Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 5 (5): 422-431, 2010
ISSN 1816-4935 / DOI: 10.3923/jm.2006.142.151

Biodegradation of Nitrate in Wastestreams from Explosives Manufacturing Plants*

A. Vijaya Chitra and P. Lakshmanaperumalsamy
Department of Environmental Sciences, Bharathiar University,
Coimbatore-641 046, Tamil Nadu, India

Abstract: The potential of the bacterial isolates to biodegrade nitrate from explosive industry effluent was tested using a batch scale process. Three bacterial species capable of biodegrading nitrate were isolated from effluent and sludge samples taken from a washwater soakaway manufacturing nitroglycerin and slurry explosives. Bacteriological analysis of the samples revealed the presence of about 58% nitrate reducing bacteria belonging to the genera *Alcaligenes*, *Corynebacterium*, *Bacillus*, *Pseudomonas* and *Micrococcus*. Among the isolates *Pseudomonas* sp.-NGS 5, *Bacillus* sp. NGS 6 and *Corynebacterium* sp.-SEE 12 were found to be efficient in nitrate reduction. Physico-chemical parameters were also analyzed for all the samples. Individual and different bacterial consortia were used for the removal of nitrate in synthetic solution. It was found that the bacterial consortium was efficient in nitrate removal in the effluent. The consortium combination-*Pseudomonas*, *Bacillus* and *Corynebacterium* was used for the removal of nitrate in nitroglycerine and slurry explosive effluents that were diluted to different concentrations viz., 25, 50, 75 and 100%. The percentage of nitrate removal by the bacterial consortium was 55 and 58% in nitroglycerine and slurry explosive effluent, respectively.

Key words: Explosive effluent, nitrate, bioremediation, bacteria

INTRODUCTION

Nitrate is one of the several inorganic pollutant contributed by nitrogenous fertilizers, organic manures, human and animal wastes and industrial effluents (Majundar and Gupta, 2000). This has exacerbated acidification of soils and water bodies, changing species composition of ecosystems, raising nitrate levels beyond acceptable levels in drinking water and causing eutrophication of lakes and the sea. Due to its high solubility in water and low retention by soil particles, nitrate is prone to leaching to the subsoil layers and ultimately to the groundwater. Ground water with high concentrations of nitrate when used for drinking cause potential risk to public health, particularly to infants (Fewtrell, 2004; Gangolli *et al*., 1994).

Health hazards associated with high nitrate, includes methemoglobinemia, gastric cancer, goiter, malformed child. Infants are susceptible to methemoglobinemia than adults as they have a lower stomach acidity, which allows growth of bacteria capable of converting nitrate to nitrite. High nitrate reduces assimilation of iodine by human body causing goiter but is yet to be proved (ECETOC, 1998). High nitrate intake with drinking water may lead to the birth of a malformed child (Dorsche *et al*., 1984; Kar *et al*., 2002). There are reports of other health disorders namely non- Hodgkins lymphoma

Corresponding Author: Dr. P. Lakshmanaperumalsamy, Department of Environmental Sciences, Bharathiar University, Coimbatore-641046, Tamil Nadu, India
Tel: 091-422-2422222 Fax: 091-422-2422387

Originally Published in Research Journal of Microbiology, 2006

(Weisenberger, 1991), increased infant mortality (Super *et al*., 1981) and hypertension (Malberg *et al*., 1978). Hence nitrate contaminated effluent must be treated by effective method before released into the land or stream. Though many chemical treatments exist, they are not efficient in the removal of nitrate. It also creates disposal problems because of the remaining solid waste and is cost effective.

Biological removal of nitrogenous compounds from industrial wastewater with high nitrogen contents has been the focus of many studies, mainly because of its potential advantage and improved removal of these compounds over physical and or chemical processes (Clifford and Liu, 1993; Kamath *et al*., 1992). Biological treatment includes both anaerobic and aerobic systems where nitrate is metabolized by microorganisms in three different ways i.e. assimilation to ammonia under aerobic conditions, dissimilation to nitrogen and dissimilation to ammonia under micro aerobic or anaerobic conditions. The latter form of dissimilation has been reported to be competitive under certain conditions depending upon available oxygen in the growth environment (Fazzolari *et al*., 1990; Rehr and Klemme, 1989).

High nitrate content is a threat to human and animal health either by nitrate accumulation or by conversion. Hence, the aim of the present research was to examine the efficiency of microorganisms individually and in consortium for the removal of nitrate from wastestreams of explosive manufacturing plants.

MATERIALS AND METHODS

Analysis of Physico-Chemical Parameters of the Effluent

The explosive industry effluent was obtained from an explosive manufacturing plant located in the Southern region of India that produces gelatin sticks and slurry explosives. Nitroglycerine and ammonium nitrate were used as a raw material for nitroglycerin and slurry explosive unit, respectively. The effluents were analyzed for the following physico- chemical parameters: pH (Cyberscan-Model-510), conductivity (Jenway-Model-4070), dissolved oxygen (Jenway-Model-9070), COD, sodium, potassium, phosphate (Saxena, 1994), nitrate (Phenol disulphonicacid method), nitrite(Manivasakam, 1987) and ammonia (colorimetric method).

Isolation and Identification of Bacterial Cultures

The explosive industry effluents from nitroglycerine and slurry explosive units were collected in sterile container from the point of release and from the pond nearer to the industry. The sludge samples were collected in sterile polyethylene bags from the bottom of the ponds. Pour plate technique using nutrient agar (beef extract: 3.0 g; yeast extract: 3.0 g ; peptone:5.0 g ; sodium chloride: 5.0 g; agar: 20.0 g ; distilled water: 1000 mL; pH: 7.0 ± 0.2) was employed to enumerate the Total Heterotrophic Bacterial (THB) count of the samples. The isolated bacterial cultures were characterized by their morphological and biochemical characteristics (Bergey's Manual, 1994). The bacteria were then subjected to nitrate test. The potent isolates were selected and used for the treatment of the effluent.

Selection of Nitrate Reducers

Nitrate reduction was tested on potassium nitrate broth (peptone- 5 g L^{-1}; beef extract-3 g L^{-1}; sodium chloride-5 g L^{-1}; potassium nitrate-5 g L^{-1}). The ability of the isolates to reduce nitrate to nitrite and ammonium was tested by the addition of 1 mL of Nessler's reagent to the cultures. The appearance of yellow orange colour indicates that nitrate/nitrite has been reduced to ammonia. The efficient isolates were selected based on the production of color and further tested for nitrate removal.

Inoculum Preparation

Nutrient broth (beef extract: 3.0 g; yeast extract: 3.0 g; peptone: 5.0 g; sodium chloride: 5.0 g; distilled water: 1000 mL; pH: 7.0±0.2) was prepared and selected bacterial isolates were inoculated separately and incubated at room temperature for 24 h. The cells were removed by centrifugation (10,000 rpm for 20 min) and were transferred to sterile saline. The cell concentration of each strain was adjusted to an OD_{600} of 1 and used as inoculum. Three efficient isolates (*Pseudomonas* sp.-NGS 5, *Bacillus* sp. NGS 6 and *Corynebacterium* sp.-SEE 12) were used for remediation.

The mixed bacterial consortium from the three isolates *Pseudomonas* sp. NGS 5-(A), *Bacillus* sp. NGS 6-(B) and *Corynebacterium* sp. SEE 12-(C), were prepared by adjusting the cell concentration of A, B and C to 1 of OD_{600}. The different combinations include A + B, B + C, A + C, A + B + C.

Nitrate Removal by Bacteria in Synthetic Solution

The selected nitrate reducers A, B and C were inoculated in Mineral salts medium-MSM (potassium dihydrogen phosphate 0.1 g L^{-1}; dipotassium hydrogen phosphate 1.0 g L^{-1}; potassium nitrate 2.0 g L^{-1}; ammonium chloride 0.5 g; calcium chloride 0.005 g L^{-1} magnesium sulphate 0.1 g L^{-1}; sodium silicate 0.05 g L^{-1}; pH-7; [Trace elements-Boron-0.025%, Copper sulphate-0.05%, Manganese sulphate-0.05%, Molybdenum chloride-0.006%, Zinc Sulphate-0.07%, distilled water-100 mL]), containing various concentrations (500, 1000, 1500, 2000 mg L^{-1}) of nitrate. They were incubated at 30°C in a shaker maintained at 120 rpm for a period of three days. Growth and nitrate concentration were monitored for every 24 h (UV-VIS Hitachi spectrophotometer – 3020) at 600 nm and 410 nm, respectively.

Nitrate was estimated by phenol disulphonic acid method. The sample was neutralized to pH 7 and evaporated to dryness on water bath. The residue was then dissolved using glass rod with 2 mL disulphonicacid reagent. The dissolved residue was diluted and transferred to Nessler's tube. Blank was prepared in the same way as sample using distilled water. The colour development was read at 410 nm with light path of 1cm which records nitrate as nitrogen in mg L^{-1}. Simultaneously conversion to nitrite and ammonia were also determined.

The bacterial consortium of different combinations A+B, B+C, A+C, A+B+C was inoculated in Mineral salts medium, containing various concentrations (500, 1000, 1500 and 2000 mg L^{-1}) of nitrate and similar procedures were followed as that of individual bacteria.

Nitrate Removal by Bacteria in Effluent

The nitroglycerine and slurry explosive effluents were diluted using sterile distilled water to different (25, 50, 75 and 100%) concentrations. The bacterial consortium of the combination A + B + C was inoculated in the nitroglycerine and slurry explosive effluent in all the above said concentrations. Growth was monitored for every 24 h and nitrate was determined as described above.

RESULTS AND DISCUSSION

Physico-Chemical Parameters of the Effluent

The physico-chemical parameters of the effluent are given in Table 1. In explosive industry effluents, the pH of nitroglycerine unit effluent was 8.9 and slurry explosive unit effluent was 6.5. The D.O was 3.7 mg L^{-1} in slurry explosive effluent followed by nitroglycerine effluent (2.5 mg L^{-1}). The Electrical Conductivity (EC) was 18.2 micro ohms in slurry explosive effluent and 15.5 micro ohms in nitroglycerine effluent.

Table 1: Physico-chemical and bacteriological parameters of explosive industrial effluent

Parameters	Nitroglycerine explosive unit	Slurry explosive unit
pH	8.86	6.5
DO (mg L^{-1})	2.5	3.7
EC (ms)	15.59	18.24
Total solids (mg L^{-1})	1,60,00	25,760
Suspended solids (mg L^{-1})	8,440	1,600
Dissolved solids (mg L^{-1})	1,51,560	24,160
COD (mg L^{-1})	1,680	1,438
Nitrate (mg L^{-1})	1,700	1000
Nitrite (mg L^{-1})	0.4648	0.38
Ammonia (mg L^{-1})	2.764	5.27
Sodium (mg L^{-1})	29,100	21,400
Potassium (mg L^{-1})	3,600	2,840
Calcium (mg L^{-1})	24,500	24.4
Phosphate (mg L^{-1})	28.7	21.0
Effluent bacteria (10^2 g^{-1})	40	100
Sludge bacteria (10^2 g^{-1})	30	18

Table 2: Nitrate reducing bacterial genera

Strain No.	Bacterial genera	Nitrate reduction
SME-1	*Corynebacterium*	-
SME-2	*Bacillus*	-
SMS-3	*Alcaligenes*	-
SMS-4	*Micrococcus*	++
NGS-5	*Pseudomonas*	+++
NGS-6	*Bacillus*	+++
NGS-7	*Bacillus*	+
NGS-8	*Micrococcus*	+
NGE-9	*Corynebacterium*	+
NGE-10	*Bacillus*	-
NGE-11	*Micrococcus*	-
SEE-12	*Corynebacterium*	+++
SEE-13	*Corynebacterium*	+++
SEE-14	*Micrococcus*	-
SEE-15	*Micrococcus*	-
SEE-16	*Bacillus*	-
SES-17	*Alcaligenes*	+
SES-18	*Micrococcus*	-
SES-19	*Corynebacterium*	++
SES-20	*Alcaligenes*	++
SES-21	*Alcaligenes*	+
SES-22	*Alcaligenes*	-
SES-23	*Alcaligenes*	+
SES-24	*Alcaligenes*	-
SES-25	*Pseudomonas*	+++
SES-26	*Alcaligenes*	-

+++ = Highly positive; + = Positive; - = Negative

In nitroglycerine effluent the amount of total solids, total suspended solids and dissolved solids were 1,60,000, 8,440 and 1,51,560 mg L^{-1}, respectively, whereas in slurry explosive effluent it was less. Similarly the parameters such as COD, nitrate, nitrite, ammonia, sodium, potassium, calcium and phosphate were found to be higher in nitroglycerine explosive effluent.

Nitrate Reducers

The THB population in nitroglycerine unit was 40×10^2 CFU mL^{-1} in effluent and 30×10^2 CFU g^{-1} in sludge. In slurry explosive unit, the THB population was 100×10^3 CFU mL^{-1} in effluent and 18×10^3 CFU g^{-1} in sludge.

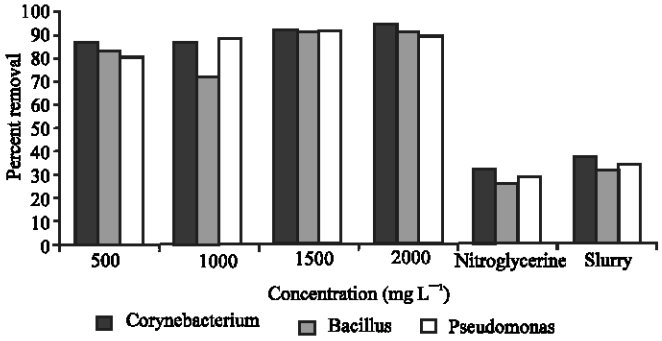

Fig. 1: Percentage of nitrate removal in aqueous solution and explosive effluents by individual bacteria

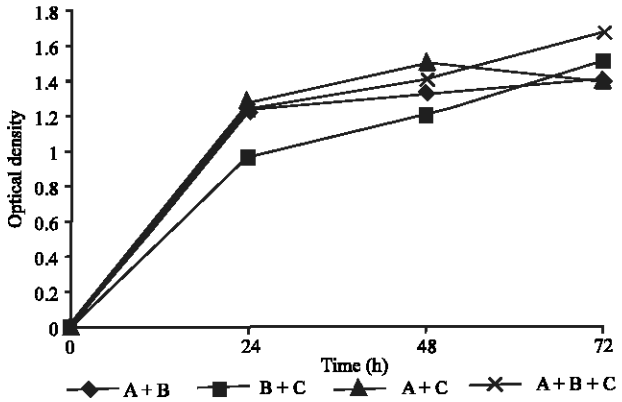

Fig. 2a: Bacterial growth at 1000 mg L^{-1} concentration of nitrate removal

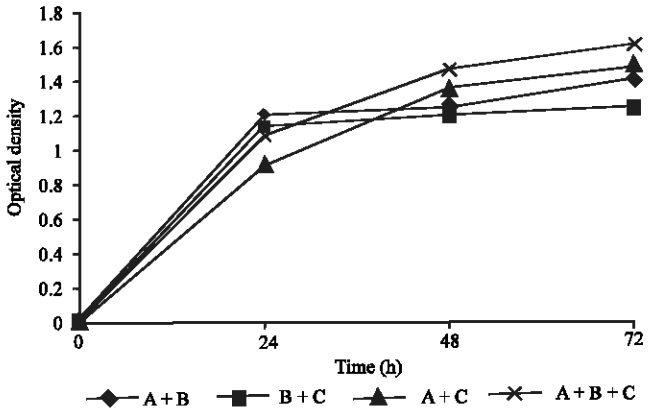

Fig. 2b: Bacterial growth at 2000 mg L^{-1} concentration of nitrate removal

The genera *Alcaligenes*, *Corynebacterium*, *Bacillus*, *Pseudomonas* and *Micrococcus* were identified as efficient nitrate reducing organisms (Table 2). Among the efficient isolates *Pseudomonas*

sp. (NGS-5), *Bacillus* sp. (NGS-6) and *Corynebacterium* sp. (SEE-12, SEE -13, SES-25) were found to be more proficient in nitrate reduction. For further study isolates *Pseudomonas* sp. (NGS-5), *Bacillus* sp. (NGS-6) and *Corynebacterium* sp. (SEE-12) were chosen.

Nitrate Removal by Bacteria in Synthetic Solution and Effluent

In synthetic solutions containing 500 mg L^{-1} nitrate concentration, *Corynebacterium*, *Bacillus* and *Pseudomonas* removed 87, 83 and 81% of nitrate, respectively. At 1000 mg L^{-1} concentration, 89% removal was shown by *Pseudomonas* followed by *Corynebacterium* and *Bacillus showing* 87 and 72% removal, respectively. Studies at 1500 mg L^{-1} concentration showed 93% removal by *Corynebacterium* sp. followed by *Bacillus* and *Pseudomonas* sp. with 92% nitrate removal. At 2000 mg L^{-1} concentration maximum of 95% nitrate removal was achieved by *Corynebacterium* sp. followed by *Bacillus* sp. (92%) and *Pseudomonas* sp. (90%) (Fig. 1). Study by Ramos *et al*. (1996) showed that microbes from the grounds of an explosive factory were relatively tolerant to high concentrations of nitrate and they were also able to grow in culture medium containing NO_3^- at concentrations up to 80 mM. Microalgae capable of growing at various concentrations of nitrate ranging from 140 to 1400 mg N L^{-1} was reported by Kwangyong and Lee. (2002). The results of this present study show that the selected bacteria strains were capable of growing at the maximum of 2000 mg L^{-1} in synthetic solution.

In the effluent, maximum percentage of nitrate removal was accomplished by *Corynebacterium* in nitroglycerine (33%) and slurry (38%) explosive effluent. The selected strains showed a higher percentage of nitrate removal in synthetic solutions, but the percentage of nitrate removal was comparatively less in the effluent (Fig. 1). So the efficiency of mixed bacterial consortium was tested for the treatment of the effluent. Growth pattern of different combination of the consortium (A+B, B+C, A+C, A+B+C) in synthetic solution at 1000 and 2000 mg L^{-1} nitrate concentrations is given in Fig. 2a and b (approximate concentration of nitroglycerine and slurry explosive effluent). The combination A+B+C recorded higher growth rate than other combinations, which indicate its resistance against nitrate.

The percentage of nitrate reduction by different consortia is given in Fig. 3. Maximum of 86% nitrate removal was noticed at 500 mg L^{-1} concentration by the consortium A+B+C, followed by B+C (67%), A+B (65%) and A+C (44%). At 1000 mg L^{-1} concentration A + B + C showed 85% nitrate removal, followed by B+C (76.60%), A+B (71.36%) and A+C (64.15%). At 1500 mg L^{-1} concentration the percentage of removal by A+B+C was 91.5% followed by B+C (87.36), A+C (80%) and A+B (75.8%). Maximum percentage of nitrate removal was achieved in the combination A+B+C (94%) followed by B+C (92.71), A+B (91.7) and A+C (88%). The consortium A+B+C removed higher percentage of nitrate than other combinations and maximum percentage reduction was noticed within 24 h (Fig. 4).

The above results clearly indicated that the consortium A+B+C was ideal for nitrate removal and so this combination was used for nitrate removal at different concentrations (25, 50, 75 and 100%) of the nitroglycerine and slurry explosive effluent. In the above said concentrations of the nitroglycerine effluent, the percentage of removal was 65, 59.3, 58.2 and 55% and in slurry explosive effluent it was 64.4, 60, 58.5 and 58%, respectively. Rate of nitrate removal in nitroglycerine and slurry explosive effluent for every 24 h is given in Fig. 5 and 6.

The consortium recorded a lower percentage of nitrate removal in the effluent compared to that of aqueous solution. This may be due to the presence of toxic organic compounds that were present in the effluent. The studies of Smith (1983) showed that the concentration above 6000 mg L^{-1} of nitrate inhibited the cell growth and thus reduced the rate of nitrate reduction. According to Francis and

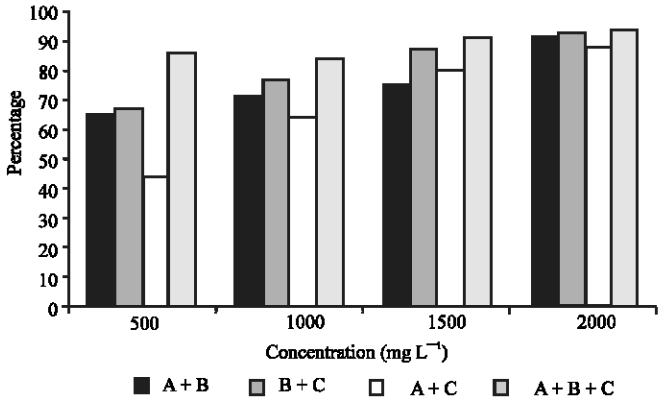

Fig. 3: Percentage removal of nitrate at different concentrations by mixed consortium

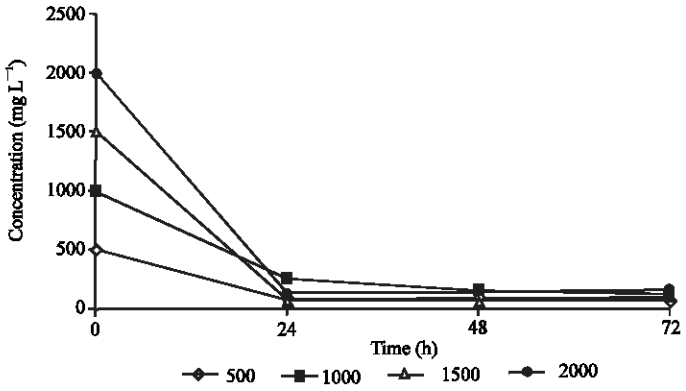

Fig. 4: Removal of nitrate by the bacterial consortium A + B + C in aqueous solution

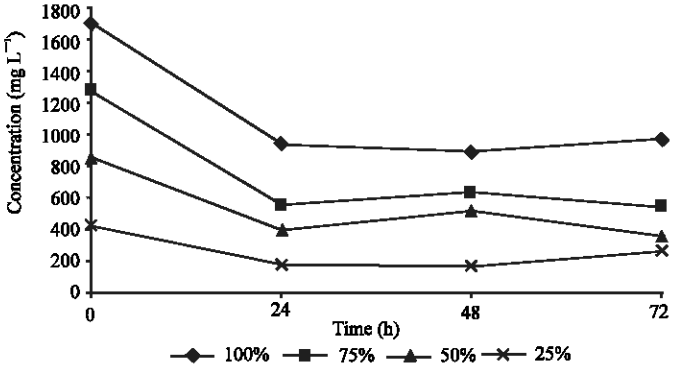

Fig. 5: Nitrate removal by bacterial consortium A + B +C at various concentrations of nitroglycerine effluent

Table 3: Nitrite and ammonia produced by the consortium after 72 h at different concentrations of nitrate, Nitroglycerine Effluent (NGE) and Slurry explosive effluent

Consortium	500*	1000*	1500*	2000*	NGE	Slurry
A+B						
Nitrite (mg L^{-1})	2.19	4.6	5.57	1.28		
Ammonia (mg L^{-1})	3.40	4.83	3.94	3.09	-	-
B+C						
Nitrite (mg L^{-1})	0.565	0.814	4.34	3.14		
Ammonia (mg L^{-1})	3.27	1.99	2.74	4.94	-	-
A+C						
Nitrite (mg L^{-1})	0.897	2.54	3.63	1.98		
Ammonia (mg L^{-1})	3.31	4.17	3.6	3.2	-	-
A+B+C						
Nitrite (mg L^{-1})	1.92	3.44	2.47	2.11	2.9	3.6
Ammonia (mg L^{-1})	3.26	4.21	3.1	2.86	3.8	4.4

* Concentration of nitrate in mg L^{-1}

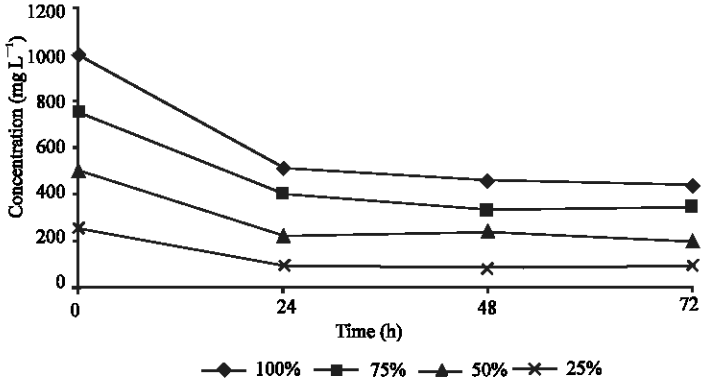

Fig. 6: Nitrate removal by bacterial consortium A + B +C at various concentrations of slurry explosive effluent

Hancher (1981) nitrate concentrations greater than 1.7 kg m^{-3} of NO_3-N of nitrate, was inhibitory. Though the consortium have used nitrate as electron acceptor, at higher concentrations of aqueous solutions, the capacity to reduce nitrate in the effluent was comparatively low. The reason could be either a competition for degradation between nitroglycerine and nitrate, as the organisms are resistant to the xenobiotic compound.

During nitrate reduction, production of nitrite and ammonia were observed in media. Concentration of nitrite and ammonia produced during nitrate reduction is given in Table 3. The accumulation of nitrite in bacterial culture may be in principle due to either assimilatory or dissimilatory nitrate reduction or due to heterotrophic nitrification. Assimilatory nitrate utilization involves the reduction of NO_3^- to NO_2^- in a two-electron reaction mediated by nitrate reductase and further reduction of NO_2^- to NH_3^+ involves six electrons in a reaction mediated by nitrite reductase. The nitrite generated can also be further reduced to nitric oxide, nitrous oxide and dinitrogen (Cole, 1988; Kuenen and Robertson, 1987). The nitrite reduction under aerobic conditions was very low and results of previous work with *Aeromonas faecalis* (Otte *et al*., 1999) indicated that the observed N_2O production may not be due to reduction of NO_2, but may be a by product of heterotrophic nitrification. The concentration of nitrite in the nitroglycerine and slurry explosive effluent was 3.8 and 4.4 mg L^{-1}.

This study evidently proved that the consortium containing *Bacillus, Corynebacterium* and *Pseudomonas* is ideal for nitrate removal.

CONCLUSIONS

The obtained results lead to the conclusion that the microbial community is capable of surviving at higher concentration of nitrate and also in the presence of the xenobiotic compound such as nitroglycerine. The consortium containing the organisms, *Pseudomonas* sp. (NGS-5), *Bacillus* sp. (NGS-6) and *Corynebacterium* sp. (SEE-12) isolated from the effluent, are found to be highly ideal for the biological treatment of the explosive industry effluent and it can also be applied for bioremediation of the contaminated site.

REFERENCES

Bergey's Manual of Determinative Bacteriology, 9th Edn., 1994. The Williams and Wilkins Co., Baltimore.

Clifford, D.A. and X. Liu, 1993. Nitrate ion exchange process with batch denitrification and reuse of spent brine. J. Am. Wat. Wks Ass., 85: 135-143.

Cole, J.A., 1988. Assimilatory and Dissimilatory Reduction of Nitrate to Ammonia. In: the Nitrogen and Sulphur Cycles (Eds.) Cole, J.A. and S. J. Ferguson Cambridge University Press, Cambridge, pp: 281-329.

Dorsche, M.M., R.K.R. Scragg, A.J. Mc Michael, P.A. Baghust and K.F. Dyer, 1984. Congenital malformations and maternal drinking water supplying rural south Australia: A case study. A.M. J. Epidemiol., 119: 473-486.

ECETOC, 1988. (European Chemical Industry Ecology and Toxicology Centre), Nitrate and Drinking Water, Technical Report, No. 27 Brussels.

Fazzolari, E., A. Mariotti and J.C. Germon, 1990. Dissimilatory ammonia production vs. denitrification in vitro and inoculated agricultural soil samples. Can. J. Microbial., 36: 786-793.

Fewtrell, L., 2004. Drinking-water nitrate, methemoglobinemia and Global Burden of Disease: A discussion. Environ. Health Perspect., 112: 1371-1374.

Francis, C.W. and C.W. Hancher, 1981. Biological Denitrification of High-nitrate Wastes Generated in the Nuclear Industry. In: Cooper, P.F. and B. Atkinson (Eds.), Biological Fluidized-bed Treatment of Water and Wastewater, Halstead Press, New York, NY, pp: 234-250.

Gangolli, S. D., P.A. Van den Brandt, V. J. Feron, C. Janzowsky, J. H. Koeman, G. J. A. Speijers, B. Spiegelhalder, R. Walker and J. S. Wishnok, 1994. Nitrate, nitrite and N-nitroso compounds. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect., 292: 1-38.

Kamath, S., D.A. Sabatini and L. W. Canter, 1992. Treatment of high nitrogen ($NaNO_2$) wastewater by biological nitrification/denitrification. In: 46th Purdue Industrial Waste Conference Proceedings. Lewis Publishers, Inc., Chelsea, Mich., pp: 623-630.

Kar, S., D.K. Khan and S.C. Santra, 2002. Ground water nitrate concentration and its impact on human health: A review. Everyman's Science, 37: 35-40.

Kuenen, J. G and L.A. Robertson, 1987. Ecology of Nitrification and Denitrification. In: The Nitrogen and Sulphur Cycles (Eds.) Cole, J.A. and S.J. Ferguson, Cambridge University Press, Cambridge, pp: 161-218.

Kwangyong, L. and C.G Lee, 2002. Nitrogen removal from wastewater by microalgae without consuming organic carbon sources. J. Microbiol. Biotechnol., 12; 979-985.

Majundar, D. and N. Gupta, 2000. Nitrate Pollution of groundwater and associated human health disorders. Indian J. Environ. Hlth., 42: 28-39.

Malberg, J.W., E. P. Savage and J. Osteryoung, 1978. Nitrate in drinking water and the early onset of hypertension. Environ. Pollut., 15: 155-161.

Manivasakam, N., 1987. Industrial Effluent, Origin, Characteristics, Effects Analysis and Treatment. Sakthi Publications, Kovaipudur, Coimbatore.

Otte, S., J. Schalk, J.G. Kuenen and M.S.M. Jetten, 1999. Hydroxylamine oxidation and subsequent nitrous oxide production by the heterotrophic ammonia oxidizer *Alcaligenes faecalis*. Applied Microbiol. Biotechnol., 51: 255-261.

Ramos, J.L., A. Haidour, E. Duque, G. Pinar, V. Calvo and J.M. Oliva, 1996. Metabolism of nitrate esters by a consortium of two bacteria. Natl. Biotechnol., 14: 320-322.

Rehr, B. and J.H. Klemme, 1989. Competition for nitrate between denitrifying *Pseudomonas stutzeri* and nitrate ammonifying enterobacteria. FEMS Microbiol. Eco., l 62: 51-58.

Saxena, M.M., 1994. Environmental analysis-Water, Soil and Air. Agro Botanical Publishers (India), 4: 121-145.

Smith, M.S., 1983. Nitrous oxide production by *Escherichia coli* is correlated with Nitrate Reductase Activity, Applied Environ. Microbiol., 45: 1545-1547.

Super, M., H. Heese, D. Machenzie, W.S. Dempster, J. Duplers and J.J. Ferreina, 1981. An epidemiological study of well water nitrates in a group of southwest African Namibian infants. Water Res., 15: 1265-1270.

Weisenberger, D.D., 1991. Potential Health Consequences of Groundwater Contamination by Nitrate in Nebraska. In: Nitrate Contamination Exposure, Consequence and Control (Ed s.) Bogaidi and R.D. Kuzeia NATO ASI Ser geological Sciences 30 Springer Verlag, Berlin, pp: 309-315.