

*Refereed Proceedings*

*The 13th International Conference on*

*Fluidization - New Paradigm in Fluidization*

*Engineering*

---

Engineering Conferences International

Year 2010

---

A STUDY ON DENITRIFICATION IN  
A FLUIDIZED BED BIOREACTOR

L. Prasanna Lakshmi\*

Y. Pydi Setty†

K. Vimala‡

\*National Institute of Technology, Warangal

†National Institute of Technology, Warangal, [psetty@nitw.ac.in](mailto:psetty@nitw.ac.in)

‡National Institute of Technology, Warangal

This paper is posted at ECI Digital Archives.

[http://dc.engconfintl.org/fluidization\\_xiii/38](http://dc.engconfintl.org/fluidization_xiii/38)

## A STUDY ON DENITRIFICATION IN A FLUIDIZED BED BIOREACTOR

K. Vimala, L. Prasanna Lakshmi and Y. Pydi Setty\*,  
Department of Chemical Engineering, National Institute of Technology,  
Warangal, India.

### Abstract:

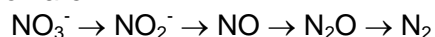
The work involves experimental investigation on biological denitrification in a fluidized bed reactor under anaerobic conditions using the microorganism *Pseudomonas Stutzeri* and plastic beads as fluidizing medium. The influence of various parameters like pH, initial nitrate concentration, Carbon/Nitrogen ratio, hydraulic retention time on nitrate-nitrogen removal rate from synthetic effluent prepared at the laboratory were studied in detail. The optimum operating conditions of pH, initial nitrate concentration, Carbon/Nitrogen ratio and flow rate obtained are 7.0–7.5, 15 mg/l, 1.5–2.0 and  $4.41 \times 10^{-5} \text{ m}^3/\text{s}$  respectively.

**Key words:** Denitrification, microorganism, fluidized bed bioreactor, biological waste water treatment, *Pseudomonas Stutzeri*, attached fermentation, immobilization.

### INTRODUCTION

Biological denitrification has been studied extensively for several reasons. Denitrification is a major natural mechanism for loss of fertilizer and can remove nitrogen from such high nitrogen waste materials as animal residues. Drinking water resources also are facing major nitrate contamination that may cause methemoglobinemia. Excess concentration in drinking water is considered hazardous to human beings, particularly, infants.

There are various methods of removing nitrogen, each with advantages and disadvantages. However, the biological treatment method is used most commonly. With this method, organic nitrogen and ammonia nitrogen are converted into nitrous and nitrate nitrogen in an aerobic environment and is dispersed into the atmosphere primarily as anaerobic nitrogen gas. The removal of nitrate nitrogen by conversion to nitrogen gas can be accomplished biologically under anoxic conditions. The conversion process is identified as anaerobic denitrification. The reactions for nitrate reduction are:



A broad range of bacteria, including many in the genera *Pseudomonas*, *Micrococcus*, *Anchromobacter*, *Thiobacillus* and *Bacillus* can reduce nitrate to nitrogen gas either in aerobic or in anaerobic or in both conditions thus causing denitrification.

The fluidized bed bioreactor has been used to carry out biological denitrification by a number of investigators. Here the waste water is sent upward with a velocity higher

\*Corresponding author: Phone: 091-870-2611, Fax: 091-870-2459547, e-mail: [psetty@nitw.ac.in](mailto:psetty@nitw.ac.in)

than the minimum fluidization velocity of solids and the intensive organism growth on the carrier particle consumes the biodegradable waste contaminants present in waste water. Particles used for fluidization can be of uniformly sized sand, expanded clay, activated carbon or plastic bead.

Fluidized bed reactors have much larger surface area per unit volume to support microorganisms, which increases the reactor micro-organism concentration. The larger surface area allows shorter hydraulic residence times for the same degree of treatment in a given volume. Each media particle eventually gets covered with biofilm and vast surface area available results in a high biomass concentration.

The fluidized bed bioreactors have been effective for both aerobic and anaerobic treatment of domestic and industrial waste water. Hence time required for treatment is reduced as compared to that in conventional processes such as trickling filters or activated sludge processes.

The superior performance of fluidized bed bioreactors is because of the following factors:

1. They offer higher productivity than stirred tank fermenters as the liquid approximates plug flow.
2. Due to immobilization of cells onto the surface of the solid particles, very high biomass concentration can be achieved. So it is possible to have high cell density and thus it reduces the treatment time.
3. The high level of mixing of solids helps in overcoming diffusion limitations in the liquid phase.
4. We can achieve intimate contact between the liquid and solid phase as a result of fluidization.
5. Washout of microbes can be prevented as a result of immobilization.
6. Since the size of the solid particles is less than that in the packed bed reactors, they offer large surface area per unit volume. Hence the volume of the reaction mixture is larger than that in other biological reactors.
7. The bed expands to accommodate the cells produced, which prevents the problem of clogging which is often encountered in a packed bed reactor.
8. We can get high heat and mass transfer rates.
9. More over higher volumetric reaction rates, lower space requirements and low investment costs add to the main advantages.

These characteristics make the fluidized bed bioreactor a good choice for large scale waste water treatment also.

But increased agitation caused at higher flow rates greatly increases the particle-particle contact and creates a significant shear force, which can result in shredding of the attached biofilm. Thus liquid velocities in bioreactors must be carefully controlled to maintain adequate bed expansion and solid liquid mass transfer that minimizes shear and particle elutriation.

## LITERATURE REVIEW

Sreekrishnan et al. (1) studied the effect of operating variables on biofilm formation and performance of an anaerobic fluidized bed bioreactor. Various operating variables such as initial inoculum circulation rate, dilution rate, COD, loading rate and quantity and quality of inoculum on the process of film formation on sand surface and reactor performance were studied using synthetic glucose based waste water. They found that a high dilution rate, a large quantity of inoculum having high methane producing capacity favor the film formation process. Experimental observations indicate that methanogenic bacteria initiate the biofilm formation process.

Jaap et al. (2) have studied the influence of volatile fatty acids on nitrite accumulation by a *Pseudomonas Stutzeri* strain isolated from a denitrifying fluidized bed reactor and denitrification characteristic of *Pseudomonas Stutzeri* with acetate as carbon and electron donor. Fraucese and Carles (3) presented a number of advantages of fluidized bed reactors that make them an attractive alternative in process involving biocatalysts. The application of fluidized bed bioreactors to different kinds of processes is also discussed.

Nirjari et al. (4) studied the effects of superficial velocity on the removal of nitrates as well as on the growth of the biofilm. They have shown that there exists an optimum velocity at which both nitrate removal and biofilm growth are at maximum.

## EXPERIMENTAL PROCEDURE

Biological denitrification is studied in a liquid-solid fluidized bed bioreactor under anaerobic conditions using *Pseudomonas Stutzeri* microorganism (5). The schematic diagram of the experimental setup and the experimental procedure are given elsewhere (6).

The parameters measured were nitrate concentration of the treated water, pH of the treated water, flow rate. The nitrate concentration was measured using UV-Spectrophotometric method.

The culture *Pseudomonas Stutzeri* obtained from National Chemical Laboratory, Pune, India was preserved in a refrigerator at a temperature of 4 °C by periodic subculture on nutrient agar.

### Slant Preparation:

The bacterium is subcultured once in a month by preparing slants using nutrient agar. The following chemicals are required per 100 ml of distilled water, for slant preparation:

Peptone: 0.5 g ; beef extract: 0.3 g ; NaCl: 0.5 g ; Agar-agar: 2.5 g

pH was adjusted at 7.0 with NaOH and HCl. All the chemicals were mixed and sterilized in an autoclave for 15 minutes at a temperature of 120 °C and a pressure of 15 psi.

The bacterium was poured upto one-third of the test tube and kept at an angle of 30 degrees and cooled to solidify agar-agar. This provides more surface area of nutrient for the micro-organism to grow. Then the slants were exposed to UV light for 30-40 minutes, which enables the total destruction of any other bacterium that is undesirable for denitrification. Then the slants were inoculated with the strain of bacterium. The slants were kept at room temperature for a period of 5 days. After the colonies were observed the slants were stored at 4 °C.

#### **Preparation of Inoculum:**

The bacterium from the slants was inoculated into liquid broth having the composition as reported in earlier work (6).

Before an experimental run was begun, the bed was seeded with a culture of denitrifying bacteria. As the reaction was carried out in anaerobic conditions, to keep the dissolved oxygen low during the reaction, Sodium Sulfite (100 mg/l) was added to the storage tank.

After the pH was adjusted the broth was sterilized in an autoclave for 15 minutes at 15 psi pressure and 120 °C temperature to kill the undesirable micro-organisms. The broth was cooled to room temperature and then colonies of *Pseudomonas Stutzeri* were introduced into the broth. After inoculating, the culture was kept for growth in an incubator for 24 hours. The temperature of the incubator was maintained at 30 °C and speed at 60 rpm.

Nitrate concentration is obtained using UV Spectrophotometer at a wave length of 220 nm to obtain the nitrate reading and a wave length of 275 nm to obtain the interference due to dissolved organic matter. The reading from UV-spectrophotometer is corrected for dissolved organic matter and the concentration of nitrates is obtained from a calibration chart prepared earlier.

### **RESULTS AND DISCUSSION**

The Effect of various parameters on the removal of nitrate-nitrogen was studied in detail to find out the optimum conditions where the nitrate removal efficiency of the given micro-organism is maximum. The parameters like initial substrate concentration, hydraulic retention time (flow rate), pH, C/N ratio were studied in detail.

#### **Initial Nitrate concentration:**

The effect of initial nitrate concentration on the nitrate removal capability of *Pseudomonas Stutzeri* was studied in detail as these micro-organisms are capable of modifying their metabolism to utilize nitrates as the source of O<sub>2</sub> and there by cause denitrification and also to see the effect of substrate inhibition..

The experiments were conducted at a constant flow rate of 0.7 gal/min with different initial nitrate concentrations i.e. 25, 20, 15, 13, 10 mg/l with initial pH of 7.0. The results shown in Fig. 1 clearly show that as the initial nitrate in the feed is decreasing, there is depletion in effluent nitrate concentration. But from the figure, it is clear that when initial concentration was at 15 mg/l, the concentration of effluent after running for 10 hours has gone as low as 0.7 mg/l. If we see in terms of amount of nitrate removed, it is at 96 %.

When the initial nitrate concentration is  $\geq 20$  mg/l, the concentration of nitrate in the effluent stream is not reducing much compared to other cases. This may be because of the substrate inhibition effect at higher concentrations of nitrate.

When initial nitrate concentration is at 10 ppm and 13 ppm, the amount of nitrate removed is less when compared to the initial concentration of 15 ppm. This may be because of more C/N ratio since the amount of methanol taken was same for all the experiments.

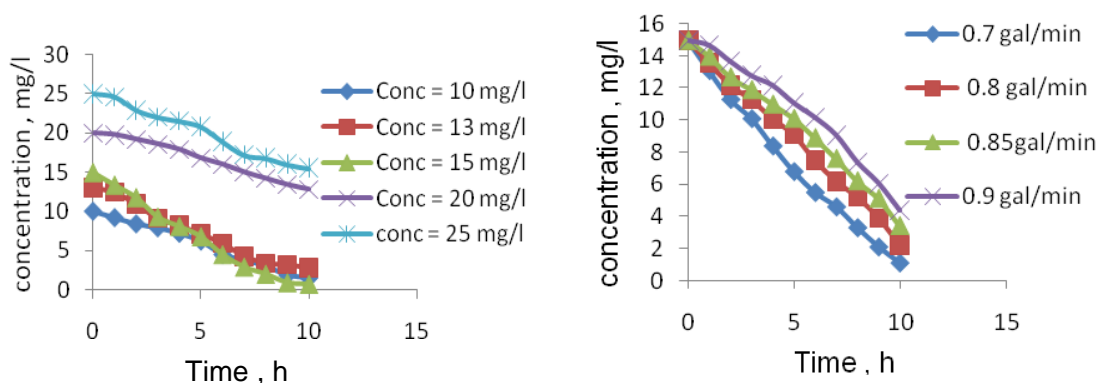


Fig. 1 Effect of initial nitrate concentration on nitrate removal

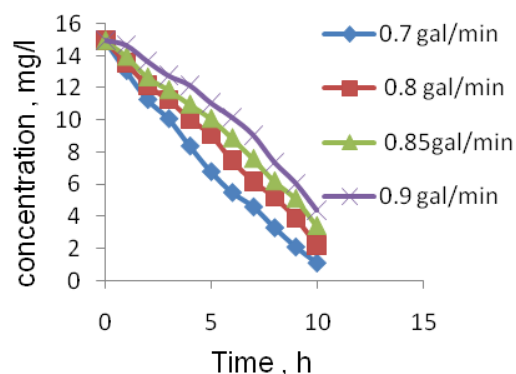


Fig 2. Effect of flow rate on nitrate removal

### Effect of flow rate

Flow rate plays an important role in the removal of nitrate from the given effluent. As flow rate to the reactor changes, the residence time of the fluid in the reactor varies. The expansion in bed height also changes with flow rate, which affects the voidage of the solid particles within the reactor.

As the bed expands it gives more space for the organism to grow on the particles, which results in more conversion, but at the same time the residence time of the fluid in the reactor will decrease which may result in the low conversion of nitrate to nitrogen. Hence the effect of flow rate on nitrate removal plays an important role.

Experiments were conducted to study the effect of flow rate on nitrate removing capability. Four different flow rates were studied viz. 0.9, 0.85, 0.8, 0.7 gal/min. with initial nitrate concentration of 15 mg/l. From the data shown in Fig. 2, it is clear that the concentration of nitrate is decreasing with time.

As the flow rate is increasing, concentration of nitrates in the effluent stream is increasing at any particular time with respect to lower flow rates. This may be due to the fact that increasing flow rate decreases the residence time of the fluid in the reactor. Hence it is noticed that at the highest value of flow rate (0.9 gal/min) in the present work, the nitrate concentration is found to reduce very slowly compared to other flow rates. Amount of nitrate in the effluent stream is minimum (1.1 mg/l) when the flow rate is maintained at its lowest i.e. 0.7 gal/min. This may be also due to decrease in mass transfer coefficient across the biofilm with increase in flow rate (6)

### Effect of pH

pH plays a vital role in all biological reactions. This is because, the activity of intracellular enzyme of micro-organism causes the biological reaction to take and will be active in a particular pH range only. Thus pH will affect the rate of reaction a lot in all biological reactions.

In denitrification, alkalinity is produced during the conversion of nitrate to nitrogen gas resulting in an increase in pH as nitrate acts as an electron acceptor in the metabolism for generation of energy. From the literature, it may be seen that *Pseudomonas Stutzeri* organisms are active around neutral pH conditions.

From Fig. 3 it is clear that the optimum pH range is between 7.0 – 7.5. When pH is deviating from neutral, i.e. at a pH of 6.5 and 8.0, the capability of organism is decreasing, may be because of the inhibitory effect of super acidity or super alkalinity on the activity of intracellular enzyme of the bacteria.

When the pH is going towards alkalinity at 8.0, the concentration of nitrate in effluent stream is almost equal to 13 mg/l and the nitrate removed is 35 % only. This may be because as the reaction proceeds the alkalinity of the reaction mixture increases and maintaining alkaline conditions initially may further reduce the activity of the micro-organism. Additionally, the  $\text{NO}_2 - \text{N}$  accumulation occurs when the pH value is higher than 8.0 and higher the pH values, more is the accumulation of  $\text{NO}_2 - \text{N}$ . This is probably due to the fact that the activity of nitrite reductase was inhibited.

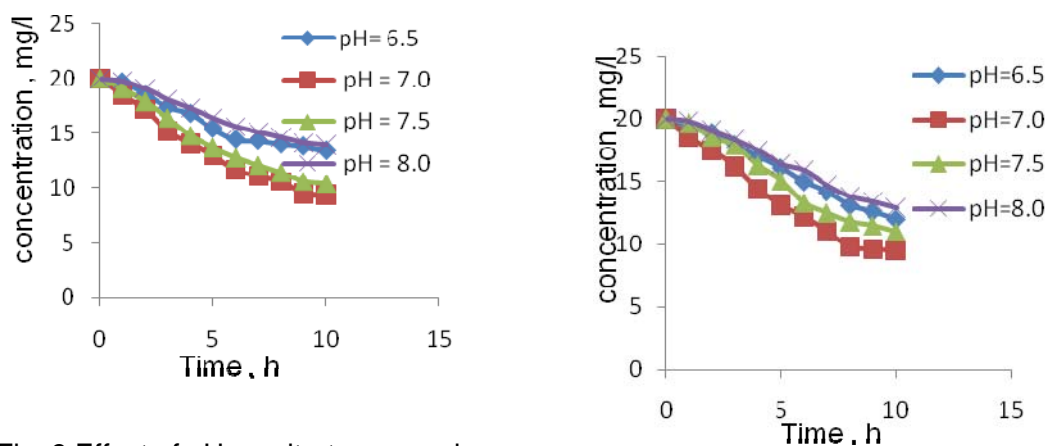


Fig. 3 Effect of pH on nitrate removal

Fig.4 Effect of pH (when pH was maintained constant throughout)

### Effect of pH (when pH was maintained constant throughout)

pH exerts remarkable influence on biological denitrification. The influence of pH values on removal rate of  $\text{NO}_x - \text{N}$  were shown in Fig. 4 at room temperature, flow rate of 0.7 gal/min and initial nitrate concentration of 20 mg/l and pH values of 6.5, 7.0, 7.5 and 8.0.

From Fig. 4, it is clear that the optimum pH range is between 7 – 7.5, when we maintain the pH constant throughout the experimental run. As the pH was maintained constant, it was observed that the reduction of nitrates was more when compared to the conditions where pH was not maintained constant. This may be because, the alkalinity of the reaction mixture is increasing as the reaction proceeds when the pH is not maintained constant throughout, which inhibits the rate of reaction.

### Effect of C/N ratio :

The amount of carbon plays a vital role in the growth of micro-organism. Organic matter is most required for the growth of any micro-organism. Methanol has been used as the carbon source for the present process.

The influence of C/N ratio on the removal of nitrate-nitrogen at the room temperature, pH value of 7.0, flow rate of 0.7 gal/min and initial nitrate concentration of 20 mg/l is as shown in Fig. 5. It can be seen that, as the methanol concentration is increasing as carbon source, the concentration of nitrates in the effluent stream is decreasing.

But when C/N ratio exceeds 2, the effluent concentration is more when compared with lower C/N ratios. This may be because of excess amount of methanol as carbon source results in waste and makes the effluent nitrate concentration more. It can be easily observed that the optimum C/N ratio range is 1.5 – 2.0.

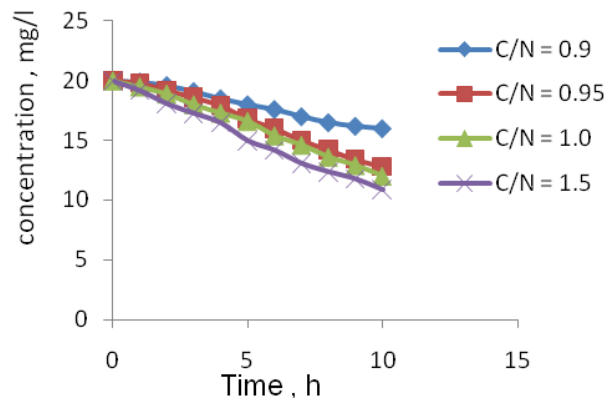


Fig. 5 Effect of C / N ratio on Denitrification



## CONCLUSIONS

It is observed that the microorganism, *Pseudomonas Stutzeri* is able to denitrify the synthetic wastewater successfully under anaerobic conditions with plastic beads as a fluidizing medium. The effect of various parameters like pH, initial nitrate concentration, C/N ratio and flow rate on removal of nitrate-nitrogen were studied in detail.

It is noticed that by taking less dense particles as a solid supporting medium for the growth of the micro-organism, the denitrification capability of the micro-organism is increased when compared with the type of dense particles used as a support as reported by Nirjari et al. (4).

It is seen from the results that the degradation of nitrates in the present work is faster compared to earlier work (4), where a feed with 20 mg/l took several days to degrade to a very low concentration. In the present work feed of 15 mg/l nitrate concentration degraded to 0.7 mg/l in 10 h. These results indicate a definite advantage of using less dense plastic particles as supporting media for biofilm growth compared to dense solids. Using plastic media also reduces fluidization velocities thus increasing the retention time of the fluid in the reactor resulting in increased degradation of nitrates (6).

From the experiments it is also clear that alkalinity is produced during the conversion of nitrate to nitrogen gas resulting in an increase pH. The optimum operating conditions are obtained as pH value of 7.0 – 7.5, initial nitrate concentration of 15 mg/l, C/N ratio of 1.5 – 2 and flow rate of 0.7 gal/min.

## REFERENCES

1. Sreekrishnan, T.R., Ramachandran, K.B and Ghosh, P (1991), "Effect of Operating Variables on Biofilm formation and performance of an anaerobic Fluidized Bed Bioreactor", *Biotechnology and Bioengineering*, Vol. 37, 557-566.
2. Jaap V. R., Yossi T and Yoran B (1996), "Influence of Volatile Fatty acids on Nitrite accumulation by a *Pseudomonas Stutzeri* Strain isolated from a Denitrifying Fluidized Bed Reactor", *Applied and Environmental Microbiology*, Vol. 62, 2615-2620.
3. Francese G and Cales S (1995), "Fluidized Bed Bioreactors", *Biotechnology Progress*, Vol. 11, 479-497
4. Nirjari, N.K., Karthik, C.K and Mahajan, S.P (1984), "Biological Denitrification in a Fluidized Bed", *Biotechnology and Bioengineering*, Vol. 26, 1445-1448.
5. Vimala, K (2004), "Parametric Studies on Biological Denitrification in a Fluidized Bed Reactor", M. Tech Thesis, National Institute of Technology, Warangal.
6. Prasanna Lakshmi, L and Pydi Setty, Y (2008) "Liquid-solid mass transfer in a two phase fluidized bed bioreactor", *Chemical Engineering Journal*, Vol.135,135-140.