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DENITRIFYING POTENTIAL OF AN ACTIVATED SLUDGE DERIVED CONSORTIUM

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Abstract

The aim of this work was the evaluation of the denitrification potential of a consortium of activated sludge adapted to anoxic conditions. As the carbon source is the main parameter influencing the denitrification process, in the first stage three carbon sources (acetate, citrate and glucose) were assayed in batch activity tests. The highest denitrification efficiency was attained with acetate, being nitrate and nitrite completely reduced. The adapted sludge was then used as a potential denitrifying consortium on an anoxic rotating biological contactor (RBC) with acetate as carbon source. Two C/N ratios were assayed (2 and 3) as well as two nitrate loads (220 and 300 mg/L). At the highest nitrate concentration, the best denitrification efficiency (nitrate removal higher than 99% without nitrite or acetate accumulation) was attained at C/N=3. From the results it can be concluded that the activated sludge possesses high denitrifying capacity and can easily be adapted to anoxic conditions. Moreover, the RBC revealed to be a good alternative solution to the conventional processes.

Key words: anoxic rotating biological contactor, biofilm, carbon source, carbon/nitrogen ratio; denitrification

1. Introduction

Presently, one of the most serious environmental problems is the discharge of effluents completely off the legislation levels, in most cases even after treatment. In Portugal, most of the wastewater treatment plants, due to the lack of denitrification systems or to their inefficiency, have strong difficulties in complying with the removal of nitrogen compounds. Besides, the increase of strict effluent discharge standards is posing significant challenges to plant operators to reduce effluent nitrate-nitrogen concentrations to levels as low as 2-3 mg /L or even lower. The lack of sufficient influent carbon in many wastewater treatment plants and its exhaustion during the nitrification stage makes it very difficult to achieve such low nitrate-nitrogen concentrations in the effluent (Ginige, 2003). An effective solution to the problem is to introduce additional external carbon sources to enhance denitrification. Some of the main requirements for a suitable external carbon source, apart from low cost, is a non toxic/non dangerous nature, a low sludge yield and the ability to stimulate a complete denitrification without the need for adaptation of the microflora, so that environmentally detrimental intermediary products such as nitrite and nitrogenous oxides can be avoided (Lee and Welander, 1996). Moreover, the nature of organic carbon source is also known to control competition between denitrification and dissimilatory nitrate reduction to ammonium (Tiedje, 1994). Several external carbon sources have been used, including methanol, acetate, propionate, acetic acid, glucose, benzoic acid, citrate, etc, being methanol the most commonly used due to its low cost (Narkis et al, 1979). However, it has been found to cause long delays until an improvement in denitrification performance is observed. On the other hand, acetate has been found to improve denitrification almost instantaneously when added, but it has a significantly higher cost (Ginige, 2003). Thus, it continues to be a challenge to select the most appropriate carbon source to be used on a denitrification process.

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Biological denitrification is currently the most widely used sustainable and cost-effective process to remove nitrogen from water and wastewater (Mateju et al., 1992). In recent years, microbial ecology involved in denitrification in wastewater treatment has been investigated for an enhancement of process performance. The majority of this bacteria are nitrate reducers, followed by the denitrifiers, while the most prevalent truncated denitrifiers reported are those deficient in nitrate reductase i.e. nitrite dependent and next are those lacking nitrous oxide reductase. For this reason a consortium of appropriate and efficient microorganisms would remove nitrate from the wastewater more efficiently (Zala et al., 1999). In this way, the utilization of activated sludge as a consortium of denitrifying bacteria can be extremely advantageous.

Accordingly, the aim of this work was to evaluate the denitrifying potential of an activated sludge consortium and to select the corresponding best carbon source.

2. Experimental

2.1. Materials and methods

2.1.1. Acclimatization of biomass

A volume of concentrated biological sludge was collected from an activated sludge tank at Esposende Wastewater Treatment Plant, Portugal. In order to get a suitable consortium, the fresh biomass was acclimatized during 3-4 weeks in a denitrifying medium, in anoxic conditions, at 30 ºC and 150 rpm, using citrate as a carbon source with the following composition: 244.8 mg/L $C_6H_5Na_3O_72H_2O$, 289 mg/L KNO_3, 93 mg/L K_2HPO_4, 18 mg/L KH_2PO_4, 24.2 mg/L $NaMoO_42H_2O$, 5.6 mg/L $FeSO_47H_2O$, 0.81 mg/L $MnCl_22H_2O$, 51.5 mg/L $CaCl_22H_2O$ and 409.2 mg/L $MgSO_47H_2O$. Due to the medium buffering capacity, no pH adjustment was performed. The acclimatized sludge was used as inoculum on denitrifying activity tests and also for seeding into the anoxic RBC.

2.2.2. Denitrifying activity tests

These assays were performed in 160 mL serum flasks containing 90 mL of the denitrifying medium referred above and were inoculated with 6 ml of adapted biomass previously prepared. Carbon sources (citrate, acetate and glucose) were used at C/N =3. Flasks were closed with butyl rubber stoppers and aluminium caps. To obtain anoxic conditions, the flasks were flushed with helium gas. Finally, the flasks were incubated at 30 ºC and 150 rpm. Aliquots of 2 mL were removed from each bottle, along the time, and immediately analysed for several parameters.

2.2.3. Synthetic wastewater

The anoxic rotating biological contactor was fed continuously with a synthetic wastewater with a composition similar to the denitrifying medium (using acetate, citrate or glucose as carbon source and a phosphorus concentration of 20 mg P/L) and 50 mg/L of nitrogen-nitrate.

2.2.4. Experimental apparatus, start-up and operation

The experimental set-up consisted on a closed rotating biological contactor (RBC) single-stage system with 8 poly-methylmethacrylate disks mounted in a rotating shaft, having a total volume of 2.5 L. The design characteristics of the RBC are listed in Table 1. The rotational speed was 4 rpm and the temperature was maintained at 30 ºC by means of a heating jacket. The reactor was covered and sealed and no special precaution was taken to maintain anoxic conditions. An influent feed tank was coupled to a previously calibrated peristaltic pump used to regulate the flow rate of the synthetic wastewater into the anoxic RBC in a direction parallel to the rotating shaft and perpendicular to the discs. A dynamic head tube resembling a vented inverted siphon on the effluent line was used to control the liquid level. The treated effluent was collected in a receiving tank.

The reactor was operated with a hydraulic retention time (HRT) of 12 h and fed, initially, with a synthetic medium containing 50 mg N-NO_3 mg/L, using acetate as the carbon source and a C/N=3. The RBC was inoculated with 2.3 L of the adapted consortium of sludge and microbial attachment onto the discs was allowed to occur in batch mode for 4 days. The initial biomass concentration in the system was 3200 mg of volatile suspended solids (VSS)/L. After that period, the anoxic RBC mixed liquor was removed, the reactor was re-filled with the synthetic wastewater and started to operate in a continuous mode. The study was conducted for a period of 23 days.

Table 1. Characteristics of the closed rotating biological contactor

Parameter	
Disk submergence (%)	71.6
Number of disks	8
Disk diameter (cm)	13
Disk thickness (mm)	3
Spacing between disks (cm)	2
Liquid volume (L)	2.5

2.2.5. Sampling and analytical methods

During the course of continuous operation, samples of the RBC influent and effluent were collected daily and analysed for various parameters such as nitrate, nitrite, acetate and biomass. For the determination of nitrate, nitrite and acetate ions concentration, samples were filtered through a 0.2 µm membrane filter (Whatman) in order to remove interfering suspended particles. Nitrate and acetate concentrations were measured by high-performance liquid chromatography (HPLC), using an organic acid column (Chrompack, 300×6.5 mm) and a mobile phase of 0.01 N sulphuric acid (H_2SO_4) at 0.5 mL

min^{-1}. Column temperature was set at 40 ºC and nitrate and acetate were detected by UV at 210 nm. Nitrite-nitrogen concentration was determined by a colorimetric method using N-(1-naphthyl)-ethylene-diamine, according to standard methods.

3. Results and discussion

3.1. Denitrifying activity

In order to test the denitrifying potential of a consortium obtained from activated sludge it was necessary to perform the sludge acclimatization. Beccari and co-workers (1983) tested the use of non-acclimatized sludge in the denitrification process and verified that when nitrite concentration increased from 20 to 25 mg/L and biomass concentration from 500 to 1000 mg VSS/L, the process was inhibited. According to Dhamole et al. (2006), sludge acclimatization is a necessary process to allow the development of an efficient consortium to treat high nitrate loads. Thus, in the present work the acclimatization process was performed during 3 –4 weeks.

This activated sludge consortium was then assayed for its denitrifying activity in batch tests and, in order to select the most efficient carbon source, three compounds were assayed: citrate, acetate and glucose. The substrate removal profiles as a function of time during the denitrifying activity tests are depicted in Fig. 1. The assays were performed in triplicate but as it was not possible to guarantee the exactly same amount of inoculum no average values were determined. Nevertheless, the graphics presented are representative of the results obtained, because all triplicate assays displayed similar trend.

From Fig. 1 (a) it can be observed that when citrate is used as carbon source it took almost 50 hours to have all organic carbon consumed but nitrate was still above 50 mg/L. Nevertheless, nitrite accumulation was not significant.

When acetate was the carbon source (Fig. 1b), nitrate consumption was faster (39 hours) but a small nitrite accumulation was observed and acetate concentration was still not null at that stage. Probably, the remaining acetate was used to convert nitrite in nitrogen gas. Several works using activated sludge in the denitrification process reported that with acetate it is possible to achieve higher denitrification rates due to its slow degradation (De Lucas et al., 2005; Gerber et al., 1986; Somiya et al., 1988; Tam et al., 1992).

In the case of glucose as carbon source, after 30 hours, only nearly 21% of glucose was consumed and 23% of nitrate was reduced (Fig.1 (c)). Some accumulation of nitrite was observed which is in accordance with the results of other authors that reported that when glucose was provided to activated sludge, the biocommunity enriched for facultative anaerobes reducing nitrate only to nitrite at the expense of true denitrifiers, causing decreased nitrite reduction rates and thus nitrite accumulation (Wilderer et al., 1987). In all assays, the C/N = 3 was not a limiting factor and, with the exception of glucose as carbon source, nitrite was simultaneously reduced with nitrate by activated sludge.

These batch denitrifying activity tests allowed to conclude that using a consortium of activated sludge and a C/N =3, acetate was the most efficient carbon source. Accordingly, this carbon source and activated sludge consortium were applied to denitrify a synthetic wastewater in a rotating biological contactor.

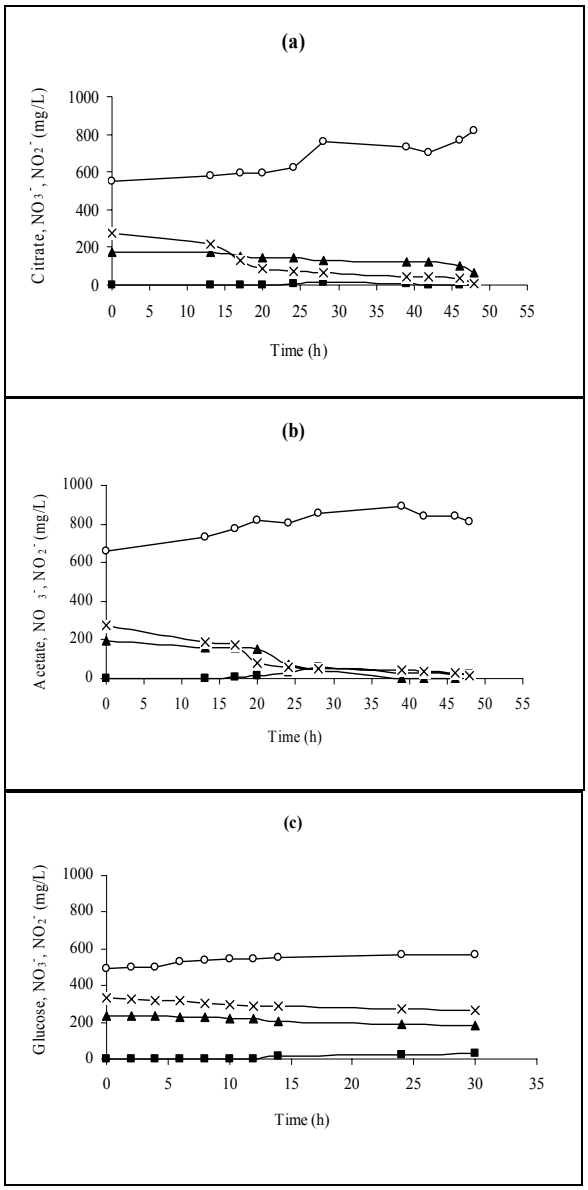

Fig. 1. Variation of nitrate (▲), carbon source (x), nitrite (■) and biomass (○) concentrations with time during a denitrifying activity test with different carbon source (a) citrate (b) acetate and (c) glucose

3.2. Denitrifying activity of activated sludge on an anoxic RBC

Carbon source and its available amount are key parameters in the denitrification process.

In fact, addition of carbon at a concentration higher than required should be avoided as it favours dissimilatory nitrate reduction to ammonium (Tiedje 1994), and leads to higher biomass and hence more sludge formation. Based in this rationale, two C/N ratios were tested to assess the denitrifying performance in an anoxic RBC, using acetate as carbon source.

The continuous experiments were carried out for 23 days and three periods can be distinguished: the first with a C/N= 3 and a nitrate load of 220 mg/L; the second with the same C/N and a higher nitrate load (300 mg/L) and the last one with the same nitrate load and a C/N= 2. These periods are differentiated in Fig. 2 by vertical lines, which indicate process disturbances. As mentioned before, the RBC operated in a batch mode for 4 days, changing then to a hydraulic retention time of 12 hours.

Nitrite accumulation is not desirable since nitrite is more toxic than nitrate (Constantin et al., 1996) and because it can lead to inhibition of the bacterial development (Hunter, 2003).

The nitrite produced during the continuous experiments was quite low, with the exception of the last stage where it slightly increases. This indicates that probably all nitrate was converted to nitrite and all nitrite was converted to nitrogen gas.

Furthermore, it suggests that the bacterial population was composed by true denitrifiers that reduce nitrate to nitrogen gas, instead of nitrate respirators that only have the enzymatic ability to reduce nitrate to nitrite. Probably, the observed increase in nitrite concentration in the last period of operation was due to some nitrite inhibition since it has been reported that a concentration of 20-25 mg/L NO_2^-N is adequate to completely and irreversibly inhibit nitrite reduction by a denitrifying population that has not been acclimatized to nitrite (Beccari et al., 1983).

Variations in the removal efficiency of nitrogen-nitrate ($N\text{-}NO_3^-$) and carbon-acetate ($C\text{-}CH_3COO^-$) as a function of operating time are shown in Fig. 3.

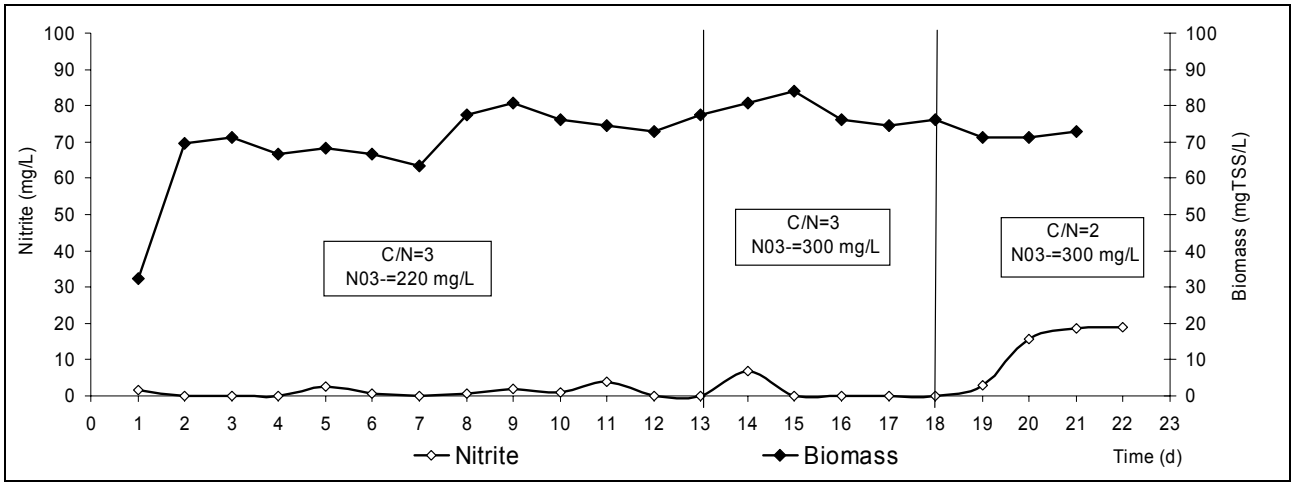

Fig. 2. Nitrite-nitrogen effluent concentration and biomass inside the reactor along the time

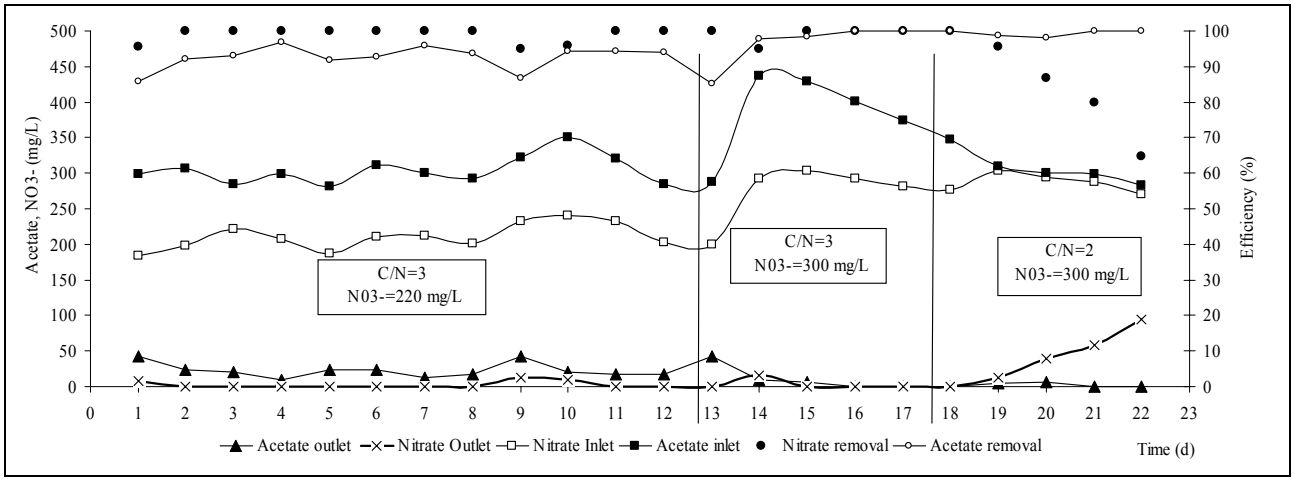

Fig. 3. Nitrogen-nitrate and carbon-acetate removal efficiency along the time

Nitrate removal efficiency was in average 99% at C/N = 3, which, obviously, represents a very good performance of the RBC as well as an excellent denitrifying ability by the activated sludge derived consortium. At C/N=2 there was a decrease to 65% at the end of the experiment but acetate removal was always around 99%. However, at C/N = 3 acetate concentration was higher than the necessary to convert all nitrate to nitrogen gas. Considering that the optimum C/N has been assumed to be the ratio that enables maximum nitrate removal with minimum excess of carbon source in the effluent (Aslan, 2005), it was decided to decrease the C/N to 2. However, in these conditions of operation, the amount of acetate was not enough to reduce all nitrate concentration to a level below the admissible value required by the European Union wastewater discharge standards considering that the receiving environment will be, for example, fresh water (10 - 30 mg $N\text{-}NO_3^-$ /L) (CEC, 1991). This suggests that an intermediate C/N value would be more efficient.

Conclusions

In this work citrate, acetate and glucose were compared for their performance as carbon source for denitrification in batch tests, also testing the denitrifying potential of an acclimatized activated sludge consortium. It was concluded that the maximum nitrate removal rate, non nitrite accumulation and non remaining carbon source were attained when acetate was used. In other words, the results also demonstrated that the denitrification ability of the sludge is highly dependent on the carbon source. It can also be concluded that it is of utmost importance to proceed to sludge acclimatization and that the sludge easily adapts to anoxic conditions and to high nitrate loads, as shown by the RBC operation. Besides, it can be stated that the RBC constitutes a very good alternative to the conventional denitrification systems. This reactor presents a very stable biofilm coping well with high changes in nitrate load.

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