# Feasibility study of a new approach to removal of nitrates from groundwater by Biological Denitrification

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Abstract— The removal of nitrate is essential for water contaminated with nitrate before being utilized, since a large amount of nitrate in drinking water often causes a disease called methemoglobinemia and other health disorders such as hypertension, increased infant mortality, goiter, stomach cancer, thyroid disorder, cytogenetic defects and birth defects. Hence nitrate removal is an important aspect of present day's wastewater treatment process. Physical and chemical processes such as reverse osmosis, ion exchange, electro dialysis and chemical denitrification have been developed for nitrate removal from water. These techniques are effective in removing nitrate from contaminated water; they are very expensive for pilot scale operation with a limited potential application. Owing to these limitations in the removal of nitrate from water and/or wastewater, the most versatile and widely used technology is biological denitrification. Hence in this research work, feasibility study was carried out for removing nitrate from ground water by biological denitrification with optimum amount of carbon source under anoxic condition.

Keywords— Biological denitrification, Bioreactor, Carbon sources, Ground water, Nitrate.

#### I. INTRODUCTION

Water, the best of all things is the nature's free gift for living organisms. It is bound up with man's evolution and doubtless destiny in countless ways. Water has been used for drinking, domestic purpose, industry, agriculture and recreation; it shows the extent to which it is an integral part of our life. Water is absolutely essential not only for human beings, but also for animals, plants and all the other living beings. The basic condition for life on earth is that water should be available in the liquid form. Threefourth of the earth's surface is covered with water of the total water resource available, about 97.25% is salt water, which is mainly in ocean and 0.68% is available as groundwater. 2.05% as ice caps, 0.001% of atmospheric moisture, 0.005% of soil moisture, 0.01% in lakes, 0.0001% in rivers and 0.00004% in the biosphere (Global Water cycle: Geochemistry and Environment, 1987). However, very little quantity of water is fit for human consumption.

The rapid urbanization, industrialization as well as agricultural activities has made environmental pollution a growing concern globally. Off all the receptor systems exposed to the contaminants, groundwater has received little attention in the past because of common belief that groundwater was pristine. Groundwater provides drinking water for more than one-half of the nation's population, and is the sole source of drinking water for many rural communities and some large cities. In India, the groundwater contamination with respect to nitrate has been observed in few areas of Andhra Pradesh, Bihar, Delhi, Haryana, Himachal Pradesh, Karnataka, Rajasthan, Tamil Nadu and West Bengal.

The studies carried out in India reveal that one of the most important causes of groundwater pollution is unplanned urban development without adequate attention to sewage and waste disposal. Industrialization without provision of proper treatment and waste and effluent disposal is another source of groundwater pollution. Excessive application of fertilizers for agricultural development coupled with over irrigation is also responsible for groundwater pollution [8].

#### 1.1 Importance of groundwater

Groundwater is important to those who have limited prescription each year. Groundwater is the primary source of water for 50% of the American population and 90% of those people in rural areas. In India, 58% of the total population uses groundwater. It plays an important role in the hydrologic cycle. Groundwater is the safest and most reliable source of available freshwater. Only 3% of earth's freshwater are located in streams, lakes, and reservoirs. The remaining 97% of freshwater is underground. Of the public supply systems in India, 43% use groundwater and of the people who live in rural areas in India, 87% use groundwater. About 500,000 individual homes, 425 public water systems and 2,500 non-community water supplies are dependent on groundwater. Groundwater is vital for Indian's industrial and agricultural growth and development. According to reports in 1985 for India, industry uses an average of 190 million gallons per day during the growing season and livestock operations depend on an average of 45 million gallons per day (Haller et al, 1996)[10]

The availability and quality of groundwater varies widely across the states of India. In general, well yields range from less than five gallons per minute in bedrock aquifers in southwest in India to several thousand gallons per minute well in aquifers beneath and adjacent to India's major rivers. Most freshwater or portable groundwater in India occurs at depths of 40 feet to 300 feet. Highly mineralized waters are usually found at greater depths[8] **1.2 Nitrate (NO3<sup>-</sup>)** 

Nitrate compounds are very soluble in water. The nitrate part (ion) is negatively charged, and since soil is also negatively charged, it is repelled by soil surfaces and stays in the solution. When excess water drains through soil, nitrate is washed out (leached). Nitrogen in the form of nitrate in surface and groundwater can be an important consequence of groundwater pollution arising from both rural and urban areas. Nitrate leaching to the water environment is contributed from the application nitrogen fertilizer in agriculture, wastes from grazing animals and soil erosion. If high rainfall occurs after ammonium nitrate fertilizer has been applied, much of the nitrate will be leached, but otherwise nitrate is taken up by plants very quickly. Nitrite (NO<sub>3</sub><sup>-</sup>) is one of the several inorganic pollutants contributed by nitrogenous fertilizes, organic manure, human and animal wastes and industrial effluents through the biochemical activities of microorganisms [8].

#### 1.3 Biological Methods

Nitrate removal through biological means is based on denitrification, a microbial process carried out mainly by facultative aerobic bacteria that, under anoxic conditions nitrate as the terminal electron acceptor in their respiratory process. Denitrifying bacteria are ubiquitous in nature; they are found in soils, activated sludge, aquatic sediments in fresh, brackish and sea water, and in living organisms such as honeybee larvae.

#### 1.4 Biochemical aspects of denitrification

The reaction requires an electron donor, as well as 10 electrons and 12 protons. The end products of the reaction are dinitrogen gas and OH<sup>-</sup>, the latter of which makes it an alkalizing process. Denitrification is an assembly of nitrate reduction, nitrite reduction, nitric oxide reduction and nitrous oxide reduction. The sequence of reactions is as follows:

 $NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$ 

This sequential pathway involves multiple enzyme systems, those of which are present in and vary among phylogenetically different organisms. The availability of carbon and energy sources plays a major role in denitrification activity as they are required for cell growth and metabolism. Denitrifiers fall into two metabolic categories: heterotrophic bacteria that utilize organic carbon and energy sources, and autotrophs which use inorganic forms. While these bacteria are ubiquitous in nature, nitrate cannot be removed intrinsically due to limited amounts of carbon and energy in unpolluted groundwater, thus creating a need for engineered denitrification processes.

#### 1.5 Heterotrophic biological denitrification

Heterotrophic biological denitrification is a wellestablished process in the realm of wastewater treatment. Numerous studies reported on the potential of using biological denitrification for nitrate reduction in groundwater supplies in laboratory-scale experiments. The results indicated that fixed-film denitrification can be expected to reduce the nitrate concentration in the influent water supply from as high as 100 mg/L (as N) to levels within the 1.0mg/L (as N) range. These removals translate into an efficiency of nearly 100percent, which is generally not matched by other processes available for nitrate reduction. However, some residual soluble as well as insoluble organic matter should be expected in the denitrified water supply. Further treatment can reduce these solids to levels sufficient to meet prevailing drinking water standards. In heterotrophic biological denitrification, facultative micro-organisms are contacted with the water supply containing nitrates and an added carbon source in an anoxic (oxygen-free) environment. Under these conditions, the bacteria utilize nitrates as a terminal electron acceptor in lieu of molecular oxygen. In the process, nitrates are reduced to nitrogen gas, which is harmless and can be directly discharged to the atmosphere. The extraneous carbon source is necessary since it supplies the energy required by the microorganisms for respiration and synthesis while serving as an electron donor. Most denitrification studies have used methanol (CH<sub>3</sub>OH) as the carbon source. If a simple carbon source is chosen such as ethanol or acetic acid, then the biomass produced during the process should be correspondingly low; a useful characteristic in that the overall excess biomass production is minimized. Since heterotrophic denitrifying bacteria require an organic carbon source for the respiration and growth, a wide variety of organic compounds have been used.

These organics include methanol, ethanol, acetic acid, glucose, and other more complex organics. While the types of organic compounds may affect the biomass yield, the choice is generally based on economic comparison. The availability of ethyl alcohol from agricultural sources could make this carbon source a strong candidate for denitrification systems. It should be noted that methanol toxicity is such that it is not recommended as electron donor and carbon source for drinking water denitrification[28].

#### II. MATERIALS AND METHODOLOGY

This section describes the procedure followed to conduct various experiments and the materials used in order to meet the objectives of the study.

#### 2.1 Details of Materials and Experiments for the Biological Denitrification Study

#### 2.1.1 Bioreactor setup:

The anoxic batch reactor of 2L of working volume was used for denitrification purpose. Synthetic water, seed material and carbon source were added to each batch. Synthetic water sample was prepared by adding a measured amount of potassium nitrate to the tap water to get the required concentration of nitrate. The reactor consisting of sample, cow dung, carbon source is kept closed to maintain anoxic condition.

Cow dung slurry was used as a seed culture since cow dung is rich in heterotrophic bacteria which are responsible for denitrification process. The seed culture was prepared by taking 100 g of fresh cow dung mixed in 1000 ml of water to get slurry from which filtered 300 ml was added to the each anoxic reactor.

In the present study we use three types of carbon sources are paddy straw, ragi straw, and wheat straw. These are agricultural by-product; the dry stalks of serial plants, after the grain and chaff have been removed. In order to find effective carbon source for denitrification paddy straw, wheat straw, ragi straw was added for three reactors separately. Since ragi straw was found to be effective as carbon source in initial studies the same continued for further studies.

Groundwater sample was collected from the bore well of Mysugar industrial area Mandya. Calculated amount of potassium nitrate was added to the groundwater samples to obtain the required amount of nitrate nitrogen, for the study purpose.

#### 2.2 Operational strategy

The entire study was done in five phases, in first phase, feasibility of removing nitrate from synthetic water containing nitrate was studied. In the second and third phase optimization of carbon source was done. In the fourth phase, nitrate removal under various nitrate loading conditions was evaluated. In the fifth phase, evaluation of a treatment system with denitrification, filtration, and disinfection for community water treatment system has been done.

#### 2.2.1 Phase - 1: Feasibility of nitrate removal

A bioreactor was started with carbon source as powdered ragi straw and cow dung slurry as seed culture to study the feasibility of nitrate nitrogen removal. Raw water used for this study was synthetic water containing 50 mg/L of nitrate nitrogen.

#### 2.2.2 Phase – 2 and 3: optimization of carbon source

Four batch reactors were started with different amount of carbon sources: 0.1, 0.2, 0.4, 0.6 gm/2L of water to be treated along with 100 ml of seed slurry. The nitrate nitrogen concentration in the synthetic water was maintained at 50 mg/L which is slightly more than the drinking water quality standard value of 45 mg/L. Synthetic water was prepared by adding 81.5 mg/L of KNO<sub>3</sub> in 100 mg/L of tap water to get 50 mg/L of nitrate nitrogen. Table 1 provides the reactor details during the optimization of carbon source.

Number of reactors	04
Total reactor volume	2.5 L
Working volume	2 L
Cow dung slurry	100 ml
Nitrate concentration	50 mg/L
Carbon source	0.1, 0.2, 0.4, 0.6 gm/L

The reactors were operated for 2 weeks and the samples were collected on alternate days for analysis. Before analysis the samples were filtered using filter paper and analyzed for nitrate, nitrite, ammonia, COD, pH.

As the COD concentration in the treated water was found more in first batch studies with carbon source concentration of 0.1 to 0.6 gm/L. To reduce this, a second set of batch reactors was started. Totally four reactors were started with 50 mg/L of nitrate concentration in synthetic water and 100 ml of seed slurry. The powdered ragi straw added was 0.025, 0.05, 0.075, 0.1 gm/ 2L in reactors 1, 2, 3 and 4 respectively. This study was conducted for 12 days and the samples were collected on alternate days and analyzed for the above mentioned parameters.

#### 2.2.3 Phase - 4: Various nitrate loading conditions

In this study the carbon source was maintained constant based on previous set of batch studies and the nitrate loading conditions were altered to know the performance of anoxic batch reactor. Four reactors were set up with specified amount of seed (100 ml) and carbon source (0.05 g/2L). The nitrate concentration was varied as 60, 70, 80, 90 mg/L. In this study each day samples were collected and analyzed for their nitrate removal efficiency. This study was conducted for four weeks with continuous cycles. When the nitrate concentration was removed then the 1 L of clarified supernatant (treated water) was decanted. The decanted volume was replaced by fresh synthetic water samples for next cycle.

### III. RESULTS AND DISCUSSION 3.1 Effective carbon sources

In the present work, in order to find effective carbon source for denitrification paddy straw, wheat straw, and ragi straw was added for three reactors

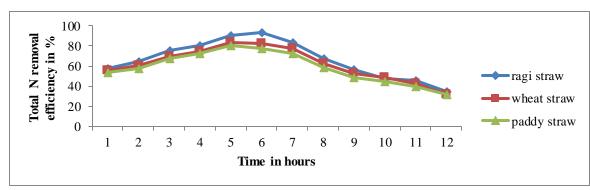


Fig. 1: Total Nitrate removal efficiency of ragi straw, wheat straw and paddy straw

Figure 1 shows, the total Nitrate removal efficiency of ragi straw, wheat straw and paddy straw on initial nitrate concentration of 50 ppm. However, the nitrate removal efficiency is more during the period 6-8 hours for three carbon sources.

From the comparison of experimental results using carbon sources, it was clearly seen that the microorganism used for the denitrification studies were active for ragi straw as carbon source compared to wheat straw and paddy straw. The use of ragi straw as the carbon source resulted in the highest nitrogen removal efficiency, followed by wheat straw and paddy straw. The results suggest that the ragi straw is the most efficient carbon source for denitrification of wastewater. Wheat straw is a satisfactory alternative carbon source for nitrogen removal as compared to paddy straw. It is observed that the nitrate removal efficiency is more than 96% with ragi straw as a carbon source. Since ragi straw was found to be effective as a carbon source in initial studies the same continued for further studies.

	R1 0.1 g/2L		R2 0.2 g/2L		R3 0.4 g/2L		R4 0.6 g/2L		
Parameters	First	Last	First	Last	First	Last	First	Last day	
	day	day	day	day	day	day	day		
Nitratenitrogen,mg/L	50.2	0	50.5	0	52.6	0	50.5	0	
Nitrite nitrogen mg/L	0.014	0.04	0.014	0.08	0.042	0.07	0.042	0	
Ammonia nitrogen, mg/L	0	15	0	10	0	12	0	12	
COD, mg/L	320	6.4	640	12.8	1280	64	1920	128	
pН	7.13	6.97	7.5	7.6	7.09	7.26	7.43	7.15	

Table.2: Performance of four anoxic reactors

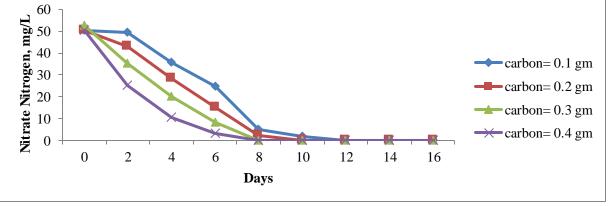


Fig. 2: Comparisons of nitrate removal in four reactors

Figure 2 shows the comparison of nitrate removal in all four reactors containing different amount of carbon source. It can be observed that the removal efficiency of nitrate is almost following the same trend but the COD concentration is more in reactor 2, 3 and 4 compared to reactor 1, in which the carbon source is 0.1 g. It can be noted that on day 8 in all the four reactors the nitrate nitrogen is nearly completely removed and with 0.1 g carbon source nitrate removal was effective. Based on the results of this study in the next batch studies carbon source of less than 0.1 g/2L has been tested.

The second batch studies were conducted for 10 days the reactors were fed with synthetic water containing nitrate nitrogen of 50mg/l and the powdered ragi

straw(carbon source) added were 0.025, 0.05, 0.075, 0.1gm/2L respectively in the four reactors. The results of the study are shown in the table 4.2. From the table it is clear that there remained some amount of nitrate nitrogen in reactor-1 fed with 0.025gm/2L of carbon source while in the other reactors it was below detection limit. The conversion of the nitrate to nitrite and ammonia was very less and this clearly shows that anoxic denitrification was taking place in all the four reactors. During denitrification process, a part of nitrate was converted into ammonia nitrogen but the amount was very less. It can be observed that the pH remains almost constant. In all the four reactors COD reduced to below detection limit at the end of reaction period.

	R1		R2		R3		R4		
	0.025 g/2L		0.05 g/2L		0.075 g/2L		0.1 g/2L		
Parameters	First	Last	First	Last	First	Last	First	Last day	
	day	day	day	day	day	day	day		
Nitratenitrogen,mg/L	50.5	6.3	54.7	0	50.5	0	52.6	0	
Nitrite nitrogen mg/L	0.01	0.15	0	0.05	0	0.028	0	0.022	
Ammonia nitrogen, mg/L	0.19	38.3	0.17	37.4	0.24	25	0.23	23.4	
COD, mg/L	80	0	150.4	0	240	0	320	0	
pH	7.02	7.88	6.92	7.77	7	7.2	6.9	7.55	

Table.3: Performance of four anoxic reactors with various Carbon loading

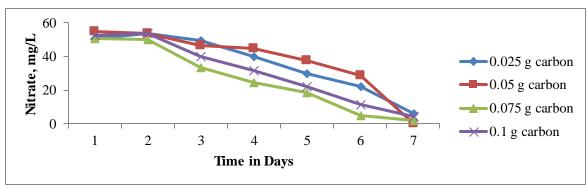


Fig.3: Comparison of nitrate removal in four reactors

From the figure 3 it can be noted that the removal efficiency of nitrate is almost following the same trend for reactor 2, 3 and 4. But the COD concentration is less in reactor-2 but the nitrate removal efficiency is

87.5% where as in reactor-2 it is 100%. Hence optimized carbon source is 0.05 gm/2L and the same was maintained in the further studies.

	R1 60 mg/L		R2 70 mg/L		R3 80 mg/L		R4 90 mg/L	
Parameters								
	First	Last	First	Last	First	Last	First	Last
	day	day	day	day	day	day	day	day
Nitratenitrogen,mg/L	56.5	1.6	66.8	3.7	72	6.3	88.3	16
Nitrite nitrogen mg/L	0.012	0.15	0	0.015	0.042	0.07	0.042	0
Ammonia nitrogen, mg/L	0.23	12	0.25	14	0.24	9.6	0.23	7.7
COD, mg/L	256	0	256	6.4	224	0	224	0
рН	7.13	6.97	7.5	7.6	7.09	7.26	7.43	7.15

Table.4: Comparison of performance of reactors operated with different nitrate nitrogen loading.

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Figure 4 and Figure 5 shows the variation of COD and nitrate nitrogen with time (Days), in the four reactors fed with synthetic water containing 60, 70, 80 and 90mg/L of nitrate nitrogen and carbon source (ragi straw) added is 0.05gm/2L of water in the reactor. In the first reactor fed with 60mg/L of nitrate nitrogen the COD and nitrate nitrogen removal was rapid and the trends were similar. It was found that at the end of the process nitrate removal efficiency was 97.16% and COD removal was 100%. In reactor-2 wherein the feed containing 70mg/L of nitrate nitrogen was fed initially the COD was 256mg/L and the nitrate concentration was 66.8mg/L. in this reactor also the trends of COD as well as nitrate nitrogen removal was similar in the three cycles. In the second and the third cycle nearly complete COD and nitrate nitrogen removal was observed. At the end of the process nitrate removal efficiency was found to be 94.4% and the COD removals observe was 97.5%. in the reactor3 the feed contain 80mg/L of nitrate nitrogen. Initially COD was 224mg/L and the nitrate concentration was 72mg/L and at the end of process nitrate removal efficiency was 91.2% and the COD removal was nearly 100%. When higher concentration of nitrate nitrogen was present in the raw water (90mg/L)it was found that in all the three cycles the nitrate nitrogen removal was not complete and there remained 16-35 mg/L of nitrate nitrogen in the treated water. Also it was found that there was a lag was observed between COD uptake and nitrate nitrogen removal in reactor 3 and 4. At the end of the process nitrate removal efficiency was found to be 81.8% and COD removal efficiency observed was nearly 100%. It is observed that at various nitrate loading conditions (60, 70,80 and 90 mg/L) nitrate nitrogen removal was effective. In the first 3 reactors the removal efficiency was above 90% whereas in the fourth reactor nitrate removal efficiency was less than 90%.

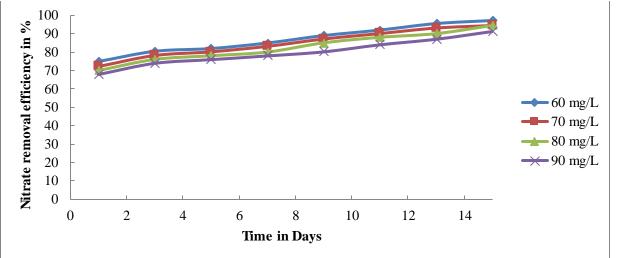


Fig 4: Nitrate removal efficiency in four reactor fed with influent containing different nitrate loading

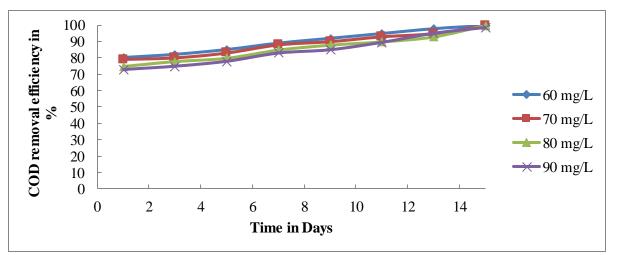


Fig 5: COD removal efficiency in four reactors fed with influent containing different nitrate loading

#### IV. CONCLUSIONS

• Among the three types of carbon source nitrate removal efficiency is more than 96% with Ragi

straw as a carbon source. Hence Ragi straw was found to be effective carbon source for denitrifying

the organisms under anoxic condition for further studies it was used as carbon source.

- Cow dung slurry was used as seed and it was effective in enriching denitrifying organisms.
- For optimization of carbon source added four anoxic batch reactors were operated with different amount of carbon source and nitrate removal was observed in the entire reactor, but the COD concentration in reactor 2, 3 and 4 was more compared to reactor 1.
- 0.05 g/2L of powdered ragi straw was found to be optimal dosage for complete nitrate nitrogen removal.
- From the overall studies biological and physicochemical methods, both proved to be efficient with their own advantages and disadvantages.
- Biological treatment proved to be very effective and economical for the removal of nitrate. Even though the time required for the treatment is comparatively quite high, it can be preferred over the physico-chemical methods as all the materials used in the system were locally and cheaply available.

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