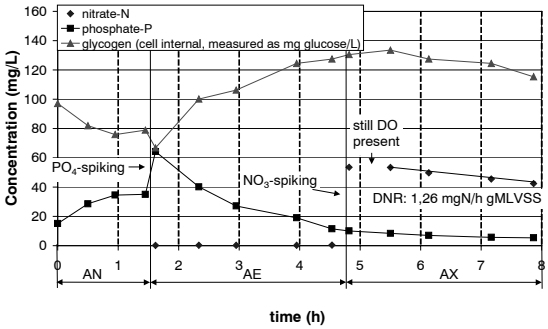

Figure 9: Evolution of glycogen, ortho-phosphate and nitrate in a batch test under anaerobic, aerobic and anoxic conditions.

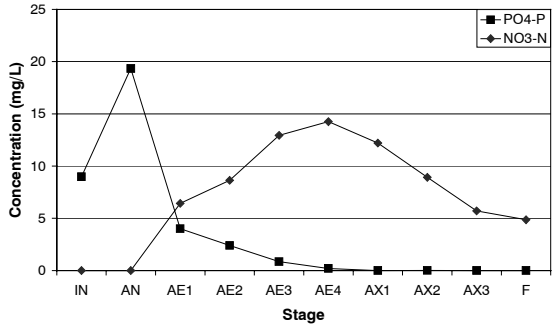

Figure 10: typical nitrate and phosphate profile in the BSP. Average calculated DNR_O= 2.6 $mgNO_3$-N/h gMLVSS for 20°C. Phosphate is below qualification limit(0.05mg/L) in all anoxic stages. IN= Influent, F= Filtration stage.