Biotechnology Letters **22:** 1789–1792, 2000.

Denitrification by *Alcaligenes denitrificans* in a closed rotating biological contactor

P. Teixeira & R. Oliveira*
Centro de Engenharia Biológica – IBQF, Universidade do Minho, Campus de Gualtar, 4709 Braga codex, Portugal
**Author for correspondence (Fax: + 351 253678986; E-mail: roliveira@deb.uminho.pt)*

Received 11 July 2000; Revisions requested 27 July 2000; Revisions received 8 September 2000; Accepted 8 September 2000

Key words: Alcaligenes denitrificans, biofilm, denitrification, phosphorous concentration, rotating biological contactor

Abstract

Biological denitrification using a pure culture of *Alcaligenes denitrificans* was investigated in a closed rotating biological contactor, which operated with a hydraulic retention time of 2 h, a carbon/nitrogen ratio of 2:1, with a dissolved O_2 concentration below 6 mg l^{-1} and under three different phosphate concentrations. *Alcaligenes denitrificans* was not repressed by O_2 limitation and the removal of nitrate was about 30% more efficient at the intermediate phosphate concentration (20 mg P l^{-1}).

Introduction

Biological processes for nitrogen removal are important in wastewater treatment for removal of nitrogen compounds that otherwise result in eutrophication, algal blooms and oxygen depletion (Okabe *et al*. 1997). These compounds are normally removed in two steps. The first step is aerobic and involves the oxidation of ammonia to nitrate by autotrophic bacteria (nitrification). In the second step (denitrification), nitrate is reduced via nitrite to nitrous oxide and finally to nitrogen gas, which is a harmless gas. Initially, this process was considered to be strictly anoxic (Payne 1973), with O_2 below 0.5 mg l^{-1} (van der Hoek *et al*. 1994). However, with a certain number of bacteria, denitrification occurs even in the presence of O_2 (Lukow & Diekmann 1997). Heterotrophic denitrifying bacteria require an organic carbon source and methanol has been the most widely used (Monteith *et al*. 1980). More recently, it became important to use carbon sources compatible with the standards imposed for drinking water. Thus, citrate and acetate are being used increasingly. Nevertheless, microbial growth is regulated by other factors, the availability of phosphorous (usually as phosphate) being one of the most important. So, in order to prevent an excessive increase in suspended biomass and for environment protection (phosphorous, beside nitrogen, is the main eutrophication agent) and economic reasons, it is important to study the optimal concentration of phosphate necessary to the denitrification process. The conventional technologies used for biological nitrogen removal are activated sludge systems, oxidation ditches and sequencing batch reactors (Kornaros & Lyderatos 1997). The use of a closed rotating biological contactor is a relatively new biological wastewater treatment process. It combines advantages of the aerobic rotating biological contactor (high biomass concentration, short hydraulic retention time, low energy consumption, operational simplicity) and the anaerobic process (low quantities of waste biological solids) (Lu *et al*. 1997).

The objective of the present work was to study the effect of phosphorous concentration in the denitrification process, in such a closed rotating biological contactor under a dissolved O_2 concentration below 6 mg l^{-1} and a carbon/nitrogen molar ratio of 2:1.

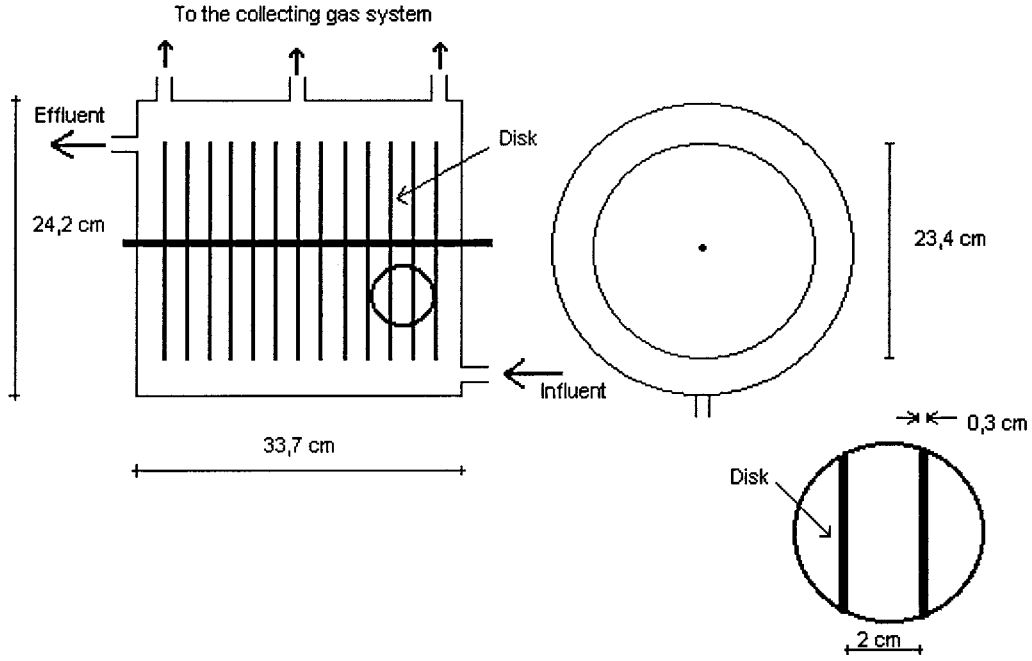

Fig. 1. Schematic diagram of the anoxic rotating biological contactor.

Materials and methods

Experimental apparatus

The experimental set-up was a closed rotating biological contactor single-stage system consisting of 13 poly-methylmethacrylate disks (diam. = 23.4 cm) mounted in a rotating shaft, having a total volume of 15.5 l. The submergence of the disks was 96%. The reactor was covered and sealed, but operated always with dissolved O_2 concentrations up to 6 mg l^{-1} (it was not taken any precaution in order to maintain anaerobic conditions). The rotational speed was 2 rpm and the temperature was maintained around 26 °C by means of a water jacket. A schematic description of the reactor is illustrated in Figure 1. The reactor was operated with a hydraulic retention time of 2 h and fed with a synthetic medium containing 50 mg N-NO_3 l^{-1}, using citrate as the carbon source.

Start-up of the RBC

The reactor was inoculated with 600 ml *Alcaligenes denitrificans* (0.5×10^8 cell ml^{-1}). For the initial formation and accumulation of the biofilm, the reactor was operated in batch mode for a week. After this period, it was fed continuously with a synthetic wastewater: 408 mg l^{-1} $C_6H_5Na_3O_7 \cdot 2H_2O$ (tri-sodium citrate-di-hydrate), 361 mg l^{-1} KNO_3, 24.2 mg l^{-1} $NaMoO_4 \cdot 2H_2O$, 5.6 mg l^{-1} $FeSO_4 \cdot 7H_2O$, 0.81 mg l^{-1} $MnCl_2 \cdot 2H_2O$, 51.5 mg l^{-1} $CaCl_2 \cdot 2H_2O$, 409 mg l^{-1} $MgSO_4 \cdot 7H_2O$ and a variable concentration of K_2HPO_4 and KH_2PO_4 in order to obtain three different concentrations (200, 20 and 2 mg P l^{-1}). Due to the medium buffering capacity, no pH adjustment was performed. It should be stressed that the medium used represents a ratio C/N = 2:1, which is slightly higher than the theoretical value of 1.86:1. This last value is obtained considering the approach of McCarty *et al*. (1969), taking the synthesis of bacterial cells into consideration. However, it is also necessary to consider that some carbon will be used for the deoxygenation of the medium.

Experimental procedure

During the course of continuous operation, parameters such as pH, temperature, dissolved oxygen (DO), biomass concentration, total and volatile solids, nitrate, nitrite and citrate concentration, were measured. For the determination of nitrite, nitrate and citrate ions the sample was filtered through a 0.2 μm membrane filter in order to remove interfering suspended particles.

Analytical methods

Citrate and nitrate concentrations were measured by HPLC in an organic acids column (Chrompack, 300 mm × 6.5 mm). Nitrite was determined by a colorimetric method using *N*-(1-naphthyl)-ethylenediamine (Clesceri *et al*. 1989).

Results and discussion

In the first run the reactor was fed with phosphate at 200 mg P l^{-1}. This was the highest concentration assayed. As it is not easy to find information on denitrification with *Alcaligenes denitrificans*, the basis for selecting this value was a study performed in a chemostat with different denitrifying strains, namely *Alcaligenes faecalis* (Robertson *et al*. 1989). With this amount of phosphorous there was an excessive biomass growth, leading to a complete clogging of the reactor. It should be stressed that 200 mg P l^{-1} is still slightly lower than the concentration proposed by Robertson *et al*. (1989) (211 mg P l^{-1}). Other authors suggest an optimal concentration of phosphorous in a significantly lower range: 0.15–0.18 mg l^{-1} (Richard & Leprince 1982). However, a study with *Alcaligenes denitrificans* in a fluidised bed reactor, using 0.16 mg P l^{-1} showed that this was not enough for the microorganism to denitrify; 95% of denitrifying activity was obtained with 1.14 mg P l^{-1} (Alves & Vieira 1998). So, it was decided to start another run with phosphate at 2 mg P l^{-1}. Under this condition the nitrate removal was higher than with phosphate at 200 mg P l^{-1} (Figure 2). In terms of carbon removal it was similar in both situations (Figure 3). However, the higher nitrate removal was not accompanied by the formation of gaseous nitrogen (results not shown), but either by undesirable accumulation of nitrite (Figure 4). Such accumulation can lead to inhibition of the bacterial development (Constantin *et al*. 1996). Moreover, a high nitrite concentration has a toxic effect in human health. In order to reduce the formation of nitrite the phosphorous concentration was increased tenfold in the 23rd day of operation (start-up with phosphate at 2 mg P l^{-1}). All the other operational conditions were kept constant. In this situation the nitrate removal was similar to that obtained with phosphate at 2 mg P l^{-1}, attaining an average value of 90%. Nevertheless, the accumulation of nitrite was drastically lowered, indicating a good performance of the reactor in this operating conditions (C/N = 2 and phosphate at 20 mg P l^{-1}).

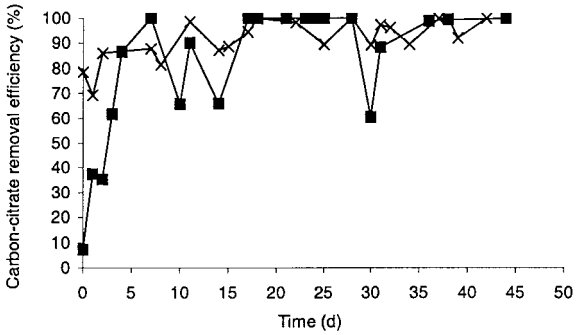

Fig. 2. Effect of different phosphorous concentrations on the carbon removal. ×, Phosphate at 200 mg P l^{-1}; ■, phosphate at 2 and 20 mg P l^{-1} (after 23rd day).

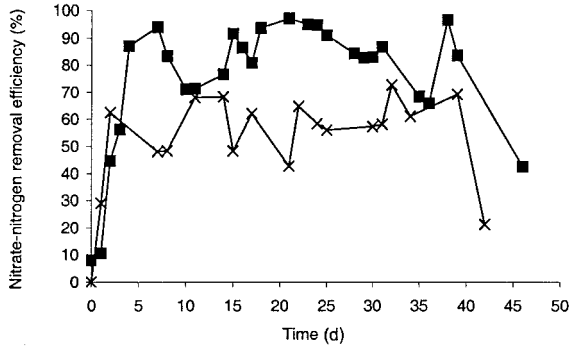

Fig. 3. Effect of different phosphorous concentrations on the nitrate-nitrogen removal. ×, Phosphate at 200 mg P l^{-1}; ■, phosphate at 2 and 20 mg P l^{-1} (after 23rd day).

As can be seen in Figure 5, the pH was always between 7 and 8.2, which is the optimum range for biological denitrification (Wang *et al*. 1995). As it was expected, the pH was lower and steadier when

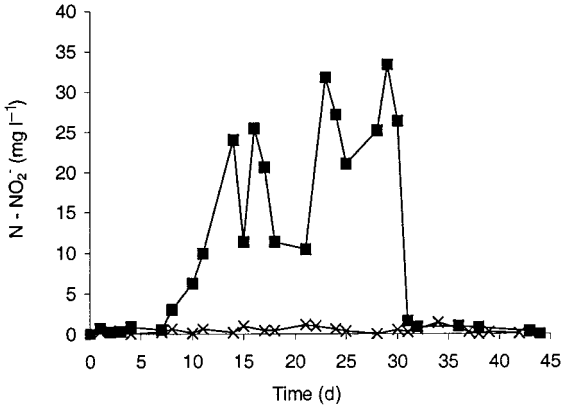

Fig. 4. Nitrite-nitrogen profile. ×, Phosphate at 200 mg P l^{-1}; ■, phosphate at 2 and 20 mg P l^{-1} (after 23rd day).

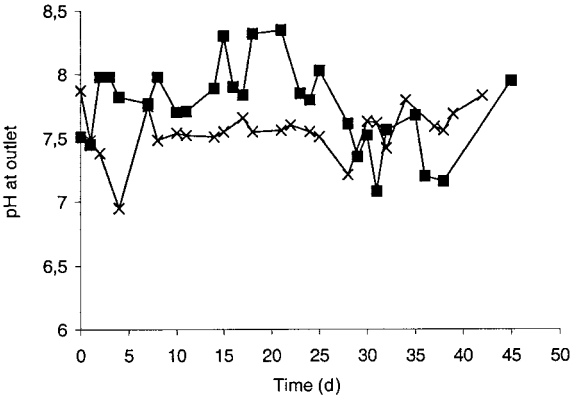

Fig. 5. pH profile. ×, Phosphate at 200 mg P l^{-1}; ■, phosphate at 2 and 20 mg P l^{-1} (after 23rd day).

the phosphorous concentration is greater, due to the buffering capacity of phosphate.

Conclusions

Probably it might be possible to optimize the phosphorous concentration in the range 2–20 mg l^{-1}. However, the closed rotating biological contactor showed an efficient performance in terms of denitrification working with a carbon/nitrogen ratio of 2:1 and with phosphate at 20 mg P l^{-1}. For such a phosphorous concentration there is some confidence that no nitrite accumulation will occur.

Acknowledgements

The authors fully acknowledge the financial support provided by Instituto de Biotecnologia e Química Fina (IBQF) and PRAXIS XXI through grant BD/9121/96.

References

Alves CF, Vieira MJ (1998) Influence of the C/N/P ratio on nirate removal in a denitrifying biofilm fluidized bed reactor. In: *Proceedings of the IV Iberian Congress on Biotechnology*, University of Minho, Braga, Portugal, p. 345 (ISBN: 972-97810-0-1).

Clesceri LS, Greenberg AE, Trussel RR, Franson LAH, eds. (1989) *Standard Methods for the Examination of Water and Wastewater*. 17th edn. Washington D.C.: American Public Health Association.

Constantin H, Raoult S, Montigny W, Fick M (1996) Denitrification of concentrated industrial wastewater: microorganism selection and kinetic studies. *Environ. Tech.* **17:** 831–840.

Kornaros M, Lyberatos G (1997) Kinetics of aerobic growth of a denitrifying bacterium, *Pseudomonas denitrificans*, in the presence of nitrates and/or nitrites. *Water Res.* **31:** 479–488.

Lu C, Lin MR, Lee LY (1997) Temperature effects on the performance of an anaerobic rotating biological contactor. *Environ. Tech.* **18:** 711–720.

Lukow T, Diekmann H (1997) Aerobic denitrification by a newly isolated heterotrophic bacterium strain TL1. *Biotechnol. Lett.* **19:** 1157–1159.

McCarty PL, Beck L, Amant PS (1969) Biological denitrification of wastewaters by addition of organic materials. In: *Proceedings of the 24th Industrial Waste Conference*, Purdue University, West Layfayette, Indiana, pp. 1271–1285.

Monteith HD, Bridle TR, Sutton PM (1980) Industrial waste carbon sources for biological denitrification. *Prog. Water Tech.* **12:** 127–141.

Okabe S, Hirata K, Watanabe Y (1997) Significance of the spatial distribution of microbial species in mixed-populations biofilms. *Biofouling* **11:** 119–136.

Payne WJ (1973) Reduction of nitrogenous oxides by microorganisms. *Bacteriol. Rev.*. **37:** 409–452.

Richard Y, Leprince A (1982) Pollution par les nitrates: traitements disponibles. *Trib. Cebedeau.* **35:** 21–33.

Robertson LA, Cornelisse R, de Vos P, Hadioetomo R, Kuenen JG (1989) Aerobic denitrification in various heterotrophic nitrifiers. *Anton. Leeuwenhoek* **56:** 289–299.

Van der Hoek JP, Kappelhof JWNM, Schippers JC (1994) The use of vaccum daeration in biological nitrate removal processes. *J. Water SRT – Aqua* **42:** 84–94.

Wang JH, Baltzis BC, Lewandowski GA (1995) Fundamental denitrification kinetic studies with *Pseudomonas denitrificans*. *Biotechnol. Bioeng*. **47:** 26–41.