ISSN: 1394 7990
Malaysian Journal of Soil Science Vol. 9: 65-74 (2005) Malaysian Society of Soil Science

The Limiting Factors of Sand Bed Reactor for Heterotrophic Denitrification Process in Tropical Conditions

SARWOKO MANGKOEDIHARDJO
Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember Surabaya, Jl AR Hakim Sukolilo, 60111 Surabaya, Indonesia

ABSTRACT

This research was carried out in a sand bed reactor for heterotrophic denitrification processes. A solution mixture of 24 mg nitrate-N/L and 64 mg methanol-CH_3OH/L was prepared for an influent of heterotrophic reactor. Influent pH level was maintained at 6.5 with the operating temperature in the range of 20 – 35°C. By adjusting nitrogen volumetric loading (NVL) and phosphorus concentrations at tropical operating conditions, it was found that the detention time in the reactor and the phosphorus concentrations of influent were the limiting factors for heterotrophic denitrification process to assure free-nitrite effluent. The maximum detention time was 1 hour and the phosphorus concentrations were 0.4 – 0.6 mg P/L for suppressed nitrite accumulation. The maximum NVL of 0.6 g N/day/L reactor achieved a maximum denitrification efficiency of 94%. The use of 64 mg glucose-$C_6H_{12}O_6$/L resulted in maximum denitrification efficiency of 98 %, while 64 mg acetic acid-CH_3COOH/L only offered a maximum denitrification efficiency of 88%.

Keywords: Sand bed, heterotrophic denitrification, nitrogen volumetric loading (NVL), phosphate, methanol, glucose, acetic acid

INTRODUCTION

The on-site system is used as a means of human waste management in urban and rural areas in Indonesia. The system ensures that the wastewater flows to septic tanks, and the effluent is disposed through the soil absorption system. The wastewater contains mostly nitrogen-rich organic matter which is biodegradable. In soil, the multiple bioconversion of organic nitrogen results in nitrate which can be converted partly to nitrite and dinitrogen gas. The nitrite is toxic to living organisms including humans (Calow 1993; Pratt and Jury 1984). The question arises as to whether the nitrite can be suppressed in the soil absorption system.

To answer the question, this research looked for an appropriate technology. Various sophisticated engineered systems for converting nitrate to dinitrogen gas (denitrification) are available especially in the field of water treatment. These are ion exchange, reverse osmosis, electrodialysis, and microbial treatment in anoxic conditions (Boley *et al.* 2000; Dries *et al.* 1988; Gommers *et al.* 1988; Mergaert *et*

al. 2001; Nakhla and Farooq 2003; Schipper and Vojvodiæ-vukoviæ 2000; Soares *et al.* 2000; Vanderhoek *et al.* 1988; Vanderhoek *et al.* 1989). If the engineered system is applied to treat wastewater in a soil absorption system, the operational and maintenance consequences create difficulties in implementation and financing the treatment technology in developing countries (Feachem *et al.* 1983), including Indonesia. Since the wastewater contains nitrogen-rich organic matter, which is biodegradable, there is a potential to develop a heterotrophic denitrification process in the soil absorption system.

The heterotrophic denitrification process can be explained as follows (Dries *et al.* 1988; Lin *et al.* 2002):

$$5\ CH_3OH + 6\ NO_3^- + 6\ H^+ \Rightarrow 3\ N_2 + 5\ CO_2 + 13\ H_2O \quad (1)$$

The requirement for methanol creates the formula (St. Amant and McCarty 1969):

$$Cm = 2{,}47\ [NO_3^-\text{-}N] + 1{,}53\ [NO_2^-\text{-}N] + 0{,}87\ [DO] \quad (2)$$

where Cm = methanol needed for energy and carbon source, mg/L; $[NO_3^-\text{-}N]$ = initial nitrate-nitrogen concentration, mg/L; $[NO_2^-\text{-}N]$ = the presence of nitrite-nitrogen concentration, mg/L; [DO] = dissolved oxygen concentration, mg/L.

Nitrite is not formed during the transformation of nitrate to dinitrogen gas because of anoxic conditions and the presence of phosphate could be maintained for nitrate concentration of 17 mg N/L (Chung and Bae 2002; Dries *et al.* 1988). Also, the nitrite is not formed in a phosphate free-heterotrophic denitrification process when the operating temperature is below 20°C (Reisinger *et al.* 1989). By maintaining operating conditions, the amount of methanol required to treat 24 mg NO_3^--N/L is 64 mg CH_3OH/L.

The heterotrophic denitrification process takes place in a temperature range of 0–50°C (Christensen and Harremoes 1977). However, the process runs optimally in a temperature range of 4 – 30°C (Claudio *et al.* 2005; Soares 2000; WPCF 1983). In addition, pH level influences the process. The optimum pH level is in the range of 6.5 –7.5 (Barnes and Bliss 1983) but pH levels between 6 and 8 are acceptable (Lin *et al.* 2002; Moore and Schrooder 1971).

The current research is particularly useful in determining the limiting factors for heterotrophic denitrification processes in tropical conditions as these can be subsequently used to assess and predict the performance of the existing soil absorption system. In addition, the limiting factors could serve as a basis for design criteria of a new soil absorption system.

MATERIALS AND METHODS

Basic Design

The research on heterotrophic denitrification of wastewater was carried out in a saturated sand bed reactor to represent anoxic conditions. Process capacity is stated as nitrogen volumetric loading (NVL) which is defined as:

$$NVL = (Q*C)/V \quad (3)$$

where NVL in g N/day/L, Q = influent flowrate (L/day), C = nitrogen concentration (g/L) in the influent, and V = reactor volume (L).

Process efficiency was based on the methanol based-denitrification (Eq.1) and was assessed as follows:

1. Various NVL rates were applied to determine the level of NVL that produces efficient denitrification and nitrite free-effluent.
2. Various phosphate concentrations were applied to determine the level of phosphate concentration that prevents nitrite accumulation.
3. Carbon sources, i.e glucose and acetic acid at the same level as methanol, were applied to compare the process efficiency based on carbon sources.

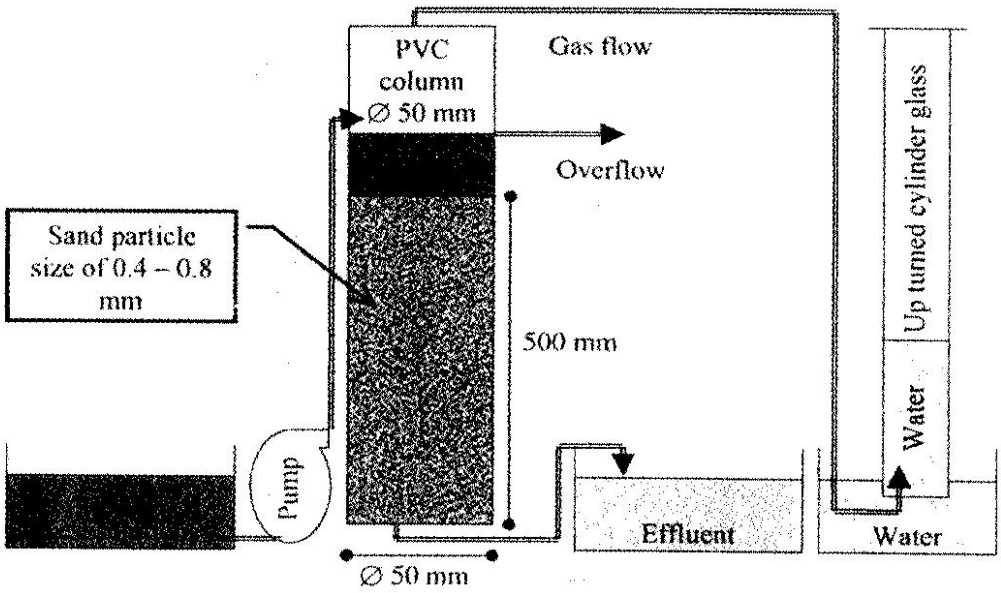

Fig. 1: Reactor installation for heterotrophic denitrification process

Reactor Installation

A saturated soil absorption system of wastewater was simulated as shown in *Fig. 1.* A container was provided to test solutions from which the solution, an influent of the sand bed reactor, was pumped into the sand bed reactor. The influent height in the PVC column was maintained by means of an overflow pipe.

A PVC column ∅ 50 mm was provided. Clean silica sand, with particle-size of 0.4 – 0.8 mm, was placed in the column up to a height of 500 mm. Porosity of

the sand bed was measured as about 0.585. Therefore, the active volume of the sand bed reactor, V_{active} = 50 cm x (3.14/4) x 5^2 cm^2 x 0.585 = 580 cm^3 = 0.58 L. This V_{active} was the volume for the calculation of the NVL (see Eq.3) and detention time of influent in the reactor.

The bottom of the PVC column was equipped with a pipe to allow the test solution to flow to the effluent container. The test solution that passed through the sand bed reacor was an effluent, a product of heterotrophic denitrification process. The up side of the PVC column was equipped with a pipe to allow gas flows to the upturned cylinder glass. Gas was produced as a result of the heterotrophic denitrification process.

Test Solutions Preparation

A test solution was prepared for 24 mg NO_3^--N/L by dissolving 173.14 mg KNO_3 into 1 L of aquadest. A preparation of 1.00 mg PO_4^{3-}-P/L was used to dissolve 4.4 mg KH_2PO_4 into 1 L of aquadest. Various concentrations of the chemical were carried out by means of dilution using aquadest.

An inoculant of denitrifiers was prepared by dissolving 10 g healthy garden soil into 200 mL physiological solution made up of 8.5 g NaCl/L. 1 mL of the soil solution was introduced into the nitrate solution in a reaction tube with a Durham tube. The reaction tube was incubated at 28°C for 1 week. The fact that the Durham tube produces gas at the end of the incubation period is an indication that denitrifiers exist; therefore the solution is said to be the inoculant.

Research Operation

A preliminary operation was carried out to assure that the heterotrophic denitrification runs properly. An influent containing 24 mg N/L, 64 mg methanol/ L and 1 mL inoculant was introduced to the reactor. The pH level of 6.5 was controlled by the addition of 0.1 N HCl. The influent was left for 3 days to allow the denitrifiers to become active in the sand bed. A drop in the water level in the cylinder glass would indicate that denitrifiers in the sand bed reactor were alive. The flowrate of the influent was kept at 0.15 L/hour. Based on the flowrate, nitrogen volumetric loading, NVL = (0.15 L/hour x 24 mg N/L)/0.58 L = 6.2 mg N/hour/L reactor = 0.15 g N/day/L reactor. NVL at various rates of 0.30, 0.60, 0.90 and 1.20 g N/day/L was tested by adjusting influent flowrate. Various concentrations of phosphate at 0.25; 0.5; 0.75 and 1.00 mg P/L were tested. Various carbon sources were tested for the most efficient NVL and for the phosphate concentrations that produced nitrite free-effluent. Carbon sources used were methanol, glucose and acetic acid at a similar concentration level of 64 mg/L. The same level of each carbon source was compared to determine carbon source efficiency.

Quality Analyses Method

Standard Methods (1995) were adopted for all chemicals analyses. Nitrate analyses for influent and effluent were carried out by means of spectrophotometry (Dries *et al.* 1988; Pekdemis *et al.* 1998) using Spectronic-20 for λ 400 nm. Nitrite was measured by the same method of nitrate for λ 540 nm.

RESULTS AND DISCUSSION

General Results

Results of triplicate testing showed that a pH influent level of 6.5 resulted in a pH effluent ranging between 7.2 – 7.8. The temperatures of influent and effluent were maintained between 20 – 35°C to represent tropical conditions. Both environmental parameters were suitable for the heterotrophic denitrification process (Barnes and Bliss 1983; Christensen and Harremoes 1977; Lin *et al.* 2002; Claudio *et al.* 2005). Therefore, pH and temperatures were not the limiting factors for various NVL, phosphate concentrations and carbon sources.

The Influentce of NVL and Phosphate to Nitrite Formation

The results of triplicate testing for various NVL and phosphate to nitrite formation in methanol based-denitrification are presented in *Fig. 2.*

Fig. 2 shows that nitrite formation was suppressed when level of NVL and phosphate concentration were increased. By keeping NVL 0.6 g N/day/L reactor to assure free nitrite and nitrate concentration 24 mg nitrat-N/L and active volume of reactor (V_{active}) 0.58 L, the detention time (td) can be calculated as follows: Rearrangement of Eq. 3 gives

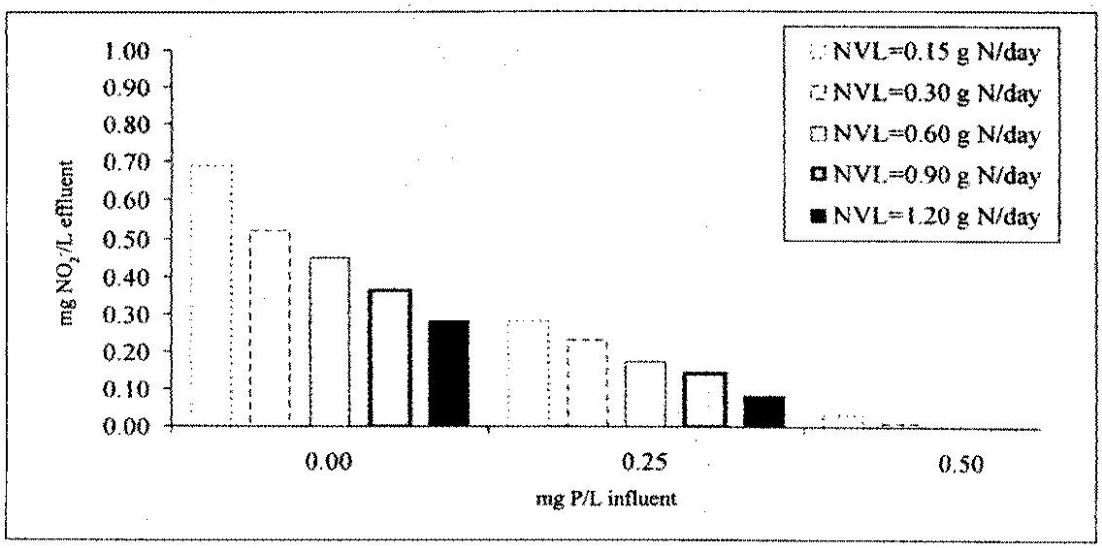

Fig. 2: Influence of NVL and phosphate in methanol-based denitrification to nitrite formation in effluent

$$Q = NVL*V/C \quad (4)$$

It follows that Q = 600 (mg N/day/L) x 0.58 (L) / 24 (mg N/L) = 14.5 L/day. Furthermore

$$td = V/Q \quad (5)$$

This results in *td* = 0.58 (L) / 14.5 (L/day) = 0.04 day = 1 hour.
A simple calculation of *td* using Eq. 3 is as follows:

$$td = V/Q = C/NVL \quad (6)$$

It follows that td = 24 (mg N/L) / 600 (mg N/day/L) = 0.04 day = 1 hour.

At constant N concentration (C in Eq. 6 is kept constant), it is clear that increasing NVL resulted in decreasing *td*. Therefore, the phenomenon of decreasing nitrite formation is due to decreasing *td*.

Phosphate free-influent nitrite was formed in the effluent. Hunter (2003) found an accumulation of nitrite when phosphate is a limiting factor. Perhaps this may be that denitrifiers are not active without phosphate and thus do not have the capability to reduce the nitrate. For phosphate-containing influent, 0.25 mg P/L and 0.50 mg P/L, the formation of nitrite in the effluent decreased as the phosphate concentration was increased. This means that phosphate is the limiting nutrient for denitrifiers and thus limits the process of heterotrophic denitrification. Phosphate free-influent resulting in nitrite free-effluent has been found at temperatures below 20°C (Reisinger *et al.* 1989; Oleszkiewicz and Berquist 1988). Results obtained by other researchers are different from that of our current research as this research was operated at a temperature of above 20°C. The difference might be due to the higher need for phosphate in denitrifiers in tropical conditions than in subtropical conditions.

Influence of NVL and Phosphate on the Efficency of Denitrification

Triplicate treatments of various NVL and phosphate concentrations resulted in various efficiency levels of methanol based-denitrification, which are presented in *Fig. 3*.

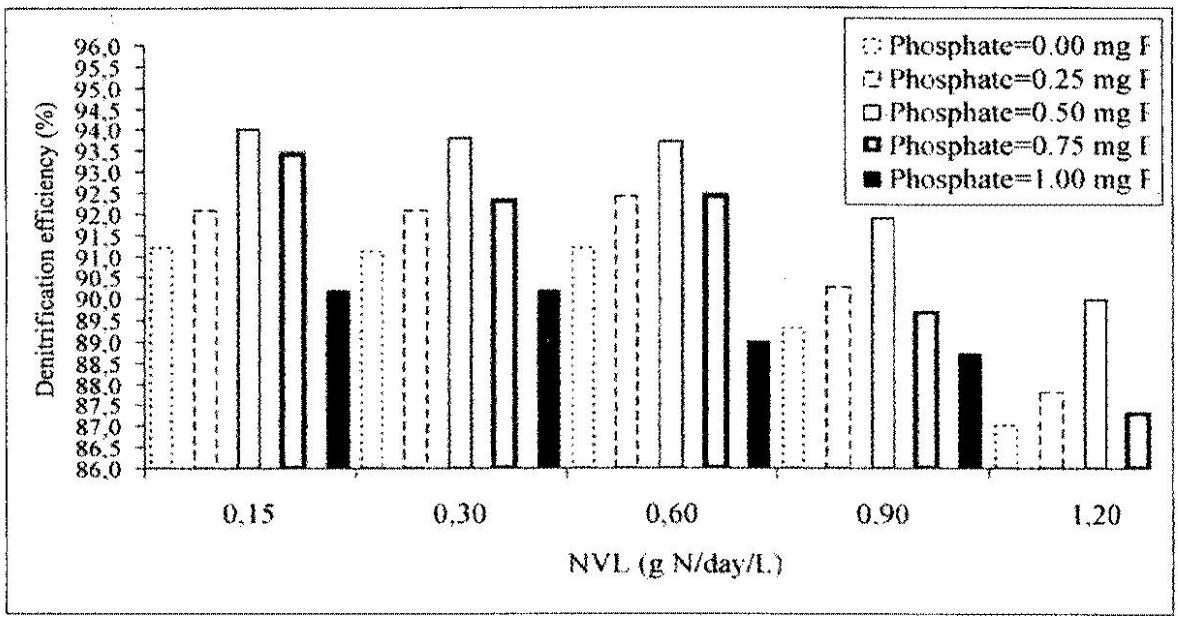

Fig.3: Influence of NVL and phosphate concentrations on the efficiency of methanol base-denitrification

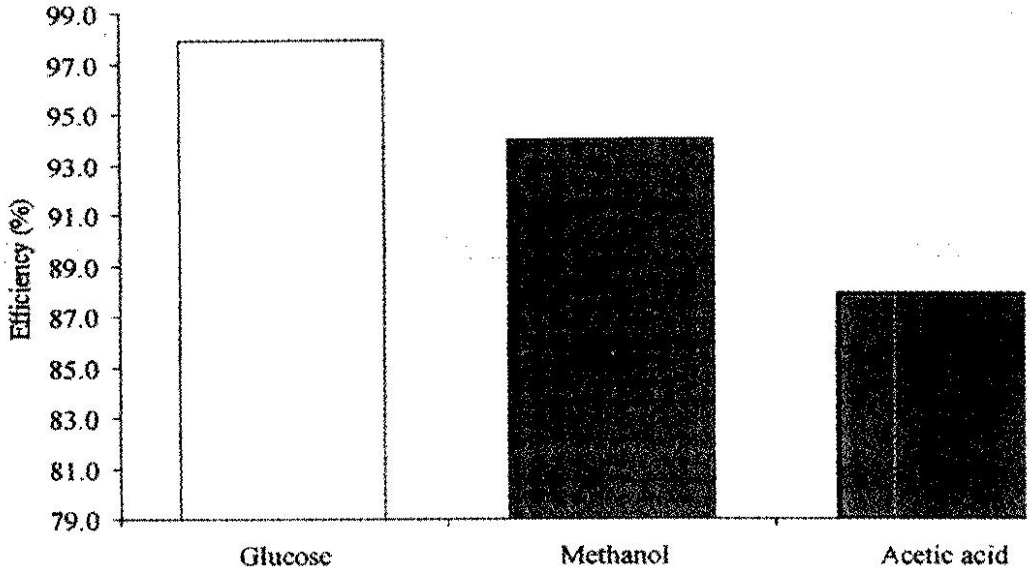

Fig.4: Influence of carbon sources on denitrification efficiency

Fig. 3 shows that for each level of NVL, increasing phosphate concentrations up to 0.5 mg P/L influent increased efficiency. Beyond the level of 0.5 mg P/L, efficiency decreased. This means that phosphate concentration, to some extent, could be promotive and destructive. Therefore, to achieve maximum efficiency, optimum phosphate concentrations should be in the range of 0.4–0.6 mg P/L influent. To maintain maximum efficiency, a maximum NVL was about 0.6 g N/ day/L reactor.

Influence of Carbon Sources on Dentirification Efficiency

Triplicate results of various carbon sources on denitrification efficiency are shown in *Fig. 4.* It can be seen that methanol resulted in the highest efficiency of 94%, glucose 98 % and acetic acid had the lowest at 88%. The results can be explained as the potential energy donation of 1 electron in each carbon source (Verstraete 1987), i.e glucose – 10.0 kJ, methanol – 8.965 kJ and acetic acid – 6.609 kJ. This means that denitrifiers would have a high affinity for carbon sources with the highest potential energy.

CONCLUSION

Results of the current experiment can be concluded as follows:

1. Nitrogen volumetric loading (NVL) at a maximum of 0.6 g N/day/L reactor resulted in an efficient heterotrophic denitrification process.
2. Importantly, increasing NVL decreases detention time. A detention time of about an hour was set as a limiting factor for suppressed nitrite formation.
3. A setting of 0.4 – 0.6 mg P/L allows phosphate to assure free nitrite at tropical conditions.

4. At a similar concentration of 64 mg/L, glucose, methanol and acetic acid had an efficiency of 98%, 94% and 88%, respectively.

ACKNOWLEDGEMENTS

The author wishes to thank the following institutions: Pasuruan Industrial Estate at Rembang (PIER) managed by PT Surabaya Industrial Estate Rungkut (SIER) Surabaya, Septage Treatment Plant Unit Keputih Surabaya managed by Surabaya Cleansing Department, and the Laboratory of Process Engineering of Department of Environmental Engineering, ITS Surabaya. Special thanks go to Mr. P. Ruthen, Team Leader of Advisor Consultants for Western Java Environmental Management Project, for language editing.

REFERENCES

BARNES, D. and P.J. BLISS. 1983. *Biological Control of Nitrogen in Wastewater Treatment.* New York: E & FN Spon. 146p.

BOLEY, A., W.R. MÜLLER and G. HAIDER. 2000. Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems. *Aquacult. Eng.* **22:** 75-85.

CALOW, P. 1993. *Handbook of Toxicology.* London: Oxford Blackwell Scientific.

CHRISTENSEN, M.H. and P. HARREMOES. 1977. Biological denitrification of sewage: a literature review. *Progress in Water Technology* **8**: 509-522.

CHUNG, J. and W. BAE. 2002. Nitrite reduction by a mixed culture under conditions relevant to shortcut biological nitrogen removal. *Biodegrad.* **13:** 163-170.

CLAUDIO, D.R., B. VINCENZO and M. SUREYYA. 2005. Cotton-supported heterotrophic denitrification of nitrate-rich drinking water with a sand filtration post-treatment. *Water SA.* **31:** 229-236.

DRIES, D., J. LIESSENS, W. VERSTRAETE, P. STEVENS, P. DE VOS and J. DE LEY. 1988. Nitrate removal from drinking water by means of hydrogenotrophic denitrifierss in a polyurethane carrier reactor. *Water Supply* **6:** 181-192.

FEACHEM, R.G., D.J. BRADLEY, H. GARELICK and D.D. MARA. 1983. *Sanitation and Disease-Health Aspects of Excreta and Wastewater Management.* John Wiley & Sons, pp.112-113.

GOMMERS, P.J.F., W. BIJLEVELD and J.G. KUENEN. 1988. Simultaneous sulfide and acetic acid oxidation in a denitrifying fluidized bed reactor – I. *Water Research* **22(9)**: 1075-1083.

HUNTER, W.J. 2003. Accumulation of nitrite in denitrifying barriers when phosphate is limiting. *J. Cont. Hydrol.* **66:** 79-91.

LIN, Y.F., S.R. JING, T.W. WANG and DY. LEE. 2002. Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. *Environ Pollut.* **119(3):** 413-20.

MERGAERT, J., A. BOLEY, M.C. CNOCKAERT, W.R. MULLER and J. SWINGS. 2001. Identity and potential functions of heterotrophic bacterial isolates from a continuous-upflow fixed-bed reactor for denitrification of drinking water with bacterial polyester as source of carbon and electron donor. *Systematic and Applied Microb.* **24(2):** 303 –310.

MOORE, S.F. and E.D. SCHROODER. 1971. The effect of nitrate feed rate on denitrification. *Water Research* **5:** 452-455.

NAKHLA, G and S. FAROOQ. 2003. Simultaneous nitrification-denitrification in slow sand filters. *J. Haz. Mat.* **B96:** 291-303.

OLESZKIEWICZ, J.A. and S.A. BERQUIST. 1988. The effect of low temperature on nitrogen removal in sequencing batch reactor. *Water Research* **22(9):** 1163-1171

PEKDEMIS, T., E.K. KCMAZOGLU, B. KESKINLER and O.F. ALGUS. 1998. Drinking water denitrification in a fixed bed packed biofilm reactor. *J. of Engineering and Environmental Sci.* **22:** 39-45.

PRATT, P.F. and W.A. JURY. 1984. *Pollution of the Unsaturated Zone with Nitrate. Pollutants in Porous Media.* Springer-Verlag. pp.52-65.

REISINGER, C., R. BRAUN and A. MOSER. 1989. Cybernetic and technical aspects for advanced biological drinking water treatment. Workshop Graz University of Technology.

SCHIPPER, L.A and M. VOJVODIÆ-VUKOVIÆ. 2000. Nitrate removal from groundwater and denitrification rates in a porous treatment wall amended with sawdust. *Ecol. Eng.* **12:** 269-278.

SOARES, M.I.M, A. BRENNER, A. YEVZOR, R. MESSALEM, Y. LEROUX and A. ABELIOVICH. 2000. Denitrication of groundwater: pilot-plant testing of cotton-packed bioreactor abd post-microfiltration. *Water Sci. Technol.* **42(1-2):** 353-359.

SOARES, M.I.M. 2000 Biological denitrification of groundwater. *Water Air Soil Pollut.* **123:** 183-193.

ST. AMANT, P.P. and P.L. MCCARTY. 1969. Treatment of high nitrate waters. *J. American Water Works Assoc.* **61**: 659-662.

STANDARD METHODS. 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, 19th Edition.

VANDERHOEK, J.P., P.J.M. VANDERVEN and A. KLAPWIJK. 1988. Combined ion exchange/ biological denitrification for nitrate removal from groundwater under different process conditions. *Water Research* **22(6):** 679-684.

VANDERHOEK, J.P., W.F. VANDERHOEK and A. KLAPWIJK. 1989. Nitrate removal from groundwater use of a selective resin and a low concentrated regenerant. *Water, Air, Soil Pollution* **37**: 41-53.

VERSTRAETE, W. 1988. Theoretical exercises environmental biotechnology. Lab of Microbial Ecol, State University of Ghent, Belgium.

WPCF. 1983. Nutrient Control. Manual of practice FD-7. Water Pollution Control Federation, USA.