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Effect of Carbon Sources on Biological Denitrification of Wastewater by Immobilized *Pseudomonas Stutzeri* Bacteria in a Fluidized Bed Bio Reactor (FBBR)

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Abstract. Nitrate contamination is one of the major problems in groundwater, which is increasingly becoming a threat to groundwater supplies. Nitrate in drinking water for animal and human consumption is not recommended for health reasons. 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. Nitrogen in groundwater results from human excreta, ground garbage and industrial effluents, particularly from food processing plants. In order to remove nitrate from wastewater the biological denitrification technology can be applied for which a carbon and energy source is needed. The addition of external carbon source helps in increasing denitrification rates and enhances the nitrogen removal. Nitrates cause cancer, methemoglobinemia (blue baby syndrome), hypertension and thyroid hypertrophy and Eutrophication in plants. Methanol, ethanol and methane were used as external carbon sources for biological denitrification of wastewater with immobilized *Psuedomonas stutzeri* bacteria for 150, 180 and 200 ppm. The total removal efficiency was examined at different carbon sources at different levels by keeping optimized parameters such as Airflow rate: 2.5 lpm, Temperature: 30^0 C, Poly propylene beads: 15 gm/lit and pH: 7. The use of methanol as the carbon source resulted in the highest nitrogen removal efficiency, followed by ethanol and methane. The results suggest that the methanol is the most appropriate carbon source for denitrification of wastewater.

Keywords: Biological denitrification, Carbon source, *Pseudomonas stutzeri*, Fluidized bed bio reactor.

1. Introduction

Nitrate, which is one of the most common groundwater contaminants world-wide. Nitrate in drinking water for animal and human consumption is not recommended for health reasons. 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. Industries are the greatest source of pollution, accounting for more than half the volume of all water pollution and nitrogen in ground water can 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 [1]. 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" [2] and also causes cancers, birth defects, abortions, hypertension thyroid hypertrophy, nitrate in excessive levels can also be potentially harmful to animals and caused for abdominal pains, muscular weakness, or poor coordination, and brown or chocolate colored blood. Hence nitrates removal is an important aspect of present days wastewater treatment process and biological denitrification is one of the most economical processes for nitrate removal from wastewater.

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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 most 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 groundwater, in which the nitrate contents 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.

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

A source of organic carbon is an important component of the denitrification process; generally most of wastewater treatment plants use Methanol as external carbon source due to economic reasons in denitrificatioin process. Other compounds can be used in denitrifcation process includes acetate, ethanol, methane, glucose, peptone, saw dust, glycerol, lactic acid, molasses, etc.

A number of investigations are reported in the literature on the nitrate removal from wastewater using carbon sources. An extensive review has been carried out by Constantin et al. 1997 [3], for denitrification of industrial wastewater with two different carbon sources (ethanol and acetic acid) with same organism and reported the experiment with ethanol has shown a greater overall denitrification rate. Guven et al., 2009 [4], have reported an overview on the effect of carbon sources (acetate, propionate, ethanol and glucose) for denitrification of wastewater. Greben et al. 2004 [5], have also made a review on removal of nitrate using saw dust as the energy source. Peter Kesseru et al. 2002 [6], directed their investigation on denitrification activity of immobilized *Pseudomonas butanovora* cell in the presence of three different carbon sources, succinic acid, ethanol and acetic acid and reported highest denitrification activity was observed with succinic acid. Khanitchaidecha et al. 2010 [7], reported the influence of carbon sources on biological nitrogen removal with three sources, acetate, ethanol and hydrolysed rice and suggested that acetate is the most appropriate carbon source for nitrogen removal followed by ethanol and hydrolysed rice. Gininge et al. 2009 [8], made a comparative study on methanol as carbon source using three lab-scale sequencing batch reactors (SBR).

Several sources of carbon have been used for denitrification including news paper [9], cotton [10], acetate, ethanol and methanol [11], rice husk [12], molasses [13].

There are different methods, which have been developed for removing nitrogen like ion exchange [14], reverse osmosis [15], electro dialysis [16], chemical and catalytic denitrification [17]. Each of them has its own advantages and disadvantages, from which biological method is found to be the most commonly used and effective method. Several studies has been carried on denitrification [18-26] but work reported on biological denitrification of waste water using a fluidized bed bioreactor is very little using *Pseudomonas stutzeri*.

2. Materials and Methods

2.1. 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 equation of carbon monoxide with hydrogen at a high temperatures and pressure. Ethanol and methane have been also used as external carbon source for wastewater treatment due to their availability and comparable cost.

2.2. 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 effects carbon sources on 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 $Na_2H_2PO_4$[27] and with different carbon sources (methanol, ethanol and methane) of 85 mg each. 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 experiment was conducted initially for 150, thereafter 180 and 200 ppm.

2.3. Experimental Set-up

The Fluidized bed bio-reactor consists of a glass column of 0.5m 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 [27].

2.4. Analytical methods

Samples of nitrate were collected for every 1 hour, filtered and were used for the analysis of final nitrate concentration using UV- Visible Spectrophotometer according to standard methods [28].

3. Results and Discussions

Parameters, which have major impact on denitrification rate, were selected by conducting different experiments. The parameters like, air flow rate, temperature, carbon source, supporting media (poly propylene beads), pH and initial nitrate concentration were selected since these parameters significantly affect the nitrate removal rate. The denitrification process was the rate limiting step of NH_4-N removal and the efficiency of the denitrification process depended on the growth rate of competitive bacteria, the carbon consumption and the carbon source. The effect of carbon source on denitrificatin and growth of competing bacteria was examined for 12 hrs.

In the present work, methanol, ethanol and methane were used as external carbon sources for biological denitrification of wastewater with immobilized *Psuedomonas stutzeri* bacteria. The total removal efficiency was examined by keeping optimized parameters (from previous work) such as airflow rate: 2.5 lpm, temperature: 30^0 C, poly propylene beads: 15 gm/L and pH: 7 with different carbon sources of 85 mg/L each.

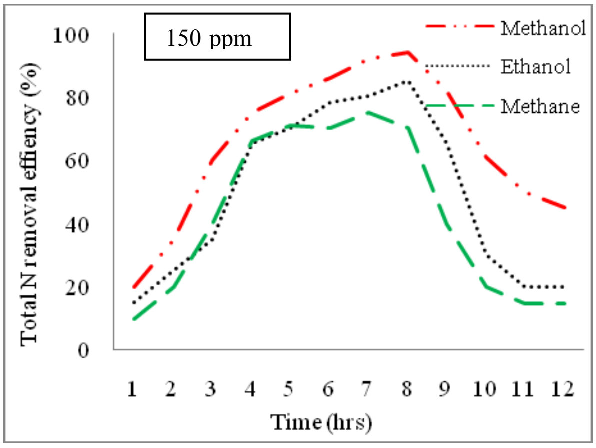

Fig. 1: Total Nitrate removal efficiency of methanol, ethanol and methane on initial nitrate concentration at 150 ppm.

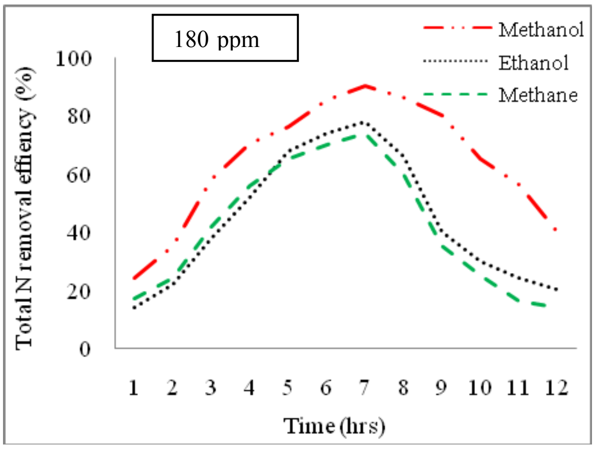

Fig. 2: Total Nitrate removal efficiency of methanol, ethanol and methane on initial nitrate concentration at at180 ppm.

Figures 1, 2, and 3 show, the total Nitrate removal efficiency of methanol, ethanol and methane on initial nitrate concentration of 150 ppm, 180 ppm and 200 ppm. It is clear from figures. 1, 2 and 3 that the total nitrate removal efficiency was highest with the methanol followed by the ethanol and methane. However, the nitrate removal efficiency is more during the period 6-8 hours for three carbon sources.

From the graphs, also observed that the performance of methane is lower than the ethanol, but over a period of time methane shows greater efficiency than ethanol in the starting period. And after 8 hours the efficiency of nitrate removal has slightly decreased with methanol as carbon source but has significantly decreased by ethanol and methane. As shown in fig. 4, the biomass concentration is more with methanol comparatively other two carbon sources.

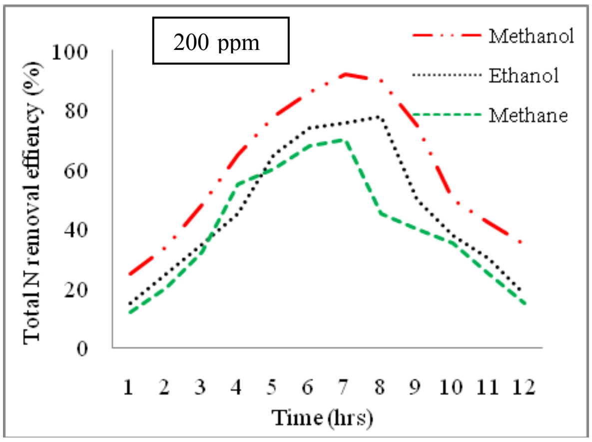

Fig. 3: Total Nitrate removal efficiency of methanol ethanol and methane on initial nitrate concentration at 200 ppm.

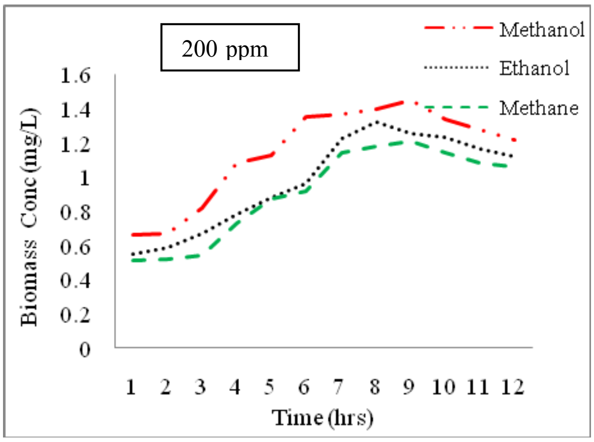

Fig. 4: Biomass concentration on initial nitrate concentration using methanol, ethanol and methane at 200 ppm.

4. Conclusions

From the comparison of experimental results using carbon sources, it was clearly seen that the microorganism used for the denitrification studies were active for methanol as carbon source compared to ethanol and methane. The use of methanol as the carbon source resulted in the highest nitrogen removal efficiency, followed by ethanol and methane. The results suggest that the methanol is the most efficient carbon source for denitrification of wastewater. Ethanol is a satisfactory alternative carbon source for nitrogen removal as compared to methane. It is observed that the nitrate removal efficiency is more than 96% with methanol as carbon source.

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