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Biological Denitrification of High-Nitrate Wastewaters: a Comparison Between Three Electron Donors

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Wastewaters discharged by several industrial activities, such as synthetic fibers, mineral processing, fertilizers, metal finishing, and ammunitions and explosives industries, have an high-salinity content and are characterized by a very high concentration of nitrates (more than 3 g/L). The treatment of these wastewaters generally deals in an anoxic biological process performed in activated sludge reactors (ASR). Due to the practical absence of an organic component, the treatment involves the addition of an external source of carbon, as electron donor for denitrification reactions. In addition, explosives industries wastewaters are characterized by low pH (2-3), since nitrates are generally discharged as nitric acid, and this induces a further difficulty in reactor operation, due to the extreme sensitivity of denitrifying biomass to pH conditions.
In this paper the results of an experimentation performed in a laboratory scale anoxic ASR treating a high-nitrate wastewater, simulating the explosives and ammunitions industries wastewaters, are presented and discussed. Three different carbon sources (methanol, acetic acid and sucrose) were compared, and the conditions to achieve the maximum removal of nitrogen were assessed. The ratio C:N to be maintained in the reactor to optimize cell growth and denitrification rate was also investigated.

1. Introduction

Several industries activities produce large quantities of nitrates as by-product. Synthetic fibers, mineral processing, fertilizers, metal finishing, and ammunitions and explosives industries wastewaters are indeed highly concentrated in nitrates (Cyplik et al., 2012), present in a concentration higher than 1 g/L and generally accompanied by large amounts of other inorganic ions (sulphates, chlorides, etc.) (McAdam and Judd, 2009).
The treatment of these wastewaters generally deals in an anoxic biological process performed in activated sludge reactors (ASR) (Shen et al., 2009), though other technologies are currently under investigation as microwave-assisted degradation (Halasz et al. 2010) and reductive transformation by pyrite and magnetite (Oh et al. 2008). Due to the practical absence of organic compounds in the wastewaters, the treatment involves the addition of an external source of carbon as electron donor for denitrification reactions. In particular, explosives industries wastewaters are also characterized by low pH (2-3), since nitrates are generally discharged as nitric acid, and this induces a further difficulty in reactor operation, due to the extreme sensitivity of denitrifying biomass to pH conditions. It was in fact already shown that under strong acidic conditions denitrification of wastewaters with high concentration of nitrates can be severely inhibited (Eusebi et al., 2009).
Several studies have been performed to assess the optimal operating conditions for biologic denitrification of high-nitrate wastewaters (Constantin and Fick, 1997; Glass and Silverstein, 1997; Glass and Silverstein, 1999; Cortez et al., 2011). Tests were performed in different types of bioreactors, such as sequencing batch reactors (SBR, Mekonen et al., 2001), membrane bioreactors (Shen et al., 2009) anoxic rotating biological contactors (Cortez et al., 2011), attached biomass systems (Oh et al., 2001), using both internal or external (Nair et al., 2007; Fernandez-Nava et al., 2010) carbon source, and also using properly selected microorganisms (Cyplik et al., 2012).

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However, there is a lack of information about the treatment of very high nitrate wastewaters (above 3 g/L), such as the explosives and ammunitions industries wastewaters, and, in particular, about the extent of nitrite accumulation and its dependence upon the type and amount of the carbon source.
This paper presents the results of an experimentation performed in lab scale anoxic ASR treating a synthetic high-nitrate wastewater, simulating the explosives and ammunitions industries wastewaters. The aim of the experiments was to investigate the behaviour of an ASR in the treatment of very high nitrate concentrations in the presence of an external carbon source. Three different carbon sources among the most widely used in wastewater treatment plants, i.e. methanol, acetic acid and sucrose (Abu Ghararah, 1996) were compared, and the conditions to achieve the complete removal of nitrogen were assessed.

1.1 Denitrification kinetic

Several models have been developed in the past to describe denitrification kinetic in both open and closed system (Wild et al., 1995; Sözen and Ohron, 1999; Wicht, 1996; Aytimur et al., 2008). The reduction pathway in biological denitrification systems involves a two step mechanism: in the first step nitrates reduction to nitrites occurs, through the reaction:

$$NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O \quad (1)$$

while, in the second step, the above formed nitrites are reduced to molecular nitrogen, by the reaction:

$$NO_2^- + 3e^- + 4H^+ \longrightarrow 0.5\ N_2 + H_2O \quad (2)$$

Both reactions require a carbon source as electron donor. The limiting substrate, at pH in the range between 8 and 8.5, and at a dissolved oxygen concentration lower than 0.5 mg/L, is then the nitrate or carbon substrate concentration.
In such conditions, denitrification follows the Monod kinetic, and the specific denitrification rate v_d can be expressed by the relationship:

$$v_d = v_{dTmax} \cdot \frac{S_n}{K_{dT}+S_n} \cdot \frac{S_c}{K_{cT}+S_c} \quad (3)$$

where:
v_{dTmax} = specific denitrification rate [mg NO_3^--N /(g VSS d)]
S_n = nitrates concentration [mg NO_3^--N /L]
K_{dT} = Monod constant for nitrates [mg NO_3^--N /L]
S_c = biodegradabile carbonaceous substrate concentration [mg COD/L]
K_{cT} = Monod constant for carbon [mg COD/L]
In the treatment of high strength wastewaters (nitrates concentration higher than 0.5 g/L), since K_{dT} values are generally low (about 0.1 mg NO_3^--N /L, Barker and Dold, 1997), the kinetic of nitrates conversion can be considered of zero order with respect to nitrates concentration.

2. Materials and methods

2.1 Experimental procedure

Microorganisms culture was developed from an inoculum taken from the anoxic reactor of a municipal activated sludge plant. The inoculum was fed in a glass batch reactor of 3 L volume with a growth medium (THB, Todd Hewitt Broth, Biolife, Milano, Italy). The composition of the growth medium was as follows: Beef Heart Infusion, 572 mg/L; tryptone, 22.8 mg/L; glucose, 2.28 mg/L, sodium chloride, 2.28 mg/L; sodium bicarbonate, 2.28 mg/L; disodium phosphate, 0.456 mg/L.

2.1.1 Continuous tests

Denitrification tests were performed using a laboratory scale continuous flow stirred tank reactor. The working volume of the reactor was 4 L. Temperature was kept at 20±1°C using a water flow system equipped with a heating resistance. The main operating parameters of the reactor are reported in Table 1.

Table 1: Operating parameters of the reactor

Parameter	Value
Reactor volume (L)	4
Influent flow rate (L/d)	3.2
Hydraulic residence time (h)	30
Sludge age (d)	10
Temperature (° C)	20±1

The feed was a synthetic wastewater prepared by dissolving in tap water HNO_3 and the necessary amount of $Ca(OH)_2$ to adjust pH at 7.5. The feed preparation was completed by the addition of small amounts of

selected nutrients for bacterial growth (Cyplik et al., 2012). All the reagents were provided by Carlo Erba Reagenti, Milano, Italy.
To achieve the acclimation of the biomass, three series of experimental tests were carried out: in each series a different carbon source was used as an electron donor. Three carbon sources were tested: sucrose, acetic acid and methanol, all provided by Carlo Erba Reagenti, Milano, Italy.
The initial nitrates concentration in the tests was 3 g NO_3^-/L for all the three carbon sources used. A carbon:nitrogen (C:N) molar ratio of 3 was adopted around the optimal values for different types of organic matter to be used for denitrification (Henze et al., 2000), to avoid both the establishing of carbon source limiting conditions, and the COD concentration in the effluent exceeding the permissible limits (Nair et al., 2007). Each test was performed until steady state conditions were reached, showed by constant values of both effluent nitrogen compounds and biomass concentration in the reactor.

2.1.2 Batch tests

Once reached steady state conditions in each continuous series, kinetic tests were performed to estimate the kinetic coefficient behaviour of denitrification reactions. Each batch reactor was inoculated with 20 mL of the mixed liquor of the acclimation reactor and added to 180 mL of synthetic wastewater at the same C:N molar ratio of the continuous tests. The initial nitrates concentration in the tests was 3 or 5 g NO_3^--N/L for all the three carbon sources used. Additional tests at 7 and 9 g NO_3^-/L were performed using methanol as carbon source.

2.2 Analyses

The pH was measured with a Crison 421 pH meter.
The total organic carbon was measured using a Shimadzu TOC-5000A TOC Analyzer.
The nitrates and nitrites concentrations were detected by means of a Dionex DX 120 ionic chromatograph equipped with a Dionex integrator and a IONPAC AS12A anionic column (length 200 mm, diameter 4 mm) combined with a IONPAC AG12A column (length 50 mm, diameter 4 mm).
Dissolved oxygen was measured by Vittadini pO_2 probe.

3. Results and discussion

3.1 Continuous tests: biomass acclimation

Figure 1 shows the trend of nitrogen removal yield in the acclimation reactor in the tests performed with an initial nitrates concentration of 3 g NO_3^-/L.

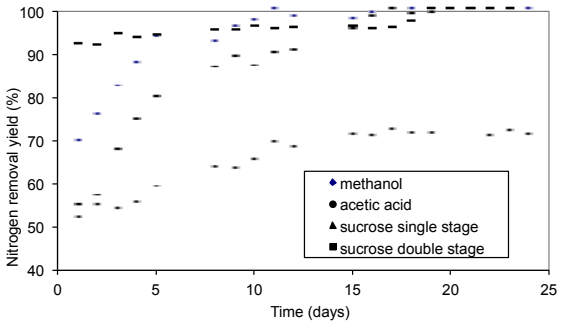

Figure 1: Nitrogen removal yield in continuous tests (influent nitrates concentration 3 g NO_3^-/L).

For all the three carbon source used, an acclimation period of about two weeks was necessary to attain a constant value in nitrates concentration in the effluent. As expected, a faster acclimation to methanol with respect to acetic acid and sucrose was observed: after three days the 82.2 % of the total nitrogen was removed using methanol as carbon source, while only 67.4 % and 53.9 % was removed using acetic acid and sucrose (one stage) respectively. An overall nitrates removal rate of 100 % was quickly achieved in the tests performed using acetic acid and methanol as carbon source, while a different behaviour was observed in the tests performed with sucrose as carbon source, where a lower nitrates removal was obtained. The overall nitrogen removal was consequently established around 70 %. In both the reactors where methanol and acetic acid were used as carbon source the measured pH was in the range between 8.0 and 8.2 during the experiments. Conversely, in the denitrification reactor where sucrose was used as carbon source a pH of 7.5-7.8 was quickly reached. At this pH the inhibiting effect of nitrites is very high: Glass and Silverstein (1998) found that nitrites concentrations of 30 and 250 mg/L inhibit denitrification at pH=6 and 7, respectively, while the inhibition is generally lower at higher pH values and becomes

negligible at pH higher than 8.5. In our experiments nitrites concentration in the tests performed with sucrose (single stage) was about 0.5 g/L and according to the above mentioned study, this concentration can inhibit denitrification kinetic. Since nitrites conversion is the limiting step of the overall denitrification pathway (Constantin and Fick, 1997), to increase the nitrogen removal yield a second reactor was added (double stage) and fed with the effluent from the first one. The composition of the solution coming from the first reactor and sent to the second one is shown in Table 2.

Table 2: Composition of the effluent from the first reactor (double stage tests with sucrose).

NO_3^- (mg NO_3^-/L)	NO_2^- (mg NO_2^-/L)	TC (mg/L)	IC (mg/L)	TOC (mg/L)
840	1154	409.8	134.88	274.92

As reported in Figure 1, the addition of the second reactor resulted in a very high nitrogen removal: the two steps of the denitrification pathway were in fact divided and two specific biomasses were selected, each one operating in a different reactor. Figure 1 shows that complete removal of both nitrogen species was observed after about three weeks of operation of the double stage system.
In the second reactor, in fact, due to the alkalinity production (3.57 g $CaCO_3$ / g NO_3^--N) during denitrification (Ohron and Artan, 1994), a further increase in pH was observed, up to about 8.5.
These pH conditions did not permit nitrite inhibition, thus allowing the denitrification pathway to be completely developed. In addition, dissolved oxygen concentration in the second reactor was less than 0.02 mg/L, thus ensuring the optimal anoxic conditions required for denitrification.

3.2 Batch tests

The results of batch tests, reported in Figures 2-4 show that biological denitrification at high nitrate initial concentration can be assumed to start following a zero order kinetic, as already observed at low nitrates concentration (Glass and Silverstein, 1999). In all the tests performed, however, nitrates conversion resulted in a growing nitrites accumulation, which, after reaching a maximum value, showed a progressive decrease until the full reduction to molecular nitrogen occurred. The growing nitrites concentration in the reactor resulted in a slower nitrates conversion: this step of the denitrification pathway began to depend upon the actual nitrates concentration, thus following a first order kinetic.
This general trend was found to be not dependent upon the used carbon source, though the nature of carbon source and the initial nitrates level strongly influenced the maximum nitrites concentration achieved in the reactor and the time required for nitrites reduction become competitive with nitrites generation from nitrates conversion, thus beginning the decrease of nitrites concentration.
Figures 2 and 3 show that increasing nitrates concentration, the peak in nitrites concentration in the reactor was observed later in the case of using sucrose and acetic acid. Sucrose did not ensure complete denitrification in the test performed at an initial concentration of 5 g NO_3^-/L (equilibrium conditions were not attained after 60 h of experiments).

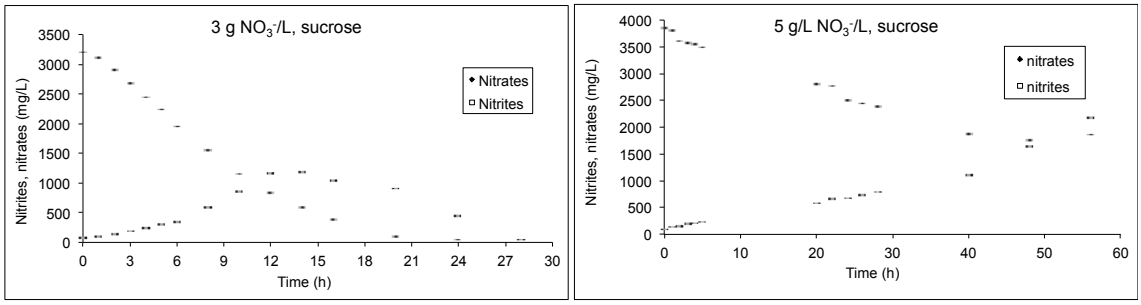

Figure 2: Results of the tests performed using sucrose as the carbon source.

Conversely, when methanol was used as carbon source (Figure 4) at 3 g NO_3^-/L, nitrates concentration respected this trend, but, from an initial nitrates concentration of 5 g NO_3^-/L, a different behaviour was observed at the beginning of the curve. A lower nitrite accumulation rate was observed, and, consequently, the change in nitrates kinetic occurred later. In the test at an initial nitrates concentration of 9 g NO_3^--N/L, the huge alkalinity production in the reactor did not permitted the pH to achieve values at which nitrites inhibition towards nitrates reduction occurs. The results obtained at 9 g NO_3^-/L are in agreement with those obtained by Dhamole et al. (2007) in a sequencing batch reactor (SBR) using sodium acetate as carbon

source. They reported complete denitrification within 6 h using acclimated sludge by means of a stepwise increase in nitrates concentration.

As a preliminary data elaboration, in Table 3 the parameters obtained in the batch tests are reported, for the zero-order (K_0) and first order (K_1) kinetics, together with the time required to achieve a complete nitrogen removal from the wastewater ($t_{100\%}$). The data obtained show that methanol ensured a faster denitrification rate with respect to acetic acid and sucrose: in particular, the results show that the use of sucrose was unfavorable due to the great nitrite accumulation.

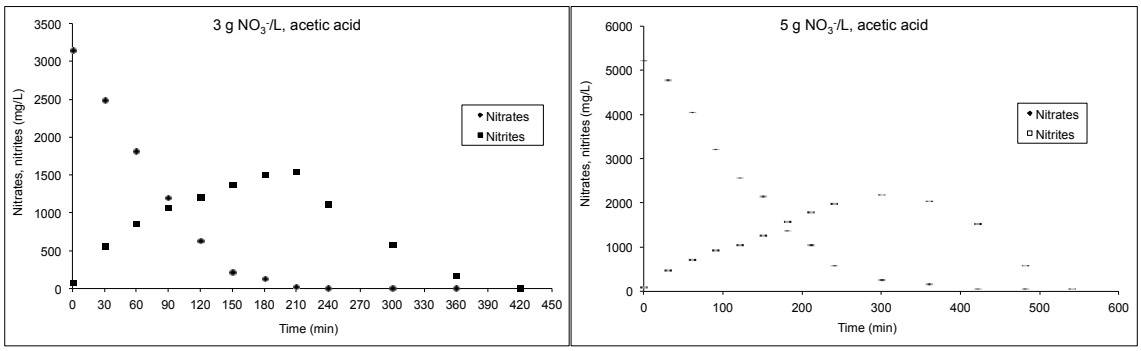

Figure 3: Results of the tests performed using acetic acid as the carbon source.

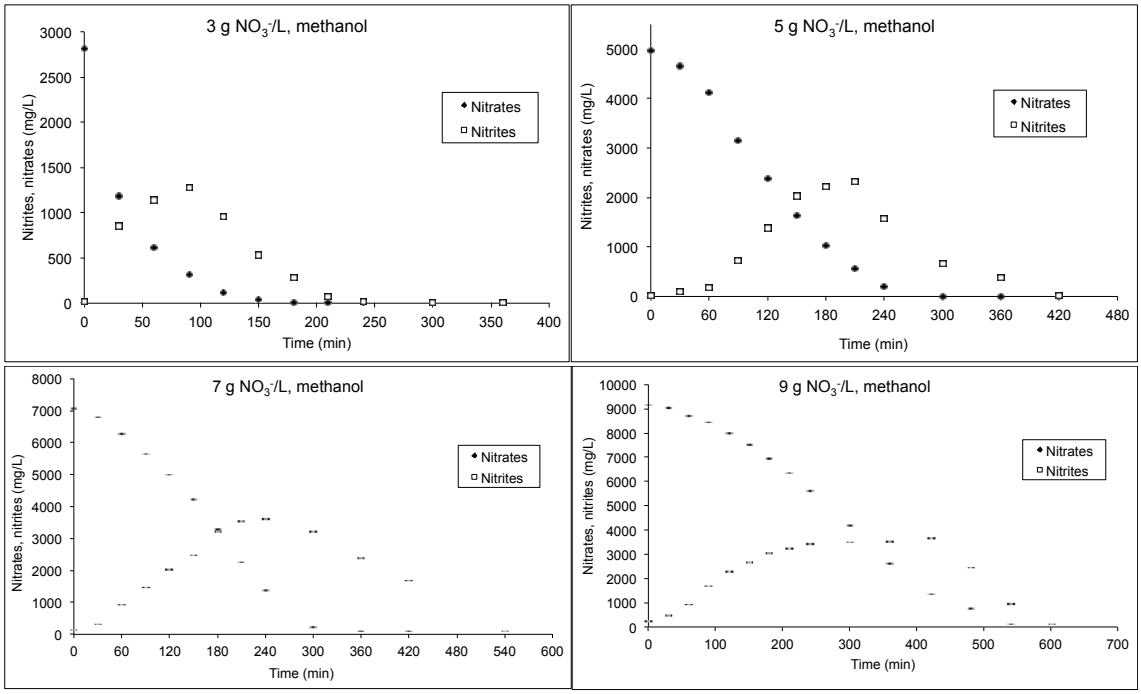

Figure 4: Results of the tests performed using methanol as the carbon source.

Table 3: Kinetic parameters calculated in batch tests.

Series	K_0 (mg NO_3 L^{-1} d^{-1})	K_1 (h^{-1})	$t_{100\%}$ (h)
Ia – sucrose 3 g NO_3^-/L	44.12	0.32	26
IIa – acetic acid 3 g NO_3^-/L	285.91	4.08	7
IIb - acetic acid 5 g NO_3^-/L	275.29	0.87	9
IIIa – methanol 3 g NO_3^-/L	333.14	4.24	3.5
IIIb – methanol 5 g NO_3^-/L	296.89	2.70	7
IIIc - methanol 7 g NO_3^-/L	287.01	2.12	9
IIId - methanol 9 g NO_3^-/L	223.21	-	10

4. Conclusions

The efficiency of the biological denitrification process in the treatment of wastewaters with high concentration of nitrates (> 3 g/L) was assessed. Using methanol, sucrose or acetic acid as carbon source, the denitrification of high nitrate wastewaters was found to follow a zero order kinetic until a nitrates concentration of about 40 mg NO_3^- /L was established. The complete removal of nitrogen from wastewater with nitrates concentrations up to 9 g/L was observed only using methanol as carbon source. Due to the optimal pH values reached, the huge nitrite accumulation does not result unfavorable to further nitrates reduction.

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