I J B A
International Journal of Biotechnology Applications
ISSN: 0975–2943 & E-ISSN: 0975–9123, Vol. 3, Issue 3, 2011, pp-106-109
Available online at http://www.bioinfo.in/contents.php?id=22

BIOLOGICAL DENITRIFICATION OF WASTEWATER IN A FLUIDIZED BED BIOREACTOR BY IMMOBILIZATION OF PSEUDOMONAS STUTZERI USING POLY PROPYLENE GRANULES

SRINU NAIK S. AND PYDI SETTY Y.*
Department of Chemical Engineering, National Institute of Technology, Warangal, Andhra Pradesh, India.
*Corresponding Author: Email- psetty@nitw.ac.in

Received: August 31, 2011; Accepted: September 27, 2011

Abstract- Nitrate is a major pollutant present in effluent wastewater from nitrogenous fertilizer, explosives, paper mills, pulp mills, and also from municipal waste. Nitrate is harmful to both mankind and animal and also to the environment. The World Health Organization has set a limit of 10 mg/L NO_3^- for human consumption and 100 mg/l NO_3^- for animals. Water above these limits requires denitrification. Nitrates causes cancer, blue-bay syndrome, hypertension and thyroid hypertrophy. Experimentation on biological denitrification was carried out in a fluidized bed bio-reactor using synthetic wastewater with polypropylene beads as supporting media for the growth of microorganism. Synthetic wastewater is taken into the reactor for biological treatment and air was fed from bottom with solids being fluidized at the top due to low density. Experiments were performed for water of different initial nitrate concentrations with *Pseudomonas stutzeri* microorganism by varying the parameters like airflow rate, temperature, carbon source, poly propylene beads and pH in the range 2 lpm to 3.5 lpm, 20 ^{0}C to 35 ^{0}C, 70 mg/L to 85 mg/L, 5 mg/L to 25 mg/L and 6 to 8 respectively. The optimum parameters at which maximum denitrification is noticed are found to be 2.5 lpm (air flow rate), 30 ^{0}C (temperature), 85 mg/L (carbon source), 15 mg/L (poly propylene beads) and 7 (pH). More than 95% removal of nitrates is noticed from the experimental work. The objective of the study is to investigate the effect of operating parameters like airflow rate, temperature, carbon source, polypropylene beads and pH.
Keywords: wastewater treatment, Fluidized bed bioreactor, microorganism, biological denitrification, immobilization, *Pseudomonas stutzeri*

INTRODUCTION

Removal of Nitrates is an important environmental issue as nitrate is one of the most common groundwater contaminants world-wide and discharge of nitrogen components into the environment can be a cause of serious problems such as eutrophication of rivers and deterioration of water sources, as well as hazard for human and animal health and also to the environment. Nitrate in drinking water for animal and human consumption is not recommended for health reasons. Industries are the greatest source of pollution, accounting for more than half the volume of all water pollution and nitrogen in ground water can be caused by human excreta, sewage disposal, cattle seepage, fertilizer industries, explosives industries, municipal waste and industrial effluents, particularly from food processing. The World Health Organization has set a limit of 10 mg/L NO_3^- for human consumption and 100 mg/L NO_3^- for animals. When large quantities of nitrate are consumed by infants, their skin appears to have a bluish tint due to the lack of oxygen, a condition called methemoglobinemia or "blue baby syndrome" and also causes cancer, birth defects, abortions, hypertension thyroid hypertrophy. Nitrate in excessive levels can also be potentially harmful to animals and can cause abdominal pains, muscular weakness and brown or chocolate colored blood. Hence nitrates removal is an important aspect of present day wastewater treatment process and biological denitrification is one of the most economical processes for nitrate removal from wastewater.

Denitrification is a process in which the oxidized nitrogen substances, i.e. nitrates and nitrites are reduced to nitrogen gas, such as N_2O and N_2, when a proton donor (energy source) is available. In many biological denitrification systems, the nitrate polluted wastewater (e.g. domestic sewage) contains sufficient carbon source to provide the energy source for the conversion of nitrate to nitrogen gas by the denitrifying bacteria. The treat groundwater, in which the nitrate content may be as high as 100 mg/L with low dissolved carbon content, an additional proton acceptor is required. The nitrate reduction reactions involve the following pathway, in this process microorganisms first reduce nitrates to nitrites and then produce nitric oxide, nitrous oxide and nitrogen gas.

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The main advantage of this process is that it takes place at mild reaction conditions. Denitrification requires an organic and inorganic substrate for energy and cell synthesis. Denitrification especially in waste water treatment is a heterotrophic process requiring an organic substrate for energy. Bacteria can use a variety of carbon sources, which is an important component of the denitrification process; generally most of the wastewater treatment plants use methanol as external carbon source due to economic reasons in denitrification process. Other compounds that can be used in denitrifcation process include acetate, ethanol, methane,
glucose, peptone, saw dust, glycerol, lactic acid, molasses, etc. In this experimental work methanol was used as external carbon source.
There are different methods, which have been developed for removing nitrogen like ion exchange [1], reverse osmosis [2], electro dialysis [3], chemical and catalytic denitrification [4]. Each of them has its own advantages and disadvantages. Biological method is found to be the most commonly used and effective method as there is no secondary pollution. The main advantage of this process is that it takes place at mild reaction conditions.
Several studies has been carried out on denitrification [5-13] but work reported on biological denitrification of waste water using a fluidized bed bioreactor is very little using *Pseudomonas stutzeri*.
Several sources of carbon have been used for denitrification including ethanol and acetic acid [14], news paper [15], cotton [16], acetate, ethanol and methanol [17], rice husk [18], molasses [19], succinic acid, ethanol and acetic acid [20], acetate, ethanol and hydrolysed rice [21].
In this experimental study fluidized bed reactor was used to investigate the effect of variables such as air flow rate, temperature and initial nitrate concentration on nitrate removal from the synthetic waste water. It was operated for the removal of nitrate at different concentrations. Methanol is used as the external carbon source and *Pseudomonas stutzeri* is used as the denitrifying bacteria for which polypropylene beads of density 600 kg/m^3 and of 1.96 mm in diameter were used as the culture medium. This type of reactor was chosen because its retention time is shorter and the carrier offers more for biological growth, therefore the greater nitrate removal efficiency than that of packed bed reactors. This study is aimed at investigating the nitrate
removal at optimum conditions of air flow rate, temperature, pH, Carbon source and polypropylene beads.

MATERIALS AND METHODS

Preparation of slant

The experimental work was carried out in a fluidized bed bioreactor as shown in "Fig. (**1**)". A pure culture of *Pseudomonas stutzeri*, a denitrifying bacterium was obtained which is capable of utilizing nitrates as the energy source. The bacterium was sub cultured once in a month by preparing slants using nutrient agar with the composition of peptone: 10 g, Beef Extract: 10 g, NaCl: 5 g, Agar-agar: 20 g per one liter of distilled water for the slant preparation [22].

Carbon sources

The most commonly used carbon source is Methanol due to its availability and its economic efficiency. Methanol is the simplest alcohol, also known as methyl alcohol, carbinol and wood alcohol. Methanol was originally produced by the destructive distillation of wood chips in the absence of air. Today, methanol is synthesized by a catalytic reaction of carbon monoxide with hydrogen at high temperature and pressure. Ethanol and methane have also been used as external carbon source for wastewater treatment due to their availability and comparable cost.

Cell immobilization and Inoculation of denitrifying bacteria

The experimental work was carried out in a Fluidized bed bio-reactor with attached growth process to investigate the removal of nitrate from the synthetic wastewater and *Pseudomonas stutzeri* with polypropylene beads of density 600 kg/m^3 and of 1.96 mm in diameter used as the supporting media. The bacterium from the slants was inoculated into liquid broth containing nitrate
concentration of 30 mg/L and was prepared by mixing: 48.9 mg of KNO_3, 6 mg of $MgSO_4.7H_2O$, 0.2 mg of $FeCl_3.7H_2O$, 430 mg of Na_2HPO_4 and 320 mg of $Na_2H_2PO_4$[25] and with different initial nitrate concentrations. The composition gives the initial nitrate concentration of 30 mg/L, to increase or decrease the nitrate composition we can vary the amount of potassium nitrate proportionately. The experiments were conducted for a concentration of 300 ppm.

Experimental Set-up

The Fluidized bed bio-reactor consists of a glass column of 0.5 m height, 93 mm of internal diameter and 100mm of outer diameter with a capacity of 3.4 liters. The setup was provided with a glass jacket of 118 mm ID and 122 mm OD, to maintain the temperature of the reactor system at the set point and also provision was made for the supply of air/N_2/O_2 based on the requirement. A gas sparger was located at the base of column for uniform distribution of gas as shown in "Fig.(1)".

Analytical methods

The experiments were conducted for different parameters by varying air flow rate, temperature, carbon source, polypropylene beads, pH and round 15 ml of the product was collected for every one hour, filtered and was used for the analysis of final nitrate concentration using UV-Visible Spectrophotometer.

RESULTS AND DISCUSSION

Parameters, which have major impact on denitrification rate, were selected by conducting different experiments. The parameters like, air flow rate, temperature, carbon source, poly propylene beads, pH were selected, since these parameters significantly affect the nitrate removal rate. From the comparison of experimental results, it was clearly seen that the micro organism used for the denitrification studies was active only under certain conditions. Effect of the parameters, on the biological denitrification using *Pseudomonas stutzeri* organism, was discussed and the optimum parameters were suggested for denitrification.

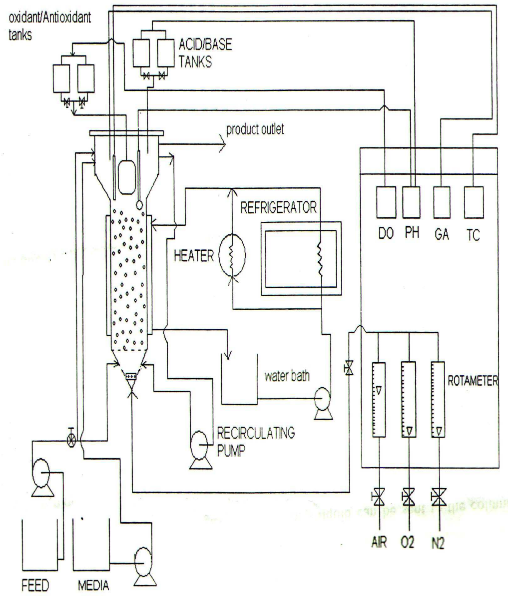

Fig.1-Fluidized bed bioreactor (FBBR)

Effect of Air flow rate

Experiments were conducted to study the effect of air flow rate on nitrate removal capability for different air flow rates like 2, 2.5, 3, 3.5 lpm. As flow rate to the reactor changes, the residence time of the fluid in the reactor varies and the expansion in the bed height also changes with the flow rate, which affects the solid particles within the reactor. As air flow rate increases, the dissolved oxygen (DO) level also increases which affects the denitrification rate of the given microorganism. The results shown in the figure clearly shows that increase in airflow rate results in slow denitrification. Hence it can be concluded that microorganism was active at low DO levels and 2.5 lpm is the suggested optimum value for the denitrification using *pseudomonas stutzeri* as shown in "Fig. (**2**)".

Effect of Temperature

The temperature has an important effect on biological denitrification. Nitrate contaminated groundwater can often be treated in natural systems, depending on many environmental factors such as microbial activity, biodegradable carbon availability and temperature. It has been demonstrated that the rate of nitrification continuously increases in the temperature range of 20 – 35 ^{0}C in agreement with the Van't Hoff- Arrhenius Law. The rate of nitrification continuously increases as the temperature increases and hence the microorganisms are sensitive to temperature and the activity of the microorganism changes with temperature. As shown in "Fig. (**3**)", 30 ^{0}C is found to be the optimum temperature for the present study.

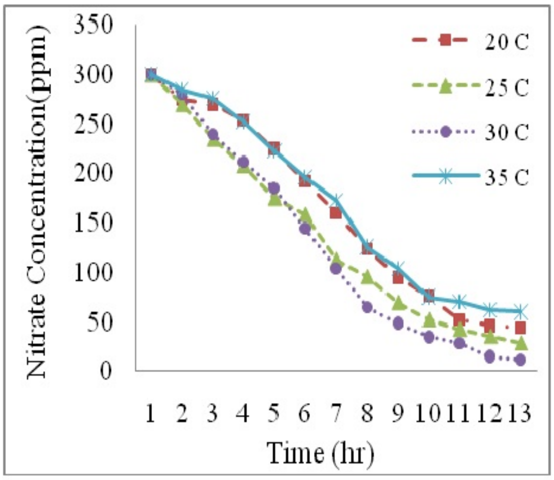

Fig.2-Effect of Airflow rate

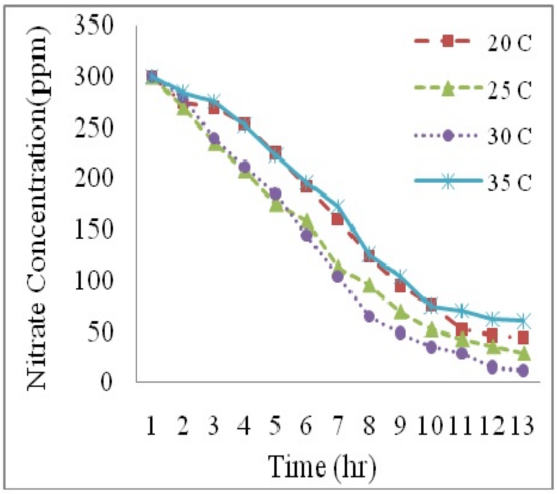

Fig.3- Effect of Temperature

Effect of Carbon source

Methanol is selected as carbon source because it has a neutral pH, contains no nutrients such as nitrogen and phosphorus, and can be considered to contain 100% readily degradable carbon. Methanol is also relatively cheap. Experiments were conducted at four levels of carbon sources i.e., 70, 75 , 80 and 85 mg/L and found that the denitrification rate is maximum at 85mg/L as shown in "Fig. (**4**)".

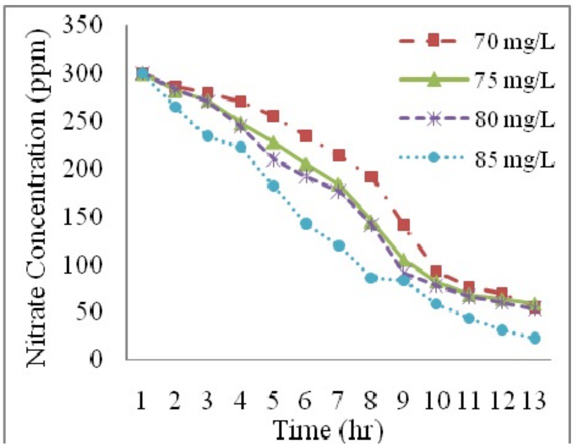

Fig.4 -Effect of Carbon source

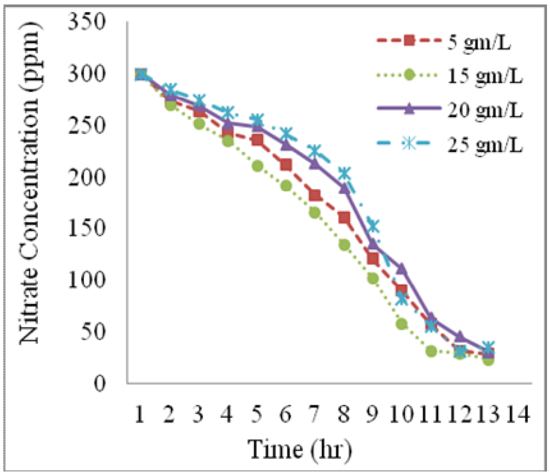

Fig.5 - Effect of Poly propylene beads

Effect of polypropylene beads

Polypropylene beads were used as the carrier for biological denitrification. In this system care must be taken to avoid the destruction and decomposition of immobilized microorganism. The particle size and quantity is an important factor for the formation of a smooth fluidized bed. As the amount of polypropylene beads increases the rate of biological dentrification decreases which may be due to decomposition of microorganism. Hence 15 grams of polypropylene per liter is found to be the optimum value for denitrifcation of wastewater as shown in "Fig. (5)".

Effect of pH

pH is one of the affecting factor for biological denitrification. The effect of pH on the rate of denitrification by *pseudomonas stutzeri* is seen in the following figure. Experiments were conducted with different pH values i.e., 6, 7, 8 and 9. The microorganism is sensitive to pH, the activity of the microorganism changes as the pH increases. It should be note that the rate of oxidation of nitrite begins to decline rapidly above pH 7. Hence from "Fig. **(6)"** it is seen that denitrification rate is high at pH 7.

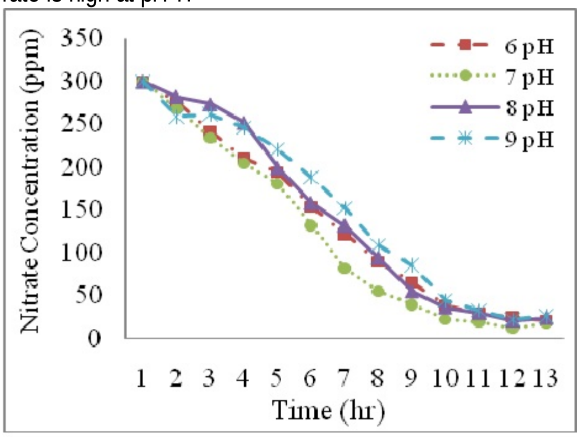

Fig.6- Effect of pH

CONCLUSION

The effect of parameters like airflow rate, temperature, carbon source, poly propylene beads and pH at different levels were conducted for denitrification of waste water using *Pseudomonas stutzeri* microorganism in a fluidized bed bio reactor at 300 ppm. Based on experimental values the optimum conditions are obtained as, airflow rate = 2.5 lpm, temperature = 30^0 C, carbon source = 85 mg/L, poly propylene beads = 15 g/L and pH = 7. It is observed that the nitrate removal efficiency is more than 96% at this optimum condition.

References

[1] Yorn T., Shon Z. H., Lee G. (2001) *Korean J. Chem. Eng*. 18, 170-172.
[2] Schoeman J.J. and Steyn A. (2003) *Desalination*, 155, 15-26.
[3] Annouar A., Mountar M., Soufiane A. (2004) *Desalination*, 168, 185.
[4] Pintor P., Batista J., Levec J. (2001) *Chemical Engineering Science,* 56, 1551-1559.
[5] Focht Dvand Chang A.C. (1975) *Advances Applied Microbiology*, 19, 153-186.
[6] Narjari Kartik C.K. and Mahajan S.P. (1984) *Biotechnology and Bioengineering,* 24, 1445-1448.
[7] Kornaros M., Zafiri C. and Lyberates G. (1996) *Water Environment Research*, 68, 934-944.
[8] Soares M.I.M. (2000) *Air and Soil Pollution*, 123, 183-193.
[9] Hirata A., Nakamura Y. and Tsuneda T. (2001) *Water Science & Technology*, 44, 171-179.
[10] Wen J., Liping D. and Ghozhu M. (2003) *Biochemical Engineering Journal*, 15, 153-157.
[11] Roaders M. and Xin-Min Z. (2004) *Bioresource Technology*, 93, 313-319.
[12] Foglar L., Briski F., Sipos L. and Vukovia M. (2005) *Bioresource Technology*, 96, 879-888.
[13] Kim J.K., Park K.S., Cho K.S., Soo-Wan N., Park T. J. and Bajpai R. (2005) *Water science & technology*, 96, 1897-1906.
[14] Constantin H. and Fick M. (1997) *Water Research*, 31, 583-589.
[15] Michal Volokita, Shimshon Belkin, Aharon Abeliovich and Soares M. (1996) *Water Research*, 30, 965-971.
[16] Michal Volokita, Aharon Abeliovich (1996) *Water Science & Technology*, 34(1-2) 379-385.
[17] Sunil S. Adav. Duu-Jong Lee, Lai J. Y. (2010) *Appl Microbiol Biotechnol*, 85, 773-778.
[18] Shao L., Xu Z.X., Yin H.L. and Chu H.A. (2008) *Journal of Biotechnology*, 1363, 647-677.
[19] Tatsuki Ueda, Yoshiyuki Shinogi, Masaru Yamaoka (2006) *Paddy Water Environ*, 4, 139-144.
[20] Pete Kesseru, Istvan Kiss, Zoltan Bihari and Bela Polyak (2002) Water Research, 36(6) 1565-1571.
[21] Wilwan Khanitchaidecha, Tatsuo Sumino, Futaba Kazama (2010) *J. Watr Resource and Protection*, 2, 527-531.
[22] Lakshi L. P. and Pydi Setty Y. (2008) *Chemical Engineering Journal*, Volume 135, 135-140.