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Crude glycerol as a carbon source at a low COD/N ratio provides efficient and stable denitritation

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ABSTRACT

The efficiency and kinetics of denitritation and sludge production in synthetic wastewater with crude glycerol as a carbon source (COD/N 3.0) and nitrite as a sole nitrogen source were determined. The influence of volumetric exchange ratio n (30, 50, and 70% cycle^{-1}) and cycle length t_c (24 and 12 h) on both processes was investigated. Denitritation effectiveness was around 100%. With $t_{c,24\ h}$, denitritation proceeded in fast and slow phases and n increased the process rate. In fast phase, denitritation rate increased from 46.59 to 112.19 mg N-NO$_2$/L h (0.28–0.51 mg N-NO$_2$/mg VSS d). With $t_{c,12\ h}$, process rate increased with n of 30 and 50% cycle^{-1}. At n of 70% cycle^{-1}, denitritation was complete less than 1 h before the end of the 12-h cycle; thus, if nitrite concentration in the influent slightly increased, $t_{c,12\ h}$ might be insufficient to complete denitritation. Both n and t_c influenced daily biomass production (24.72–111.63 mg MLSS/L d) but did not influence experimentally determined heterotrophic sludge yield Y_{HDexp} (0.14 ± 0.02 g VSS/g COD).

Keywords: Denitritation; Crude glycerol; Heterotrophic sludge yield; Nitrite reduction; Sludge production

1. Introduction

One of the main problems in wastewater treatment is the elimination of nitrogen. Despite the development of effective technologies for the removal of nitrogen from municipal wastewater (nitrification followed by denitrification), there remain difficulties with nitrogen removal from wastewater with a low COD/N ratio (e.g. landfill leachate, reject water) because of lack of available carbon for denitrification. To address this lack, nitrite denitrification can be used, as the demand for organics is up to 40% lower in nitrite reduction than in nitrate reduction when methanol is used as a carbon source [1]. Other advantages of short-cut nitrification and short-cut denitrification over traditional complete nitrification and denitrification include 25% lower oxygen consumption during nitrification, and nitrite denitrification rates that are up to 2 times faster than those of complete denitrification [1]. The short-cut processes are especially advantageous in the treatment of wastewater with a high ammonium concentration or a low COD/N ratio [2,3]. Because successful procedures for the short-cut nitrification process have been reported, the present study focuses on short-cut denitrification in wastewater with a low COD/N ratio.

The lack of available carbon in such wastewater makes it necessary to supply carbon from external sources during treatment. The most commonly used

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external carbon sources are low molecular weight alcohols (e.g. methanol and ethanol) and volatile fatty acids (e.g. acetic acid), all of which are commercially available. These carbon sources are often chosen because they enable high denitrification rates. The disadvantage of these carbon sources is that they must be purchased, which increases treatment costs. To lower the costs incurred when adding carbon, the following two different strategies can be used: (i) technological solutions involving processes based on short-cut denitrification (denitritation) [4,5], which allows complete reduction of nitrite at an organics concentration that is 22–38% lower than that used for nitrate reduction [6] and (ii) the use of waste products as carbon sources such as industrial effluents, primary sludge, the supernatant of thermally treated wastewater sludge, or the organic fraction of municipal solid waste with molasses added [7–13]. To further reduce costs, both the above-mentioned strategies can be used. Thus, crude glycerol, an industrial effluent, was used for nitrogen removal by denitritation in this study.

Crude glycerol, a by-product of biodiesel production, has great potential as a carbon source. The rising international use of biodiesel means not only is crude glycerol increasingly available but its production currently exceeds demand [14]. This makes it potentially cost-effective. However, factors other than just costs must be taken into account when selecting an external carbon source.

In addition to the rate and effectiveness of denitritation, sludge production is a factor that should be considered because sludge treatment has become more challenging and costly due to increasing restrictions. Until now, however, most studies have focused on effective nitrogen removal but have given very little attention to biomass production, especially in anoxic conditions. Instead, aerobic conditions have usually been used in research on biomass yield, which is the combination of biomass growth, accumulation and storage. Thus, information about biomass yield in anoxic conditions during denitritation is relatively sparse.

Although sludge production has been reported to be lower under anoxic conditions than under aerobic conditions, this has rarely been quantified experimentally with daily measurements of the biomass concentration in a bioreactor. In ASMs, it is assumed that the aerobic yield will be 0.67 g COD/g COD, whereas the anoxic yield will be about 0.53 g COD/g COD [15]. The anoxic yield is lower because when nitrate serves as an electron acceptor only 2 mol of ATP are formed per pair of electron moles transferred to nitrate in the electron transport chain, instead of the 3 mol that are formed when the electrons are transferred to oxygen under aerobic conditions. Thus, less energy is captured by the microorganisms when nitrate serves as the electron acceptor during oxidation of the substrate, which should lead to an anoxic yield coefficient that is lower than the aerobic yield coefficient [16,17]. In fact, the heterotrophic anoxic yield has been found to be even lower than assumed in ASMs when using respirometric batch-test protocols during treatment of municipal wastewater [17]. In other studies, the sludge yield under anoxic conditions has been determined on the basis of COD balance and stoichiometric calculations [18,19]. It should be emphasized that studies on biomass yield on the basis of daily biomass production are not common. Moreover, all of the studies on biomass yield in anoxic conditions that are mentioned here were conducted with nitrate, not nitrite, as the electron acceptor, and with municipal wastewater or simple carbon sources such as acetate, but not with complex carbon sources like waste products. Therefore, there is a need to experimentally determine biomass production and the effectiveness of nitrite removal with waste products as a carbon source.

In an earlier work [20], the authors investigated how adjusting the COD/N ratio affects effectiveness of denitritation with nitrite as a sole nitrogen source. It was found that when using crude glycerol as an external carbon source, a COD/N ratio of 3.0 results in high nitrogen removal efficiency with low biomass production. However, it is not known how adjusting the volumetric exchange ratio and cycle length when using crude glycerol at this COD/N ratio will influence the effectiveness of nitrogen removal and sludge production. Therefore, this study aimed to determine how these factors affect the efficiency and kinetics of denitritation and biomass yield in anoxic conditions.

2. Materials and methods

2.1. Process configuration

The activated sludge was obtained from the denitrifying tank of the municipal wastewater treatment plant in Olsztyn, Poland. This sludge was used in a previously presented study [20], then mixed and put in the three SBRs used in this study. These reactors were operated in parallel with a working volume of 5 L each. The reactors were equipped with stirrers rotating at 50 rpm and an air supply system. Air was supplied by porous diffusers placed at the bottom of the tank. The system was operated at room temperature (20–22°C). During the adaptation period, to maintain a stable biomass concentration at ca. 3.5 g MLSS/L and SRT 20 d in all three reactors, the defined purge of biomass was performed. During the period in which the increase in biomass concentration was investigated, there was no purging of biomass from the SBRs. There were two stages. In both, the COD/N ratio in the influent was maintained at 3.0. In Stage I, the SBRs were operated with a 24 h working cycle. A 12-h cycle was used in Stage II. Each cycle consisted of the following phases: filling (5 min), mixing (22 h of the 24-h working cycle, 10 h of the 12-h working cycle), aeration (1 h), settling, and decantation (1 h). In both stages, the volumetric exchange ratios were 30, 50, and 70% per cycle (Table 1). In the notation that will be used here, the subscript indicates the number of hours in the operational cycle and the volumetric exchange ratio, e.g. SBR_{24_30} means the reactor with a cycle length of 24 h and volumetric exchange ratio of 30% per cycle. Thus, in Stage I, there were SBR_{24_30}, SBR_{24_50}, SBR_{24_70}. In Stage II, there were SBR_{12_30}, SBR_{12_50}, SBR_{12_70}.

2.2. Characteristics of the influent

The reactors were fed with synthetic, high nitrite wastewater (nitrite was a sole nitrogen source), with the addition of crude glycerol as an external carbon source. The synthetic wastewater used in this study contained the following concentrations of solutes: NaCl, 1.01 mg/L; $Na_2HPO_4 \cdot 12H_2O$, 184.8 mg/L; $MgSO_4 \cdot 7H_2O$, 1.67 mg/L; $CaCl_2 \cdot 2H_2O$, 0.35 mg/L; $MnCl_2 \cdot 4H_2O$, 0.117 mg/L; $FeCl_3 \cdot 6H_2O$, 0.2 mg/L, $NaHCO_3$, 60.8 mg/L, $ZnSO_4$, 0.4 mg/L. Sodium nitrite ($NaNO_2$) was the source of nitrite. The chemical composition of crude glycerol was as follows: glycerol 80–85%, ash (NaCl) < 7%, M.O.N.G. (matter organic non glycerol) < 2%, methanol < 0.5%, with the remaining portion consisting of water (product specification from Biodiesel Manufacturing Plant, Poland). The solution of crude glycerol was prepared in the following way: 113 g of crude glycerol were dissolved in 1 L of distilled water resulting in 100 ± 7 mg COD/ml. Nitrite and COD concentrations in the synthetic wastewater were 200 ± 18 mg N-NO_2/L and 600 ± 32 mg COD/L to give the COD/N ratio of 3.0.

2.3. Chemical analyses

Daily measurements of pollutant concentration in the effluent from the reactors included chemical oxygen demand (COD) and nitrite. The concentrations of mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) in activated sludge were determined. For kinetic analyses in steady-state conditions, COD and nitrite were measured during the SBR cycle. All these determinations were performed according to APHA [21].

3. Results and discussion

3.1. Nitrite and organics removal

3.1.1. The effectiveness of nitrite and COD removal in activated sludge with denitritation

During Stage I, with a 24-h operational reactor cycle, the adaptation period in all reactors lasted about 36 d. In the reactor with a volumetric exchange ratio of 30% $cycle^{-1}$, with an initial N-NO_2 concentration of 60 ± 4 mg/L, the N-NO_2 concentration in the effluent dropped below 2 mg/L after 20 d. In reactors 2 and 3, with volumetric exchange ratios of 50% and 70% $cycle^{-1}$, and initial N-NO_2 concentrations of 100 ± 8 and 140 ± 11 mg/L, respectively, the N-NO_2 concentration in the effluent dropped below 2 mg/L after 30 d. In all reactors, N-NO_2 concentrations in the effluent remained close to 0 mg/l after 36 d, indicating almost 100% effective nitrite removal (Fig. 1). This means about 40 d were needed for acclimation during denitritation with crude glycerol as a carbon source. In this study, despite differences in the initial concentration of

Table 1
Organization of the experiment

Parameters	Stage I			Stage II		
	SBR_{24_30}	SBR_{24_50}	SBR_{24_70}	SBR_{12_30}	SBR_{12_50}	SBR_{12_70}
Volumetric exchange ratio per cycle (*n*) (%)	30	50	70	30	50	70
Hydraulic retention time (HRT) (d)	3.33	2.0	1.43	1.66	1.0	0.71
COD concentration at the beginning of the SBR cycle (mg/L)	180 ± 14	300 ± 17	420 ± 23	180 ± 14	300 ± 17	420 ± 23
N-NO_2 concentration at the beginning of the SBR cycle (mg/L)	60 ± 4	100 ± 8	140 ± 11	60 ± 4	100 ± 8	140 ± 11
COD/N	3.0 ± 0.3					

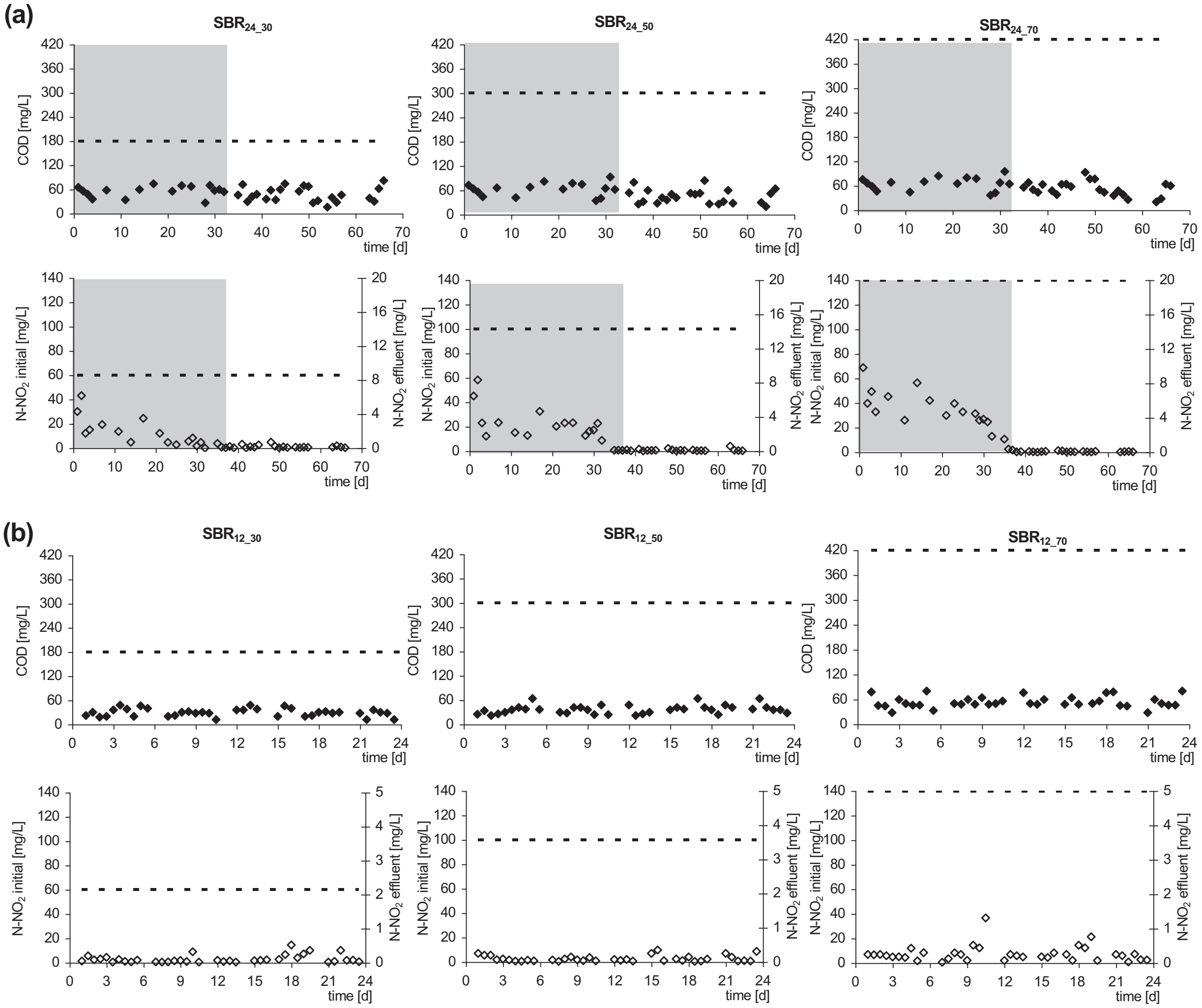

Fig. 1. Changes in nitrite and organics (COD) concentration in the reactor effluent (a) Stage I and (b) Stage II; dashed lines indicate the nitrite and organics (COD) concentrations in wastewater at the beginning of the reactor cycle, gray shaded areas show the adaptation period in Stage I.

COD, due to differences in the volumetric loading ratio, the COD concentrations in the effluent from all three reactors were similar. The averages of these concentrations ranged from 43 ± 5 to 52 ± 7 mg COD/L, and the effectiveness of COD removal increased with an increase in volumetric exchange ratio from 72% at 30% cycle^{-1} to 87% at 70% cycle^{-1} (Fig. 1).

In Stage II, cycle length was reduced to 12 h, which means that nitrogen and COD loadings were doubled. In spite of this, nitrite was still effectively removed and process effectiveness was higher than 99%. N-NO_2 concentrations in the effluent did not differ greatly between the three SBRs, and even in SBR_{24_70}, the nitrite concentration remained below 0.6 mg/L. This was despite this SBR having the highest volumetric exchange ratio, giving the highest initial concentration of N-NO_2. However, in this stage, the volumetric exchange ratio affected the concentration of COD in the effluent. The average concentration of COD increased with greater initial concentrations of COD: with an initial COD concentration of 180 ± 14 mg/L, the concentration in the effluent averaged 29 ± 4 mg COD/L; with an initial concentration of 300 ± 17 mg COD/L, effluent concentration averaged 37 ± 6 mg COD/L; with an initial concentration of 420 ± 33 mg COD/L, it was 53 ± 7 mg COD/L. The efficiency of COD removal in all three reactors ranged from 82 to 87%.

Our results indicate that at a COD/N ratio of 3.0, nitrogen is effectively removed via denitritation with crude glycerol as a carbon source, regardless of the volumetric exchange ratio. Similarly, Torá et al. [13]

showed that denitritation was effective with a COD/N ratio of 3.0, but using ethanol as a carbon source. In the case of using glycerol, to obtain complete denitritation, demand for organic was higher (COD/N 3.8). In contrast, Fernández-Nava et al. [22] found that during denitrification with three alternative sources of carbon, nitrogen removal was most effective at higher COD/N ratios. They used two sugar-rich carbon sources (residue from a soft drinks factory and wastewater from a sweets factory) and a carbon source rich in lactic acid (residue from a dairy plant). The optimum COD/N ratios were 5.5–6.5 for the sugar-rich carbon sources and ca. 4.6 for the lactic-acid-rich carbon source.

The results of the present study show effective denitritation even at a high volumetric exchange ratio (70% $cycle^{-1}$) with a 12-h cycle. However, in order to check whether the technological process is stable in these conditions, and whether a possible increase in nitrite concentration in the raw sewage affects the efficiency of the process, the kinetics of the process were studied.

3.1.2. The rates of denitritation and organics removal

To find the optimal cycle length, it was necessary to measure the rates of removal of nitrite and carbon compounds. To do this, the kinetics of changes in the concentration of nitrite and carbon compounds were determined. The rate constants for denitritation and COD removal were found. These processes proceeded according to zero-order kinetics, involving linear changes in nitrite and COD concentrations with time. The process rates (r_D—short-cut denitrification rate, r_{COD}—organics removal rate) were equal to the rate constants. With a 24-h cycle length, these processes proceeded in two phases: fast and slow (Fig. 2(a)). For both nitrite and COD removal, the fast phases all lasted about 1 h and were characterized by higher rate than the slow phases. In the fast phases of denitritation, the rate was 46.6 mg $N\text{-}NO_2$/L h (0.28 mg $N\text{-}NO_2$/mg VSS d) at a volumetric exchange ratio of 30% $cycle^{-1}$; the rate increased with increases in the volumetric exchange ratio, up to 112.2 mg $N\text{-}NO_2$/L h (0.67 mg $N\text{-}NO_2$/mg VSS d) at n 70% $cycle^{-1}$. There was a similar increase in the denitritation rate of the slow phases as volumetric exchange ratios increased. Similar trends were observed for COD removal: the rate of the fast phase of COD removal (102.9–319.5 mg COD/L h) were higher than the slow phases (9.4–18.23 mg COD/L h), and higher the volumetric exchange ratio, the higher the rate of the fast and slow phases.

Although with a 24-h cycle length, the rate of nitrite removal in the slow phase was a few times lower than in the fast phase, nitrite was completely removed shortly after 4 h. Similarly, Fernández-Nava et al. [22] showed that effluents were nitrate free and had very low COD concentrations with a 4–6 h, especially with sugar-rich carbon sources (wastewater from a sweet factory and residue from a soft drinks factory). However, their research was on denitrification, not denitritation.

In this study, because the time needed for nitrite removal was short (~4–5 h), the cycle was shortened to 12 h. With the 12-h cycle length, there were two phases at n of 70% $cycle^{-1}$, whereas there was only a fast phase at the two lower n (30 and 50% $cycle^{-1}$) (Fig. 2(b)). At n of 30 and 50% $cycle^{-1}$, nitrite and COD removal were complete after about 1 h (0.36–0.50 mg $N\text{-}NO_2$/mg VSS d; 0.76–1.21 mg COD/mg VSS d). This indicates that the cycle could be further shortened at these volumetric exchange ratios. However, at n of 70% $cycle^{-1}$, two-phase denitritation and organics removal was complete less than 1 h before the end of the 12-h cycle. This indicates that the cycle length should be longer at n of 70% $cycle^{-1}$ because a slight increase in nitrite concentration in the influent may render the cycle length insufficient to complete nitrate reduction, which would lead to a loss of process stability.

An increase in n corresponds to an increase in the initial substrate concentration, and in turn, the initial nitrite concentration influences the specific nitrite removal rate (according to Michaelis–Menten kinetics). In this study, the denitritation rate in the fast phase increased from 0.28 to 0.51 mg $N\text{-}NO_2$/mg VSS d when the initial nitrite concentration was increased (as a result of increasing n) with a 24-h cycle length. When the cycle length was halved to 12 h, this doubled the nitrite loading per day. With the 12-h cycle, the denitritation rate increased from 0.34 to 0.38 mg $N\text{-}NO_2$/mg VSS d only at n of 30 and 50% $cycle^{-1}$. At n of 70% $cycle^{-1}$, the nitrite removal rate decreased, which may be connected with the high daily nitrite loading. Chung and Bae [6] reported that the maximum rate of nitrite reduction decreased with a higher initial nitrite concentration, although complete removal eventually occurred. Their smallest maximum specific nitrite removal rate was approximately 0.09 mg $N\text{-}NO_2$/mg VSS d when the initial nitrite concentration was 200 mg $N\text{-}NO_2$/L or higher.

Most reports concern denitrification, and literature data on nitrite removal rates with the use of alternative carbon sources are few. Barlindhaug and Ødegaard [7] reported a denitrification rate of 1.4 kg $N\text{-}NO_3/m^3$ d at a $COD/N\text{-}NO_3$ ratio of 8.0 in a biological bed with

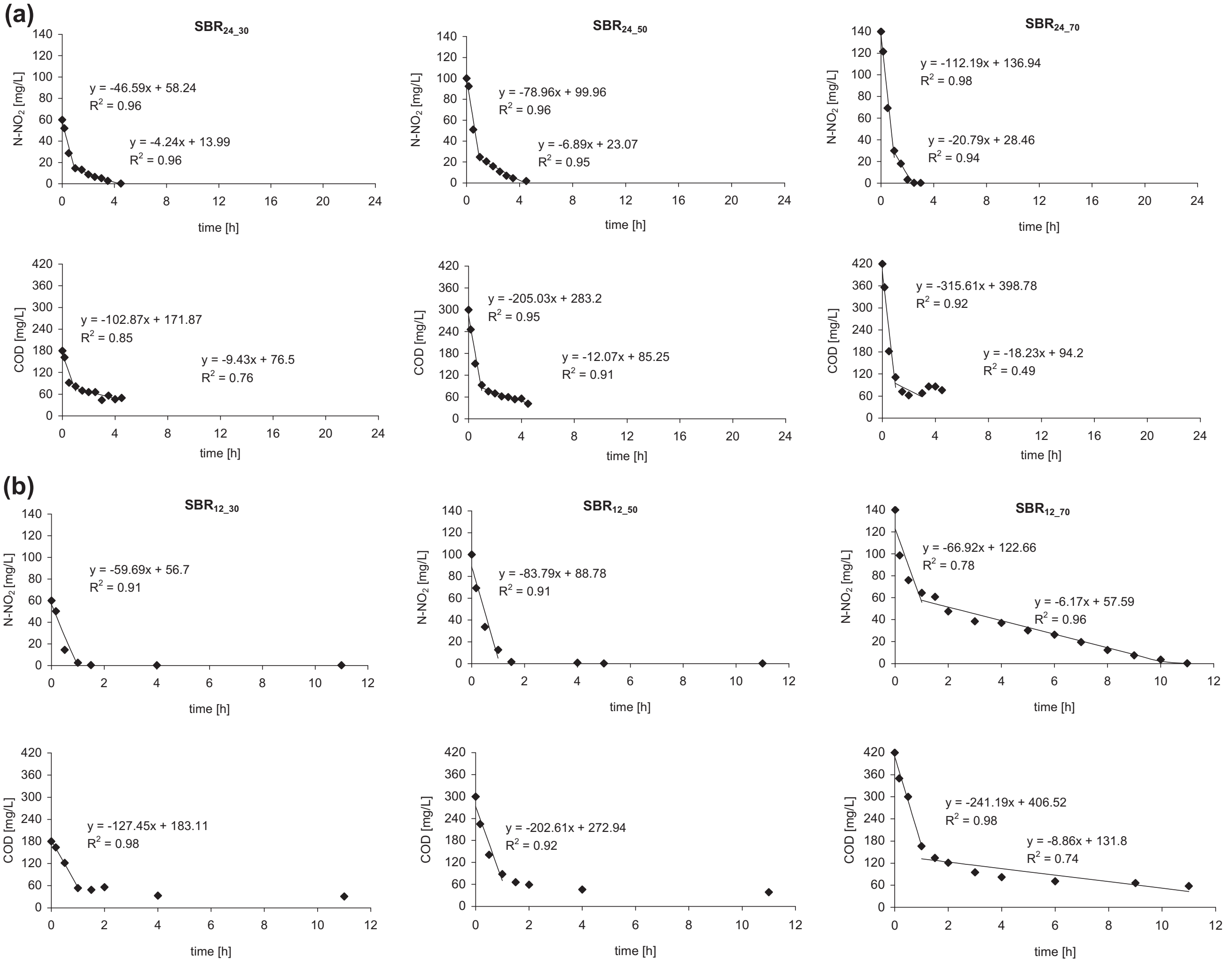

Fig. 2. Changes in nitrite and organics (COD) concentration during SBR cycles (a) Stage I and (b) Stage II.

hydrolyzed excess sludge as a carbon source. Æsøy et al. [23] found a denitrification rate nearly two-times higher with a similar carbon source (a mixture of hydrolyzed sludge and the organic fraction of municipal waste). Elefsiniotis et al. [24] reported a mean denitrification rate of 0.0111 g $N\text{-}NO_x$/g VSS d with VFA as a carbon source that was generated from an anaerobic digester (treating a mixture of starch-rich industrial and municipal wastewater). These differences may result from the fact that these experiments differed in operational conditions, dosage of organic carbon, reactor type, and kind of wastewater being treated (industrial, municipal, or synthetic). A few studies report denitritation rates with simple commercial carbon sources instead of waste products. For example, Chung and Bae [6] reported denitritation rates from 0.76 to 1.59 mg $N\text{-}NO_2$/mg VSS d with glucose. Queiroz et al. [25] found that the volumetric denitritation rate was 0.091–0.111 kg $N\text{-}NO_2/m^3$ d with phenol as a carbon source. Torá et al. [13] used both simple carbon sources and waste products in denitritation of high-strength nitrite wastewater. With simple organics, they found a denitritation rate of 0.17 mg N/mg VSS d with ethanol as a carbon source, and 0.25 mg N/mg VSS d with glycerol. With waste products, they reported 0.13 mg N/mg VSS d with fermented primary sludge centrate, and 0.16 mg N/mg VSS d with landfill leachate.

3.2. Sludge production

A net increase in biomass concentration during wastewater treatment occurs when the internal storage of substrate in microbial cells and new cell biosynthesis exceed the loss of biomass due to storage polymer degradation, cell lysis, and death. Although it is known that biomass growth occurs simultaneously with wastewater treatment, biomass production is often overlooked in technological studies. Therefore, to address this issue, the biomass concentration during steady state conditions was measured in the present study.

With a 24-h cycle length, the initial concentration of biomass in the reactors was about 3.5 g MLSS/L at all volumetric exchange ratios (Fig. 3(a)).

As the experiment progressed, the concentration of biomass increased. On the basis of these linear changes in concentration, the rate of biomass increase was determined. Sludge production proceeded according to zero-order kinetics. The rate of biomass increase was highest (69.11 mg MLSS/L d) with the highest *n* (70% cycle^{-1}), and it was lowest (24.72 mg MLSS/L d) with the lowest *n* (30% cycle^{-1}). When the cycle length was changed to 12 h, excess sludge was removed to obtain similar initial concentrations of biomass in all reactors. Sludge production again proceeded according to zero-order kinetics and rates of biomass production also increased with increases in the volumetric exchange ratio (Fig. 3(b)). However, at each *n*, the rate of change in biomass concentration was higher with a 12-h cycle than with a 24-h cycle.

The literature rarely reports actual measurements of biomass concentration like those presented here, even though these measurements provide precise information about daily increase in biomass concentration. Instead, this increase is often reported as the heterotrophic sludge yield (Y_{HD}), which is estimated on the basis of respirometric measurements or on the basis of the stoichiometric organic carbon demand for nitrite/nitrate reduction in anoxic conditions [17–19]. When making this estimate, the difference between the total amount of organic compounds consumed for both biomass production and nitrite reduction ($\text{COD}_{\text{consumed}}$), and the amount of organics consumed to reduce 1 gram of nitrite ($\text{COD}_{\text{consumed,N-NO}_2}$) is regarded as the amount of organics used to produce biomass ($\text{COD}_{\text{consumed,biomass}}$). Using the value of $\text{COD}_{\text{consumed,N-NO}_2}$, the heterotrophic sludge yield (Y_{HD}) can be calculated as follows:

$$Y_{\text{HD}} = 1 - \frac{1.72\text{N} - \text{NO}_{2\text{red}}}{\text{COD}_{\text{consumed}}} \ (\text{g VSS/g COD}) \qquad (1)$$

where 1.72 is the assumed (approximate) amount of organics used to reduce nitrite ($\text{COD}_{\text{consumed,N-NO}_2}$). This value of 1.72 g COD/g N-NO_2 used by Frison et al. [19] was obtained on the basis of the stoichiometric equation for nitrite reduction with methanol as a carbon source. In theory, the stoichiometric demand for methanol as a carbon source during denitrification would amount to 2.47 mg CH_3OH/g N-NO_3, which gives 2.86 g COD/g N-NO_3. When nitrite is denitrified, the stoichiometric demand for methanol is lower, 1.48 mg CH_3OH/gN-NO_2 (1.72 g COD/g N-NO_2). The theoretical value of 1.72 g COD/g N-NO_2 corresponds only to methanol, however in practice may be different even for this substrate. For other carbon sources, such as ethanol, acetic acid or waste products like crude glycerol, this estimate is even less accurate, because the stoichiometric demands are different [26,27].

In the present study, regardless of the volumetric exchange ratio and cycle length, Y_{HD} calculated on the basis of Eq. (1) averaged 0.3 ± 0.02 g VSS/g COD

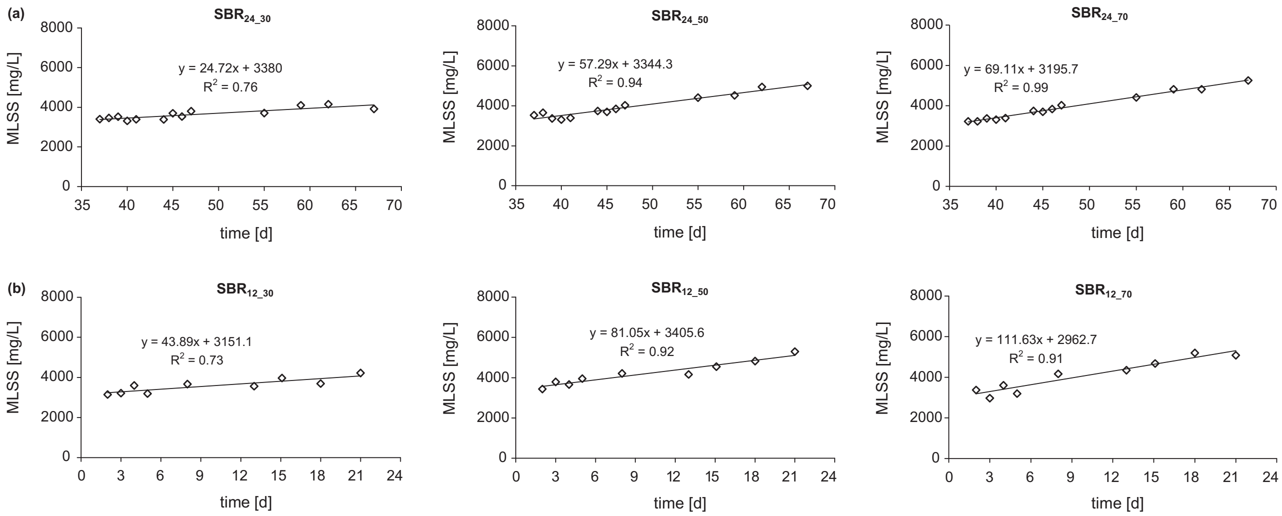

Fig. 3. Changes in MLSS concentration in Stage I at 24-h cycle length and in Stage II at 12-h cycle length.

(0.44 ± 0.03 g COD/g COD). However, because this calculation used only an approximate value of organics consumed to reduce 1 gram of nitrite ($COD_{consumed,N\text{-}NO_2}$), it should be stressed that this value of Y_{HD} is approximate. This potential for error is why it is important to experimentally determine the daily production of sludge by measuring biomass concentration in the reactor.

In this study, the concentration of the organic fraction of the biomass (VSS) was 70% of the MLSS. The experimental sludge yield (Y_{HDexp}) was determined on the basis of the rate of changes in biomass concentration (mg VSS/L d), the biomass concentration in the effluent (mg VSS/L d) and the amount of COD removed during the cycle of the SBRs (mg COD/L d). At a COD/N ratio of 3.0, and regardless of the volumetric exchange ratio and cycle length, Y_{HDexp} was 0.21 ± 0.03 g COD/g COD (0.14 ± 0.02 g VSS/g COD), which was approximately 2 times lower than the value calculated from the equation given by Frison et al. [19] (0.44 ± 0.03 g COD/g COD) and lower than in other studies. However, it should be emphasized that most of those studies were conducted with nitrate as an electron acceptor, not nitrite, and with pure carbon sources, not waste products. For example, Majone et al. [28] investigated sludge production during denitrification with acetate, ethanol, glucose, and glutamic acid. They found that the biomass yield coefficient was highest with glucose as an electron donor (0.74 g COD/g COD), followed by ethanol (0.7 g COD/g COD), acetate (0.65 g COD/g COD), and glutamic acid (0.56 g COD/g COD). Dionisi et al. [18] found that, at a stable COD/N ratio of 3.5, both increasing organic loading rate and increasing the duration of feeding mainly affected storage yield but did not substantially affect overall yield (0.62–0.68 g COD/g COD). The growth of the biomass expressed as the observed growth yield (0.01–0.08 g COD/g COD) contributed from 1.63 to 25% to the overall biomass yield, and the rest was contributed by accumulation and storage. In their study, the overall yield was higher than the biomass yield in the present study, but their initial concentration of COD was several times higher than that used here (1,067–4,267 mg COD/L vs. 180–420 mg COD/L). Muller et al. [17] used municipal wastewater and found that the heterotrophic anoxic yield was 0.42 g COD/g COD (0.28 g VSS/g COD), although they used respirometric batch-test protocols. However, it must be emphasized that in the case of mentioned studies there was no ammonium limitation, in contrary to this study where nitrite was a sole nitrogen source. During wastewater treatment, when ammonium and nitrate/nitrite are present in wastewater, ammonium is preferable for biomass synthesis. When ammonium is not available, nitrate/nitrite are used for biomass growth. In order to use nitrate/nitrite as a nutrient, these oxidized forms of nitrogen must be reduced. This means that oxygen must be removed and hydrogen must be added to the nitrogen atom to form ammonium inside the bacterial cell. Changes in oxidation state of nitrogen from +5 (nitrate) or +3 (nitrite) to −3 (ammonium) are essential because in biosynthetic pathways (processes for producing cellular material) nitrogen is represented in an inorganic form and an oxidation state of −3. The use of nitrite and nitrate as a nutrient source for nitrogen is referred to as assimilatory nitrate or nitrite reduction. The reduction in oxidation state for each oxidized nitrogen forms requires cellular energy. Therefore, less bacterial growth or MLVSS production is achieved using nitrate/nitrite as compared to the bacterial growth obtained by using ammonium [29].

On the basis of Y_{HDexp} and the amount of $N\text{-}NO_2$ and COD removed during the cycle, the amount of organics used to reduce nitrite ($COD_{consumed,N\text{-}NO_2}$) with crude glycerol as a carbon source was determined:

$$COD_{consumed,N\text{-}NO_2} = \frac{\text{CODconsumed} - Y_{HDexp}\ \text{COD consumed}}{N\text{-}NO_{2red}}\ (\text{g COD/g N-NO}_2)$$

Regardless of the volumetric exchange ratio and cycle length, the value of $COD_{consumed,N\text{-}NO_2}$ was 2.0 ± 0.09 g COD/g $N\text{-}NO_2$ at the COD/N ratio of 3.0 used in this study.

Another value that is sometimes reported is the specific COD consumption ($COD_{specific}$). This is the total amount of organic compounds removed in the cycle divided by the amount of nitrite/nitrate reduced (g COD/g $N\text{-}NO_2(N\text{-}NO_3)$). Chung and Bae [6] found that the specific consumption of COD ($COD_{specific}$) was 22–38% lower for nitrite reduction than for nitrate reduction when glucose was the carbon source. $COD_{specific}$ for nitrite reduction was 3.8–4.3 g COD/g $N\text{-}NO_2$; for nitrate reduction, 5.3–6.3 g COD/g $N\text{-}NO_3$. In theory, the reduction in COD consumption should be around 40%, because nitrite is reduced by three electron equivalents per mol of N, while nitrate is reduced by five electron equivalents per mol of N.

In the present study, $COD_{specific}$ was 2.5 ± 0.06 g COD/g $N\text{-}NO_2$ with all volumetric exchange ratios and both cycle lengths. $COD_{specific}$ was higher than $COD_{consumed,N\text{-}NO_2}$ because the former does not distinguish between the amount of organic compounds used for biomass production and for nitrite reduction. Thus, determining Y_{HDexp} allows the calculation of $COD_{consumed,N\text{-}NO_2}$. This allows the calculation of the difference between $COD_{specific}$ and $COD_{consumed,N\text{-}NO_2}$, which indicates the amount of organic compounds used for biomass production. Frison et al. [19] found a higher specific COD consumption (2.7–3.1 g COD/g $N\text{-}NO_2$) than in the present study. This was probably because they used different waste products: raw and fermented drainage liquids from the organic fraction of municipal solid waste, cattle manure, and maize silage. Torá et al. [13] reported higher values of $COD_{specific}$ than in the present study, both with simple carbon sources, 3.0 g COD/g $N\text{-}NO_2$ for ethanol and 3.8 g COD/g $N\text{-}NO_2$ for glycerol, and with waste products, 5.5 g COD/g $N\text{-}NO_2$ with fermented primary sludge centrate and 8.8 g COD/g $N\text{-}NO_2$ with landfill leachate.

The rates of biomass increase, determined experimentally, were used to calculate the total daily sludge production in the reactors (ΔX_{exp}). This was compared with daily sludge production calculated on the basis of the heterotrophic sludge yield (ΔX_{YHD}) (Fig. 4).

Higher volumetric exchange ratios gave higher ΔX_{exp}; for example, with a 24-h cycle and at a volumetric exchange ratio of 70% cycle^{-1}, daily biomass production was 0.2 g VSS/d, and at a volumetric exchange ratio of 30% cycle^{-1}, ΔX_{exp} was 0.03 g VSS/d. With a shorter cycle length, the daily production of biomass for each volumetric exchange ratio increased; for example, at a volumetric exchange ratio of 50% cycle^{-1}, daily biomass production was 0.11 g VSS/d with a 24-h cycle, and 0.17 g VSS/d with a 12-h cycle.

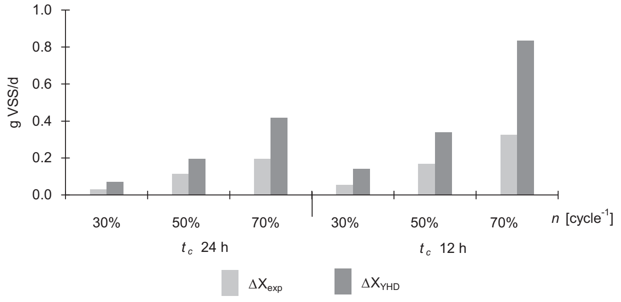

Fig. 4. Daily biomass production on the basis of daily measurements of biomass concentration in the reactors and biomass concentration in the effluent Y_{HDexp} (ΔX_{exp}) and calculated on the basis of heterotrophic sludge yield Y_{HD} (ΔX_{YHD}).

From the comparison of the daily biomass production on the basis of daily measurements of biomass concentration (ΔX_{exp}) and calculated on the basis of heterotrophic sludge yield (ΔX_{YHD}), it can be conclude that the values of ΔX_{YHD} are 1.7–2.5 times higher than the values of $\Delta X_{exp.}$ Thus, ΔX_{YHD} is only an approximate value, and daily biomass concentration should be monitored to determine real daily biomass production.

4. Conclusions

Crude glycerol can be used to provide efficient denitritation with a COD/N ratio of 3.0 in synthetic wastewater with nitrite (200 ± 18 mg $N\text{-}NO_2$/L) as a sole nitrogen source at n of 30, 50, and 70% cycle^{-1}, and t_c of 24 and 12 h. n and t_c influence both denitritation and biomass production rates. At $t_{c,12\ h}$ and n of 70% cycle^{-1}, denitritation finished less than 1 h before the end of the cycle. This means that variations in the influent can destabilize the process. Daily biomass production (24.72–111.63 mg MLSS/L d) was influenced by both n and t_c. These parameters did not affect experimentally determined heterotrophic sludge yield Y_{HDexp} (0.14 ± 0.02 g VSS/g COD). Both low daily biomass production and Y_{HDexp} may be a result of a lack of ammonium in wastewater.

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References

[1] Y. Peng, G. Zhu, Biological nitrogen removal with nitrification and denitrification via nitrite pathway, Appl. Microbiol. Biotechnol. 73 (2006) 15–26.

[2] J. Surmacz-Gorska J, A. Cichon, K. Miksch, Nitrogen removal from wastewater with high ammonia nitrogen concentration via shorter nitrification and denitrification, Water Sci. Technol. 36 (1997) 73–78.

[3] B. Schina, A.P. Annachhatre, Partial nitrification—Operational parameters and microorganisms involved, Rev. Environ. Sci. Biotechnol. 6 (2007) 285–313.

[4] C. Fux, S. Velten, V. Carozzi, D. Solley, J. Keller, Efficient and stable nitritation and denitritation of ammonium-rich sludge dewatering liquor using an SBR with continuous loading, Water Res. 40 (2006) 2765–2775.

[5] S. Aslan, M. Dahab, Nitritation and denitritation of ammonium-rich wastewater using fluidized-bed biofilm reactors, J. Hazard. Mater. 156 (2008) 56–63.

[6] J. Chung, W. Bae, Nitrite reduction by a mixed culture under conditions relevant to shortcut biological nitrogen removal, Biodegradation 13 (2002) 163–170.

[7] J. Barlindhaug, H. Odegaard, Thermal hydrolysate as a carbon source for denitrification, Water Sci. Technol. 33 (1996) 99–108.

[8] R. Moserengeler, K.M. Udert, D. Wild, H. Siegrist, Products from primary sludge fermentation and their suitability for nutrient removal, Water Sci. Technol. 38 (1998) 265–273.

[9] P. Pavan, P. Battistoni, P. Traverso, A. Musacco, F. Cecchi, Effect of addition of anaerobic fermented OFMSW (organic fraction of municipal solid waste) on biological nutrient removal (BNR) process: Preliminary results, Water Sci. Technol. 38 (1998) 327–334.

[10] T. Aravinthan, S. Mino, H. Takizawa, H. Satoh, T. Matsuo, Sludge hydrolysate as a carbon source for denitrification, Water Sci. Technol. 43 (2001) 191–199.

[11] T. Ueda, Y. Shinogi, M. Yamaoka, Biological nitrate removal using sugar–industry wastes, Paddy Water Environ. 4 (2006) 139–144.

[12] D. Kulikowska, K. Dudek, Molasses as a carbon source for denitrification, Arch. Environ. Protect. 36 (2010) 35–45.

[13] J.A. Torà, J.A. Baeza, J. Carrera, J.A. Oleszkiewicz, Denitritation of a high-strength nitrite wastewater in a sequencing batch reactor using different organic carbon sources, Chem. Eng. J. 172 (2011) 994–998.

[14] G.P. da Silva, M. Mack, J. Contiero, Glycerol: A promising and abundant carbon source for industrial microbiology, Biotechnol. Adv. 27 (2009) 30–39.

[15] M. Henze, C.P.L. Grady Jr., W. Gujer, Gv.R. Marais, T. Matsuo, Activated Sludge Model No. 1. IAWPRC Scientific and Technical Report No. 1, IAWPRC, London, 1987.

[16] W.J. Payne, Denitrification, Wiley, New York, NY, 1981.

[17] A. Muller, M.C. Wentzel, R.E. Loewenthal, G.A. Ekama, Heterotroph anoxic yield in anoxic aerobic activated sludge systems treating municipal wastewater, Water Res. 37 (2003) 2435–2441.

[18] D. Dionisi, M. Majone, R. Ramadori, M. Beccari, The storage of acetate under anoxic conditions, Water Res. 35 (2001) 2661–2668.

[19] N. Frison, S.D. Fabio, C. Cavinato, P. Pavan, F. Fatone, Best available carbon sources to enhance the via-nitrite biological nutrients removal from supernatants of anaerobic co-digestion, Chem. Eng. J. 215–216(216) (2013) 15–22.

[20] K. Bernat, D. Kulikowska, K. Żuchniewski, Glycerine as a carbon source in nitrite removal and sludge production, Chem. Eng. J. 267 (2015) 324–331.

[21] A.E. Greenberg, L.S. Clesceri, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, eighteenth ed., APHA, Washington, DC, 1992.

[22] Y. Fernández-Nava, E. Marañón, J. Soons, L. Castrillón, Denitrification of high nitrate concentration wastewater using alternative carbon sources, J. Hazard. Mater. 173 (2010) 682–688.

[23] A. Æsøy, H. Ødegaard, K. Bach, R. Pujol, M. Hamon, Denitrification in a packed bed biofilm reactor (Biofor) —Experiments with different carbon sources, Water Res. 32 (1997) 1463–1470.

[24] P. Elefsiniotis, D.G. Wareham, M.O. Smith, Use of volatile fatty acids from an acid-phase digester for denitrification, J. Biotechnol. 114 (2004) 289–297.

[25] L.M. Queiroz, M.V. Aun, D.M. Morita, P.A. Sobrinho, Biological nitrogen removal over nitritation/denitritation using phenol as carbon source, Braz. J. Chem. Eng. 28 (2011) 197–207.

[26] Y.H. Ahn, Sustainable nitrogen elimination biotechnologies: A review, Process Biochem. 41 (2006) 1709–1721.

[27] F. Sun, S. Wu, J. Liu, B. Li, Y. Chen, W. Wu, Denitrification capacity of a landfilled refuse in response to the variations of COD/NO_3^--N in the injected leachate, Bioresour. Technol. 103 (2012) 109–115.

[28] M. Majone, M. Beccari, D. Dionisi, C. Levantesi, V. Renzi, Role of storage phenomena on removal of different substrates during pre-denitrification, Water Sci. Technol. 43 (2001) 151–158.

[29] M.H. Gerardi, Nitrification and Denitrification in the Activated Sludge Process, John Wiley and Sons Inc, New York, NY, 2002.