Nitrate Removal from Wastewater Using Different Carbon Sources

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Abstract: ***Nutrient loading in water bodies has known to cause eutrophication and nutrient leaching. These are identified as a major global environmental issue, contributing to health hazards and various environmental threats. There are many sources of nitrogen and phosphorus which have been increased largely over the years. Instead of controlling the source, the main processes involved is the reduction of nitrate produced, which can be done by various physiological and biological methods. But the most efficient method identified is microbial denitrification, where the reactive nitrate is sequentially reduced to non-reactive nitrogen in the presence of facultative aerobic bacteria. It requires a major carbon source and many studies have been done to identify the most efficient external carbon source that can be used. This study mainly deals with comparing the efficiency of simple carbon sources (wood chips, paddy straw and newspaper) for denitrification. All the three sources are easily available indigenously. The study is done in synthetic wastewater with an initial NO_3-N of 50 mg/L inoculated with Pseudomonas aeruginosa in packed bed reactors of 5 L capacity as batch flow. The study has identified newspaper as the most efficient among the three.***

Keywords: Batch flow reactor, Denitrification, Efficiency, External carbon source, Microbes, Nitrate reducing bacteria, Synthetic wastewater

1. Introduction

1.1 General

Nutrients inputs (especially nitrogen and phosphorus) contribute much to water pollution and can cause eutrophication in natural water bodies. Eutrophication refers to enhanced biological production associated with a reduction in the available dissolved oxygen in the water column [1]. Nitrate and phosphorus in wastewater is identified as a global environmental issue that contribute to health and environmental threats as they are linked to illness as well as ecosystem disruption via algal blooms in contaminated water bodies. Nitrogen release to soils, surface water and groundwater systems, originating mainly from excessive application of fertiliser, manure and sewage sludge has increased over recent decades. USEPA has reported in its manual that toilet use is the main source of nitrogen compared to basin, sink and appliance use. The second biggest source, according to the report, is food waste disposal. The significant phosphorus content in wastewater is largely due to detergent use, which has also increased recently [4].

Various means of reducing the nutrient load of wastewater has been introduced and implemented, whether physiological or biological. Techniques like ion exchange, gas stripping and breakpoint chlorination for nitrogen, and coagulation/sedimentation by metal salt or lime for phosphorus are available for its removal. Yet the employment of biological agents proves to be an intriguing and attractive approach for researchers as it uses the simple principle of letting natural, living agents capable of functioning and reproducing independently and process the pollutants out of the water [3]. Hence among the various techniques available, microbial denitrification stands out for being the most economical and environmentally sound, as well as for being feasible on a large scale.

Denitrification, one of the most important process taking places in the natural environment, has been defined in many ways by various sources. Whatever be the source, denitrification is undoubtedly the reduction of nitrate or the loss or removal of nitrogen compounds in the presence of a carbon and energy source and the absence of oxygen, by facultative aerobic bacteria using nitrates as terminal electron acceptor [6]. Denitrification has been broadly divided into assimilatory and dissimilatory nitrate reduction. The assimilatory process converts nitrate to ammonia, via nitrite, and the product is incorporated by proteins and nucleic acids. The latter reduction process reduces the nitrate to atmospheric nitrogen gas via nitrite, nitric acid and nitrous acid. All the reductions are catalysed by respective reductases. Studies always surround the dissimilatory reaction, since the end product is nitrogen gas [1].

Denitrification requires an anoxic environment and a carbon source. The latter is important as an electron donor is essential to carry out the process and the denitrification potential of wastewater is a function of available organic carbon (COD/N or BOD/N). In case of insufficient COD, external sources of carbon are applied to the required water. This increases the denitrification rate and enhances nitrate removal. Solid substrates were used as an alternative to liquid carbon sources and the common sources of solid carbon can either be natural materials (cellulose-rich material, wheat straw, cotton, newspaper, pine bark) or synthetic polymers (thermoplastic). These can be simultaneously used as biofilm carrier and carbon source. An anoxic environment is much preferred because in the presence of oxygen, instead of accepting electron from nitrate, the organic matter might utilise oxygen. The presence of oxygen inhibits nitrate reductase or nitrous oxide reductase which catalyzes the first and fourth step of denitrification, respectively. The inhibitory effect stops the enzymatic process, giving rise to a progressive reduction of molecular nitrogen production and the consequent accumulation of the different intermediates, principally nitrite and nitrous oxide.

Denitrifiers belong to several physiological and taxonomic groups and can use various energy sources. The microorganisms capable of denitrification belong to the following genera: Pseudomonas, Bacillus, Spirullum, Hyphomicrobium, Agrobacterium, Acinetobacter, Rhizobium, Thiobacillus, Alcaligenes, etc. Different species have been isolated in different samples of soils, water and wastewater, but the most widespread genera are probably Pseudomonas and Alcaligenes. Along with denitrifiers, fungi provides important enhancement due to its ability to release soluble carbon substrates [2]. Many organisms like Escherichia coli carry out partial denitrification, which results in production of nitrous acid, a major air pollutant.

The related case studies on denitrification that have been reviewed for this study are the following.

Willie Jones B. Saliling et. al., [10] evaluated wood chips and wheat straw as inexpensive and readily available alternatives to more expensive plastic media for denitrification processes in treating aquaculture wastewaters or high nitrate waters. They used bench-scale upflow biofilters for synthetic wastewater with wood chips, wheat straw and Kaldness plastic as media. The characteristic study of the three media was done. Analysis was done for NO_3 + NO_2-N, NO_2-N, NH_3-N, COD, alkalinity, pH, temperature and COD. A 140 day experiment was done with methanol as external carbon source.

Michael Volokita et. al., [6] conducted a study in a laboratory column packed with shredded newspaper which served as the sole carbon and energy substrate and also the only physical support of microbial population. The study showed that the pre-treatment of newspaper by any chemical or by autoclave did not improve its efficiency, and characteristic study of the media was conducted. Samples were collected as assayed for NO_3, NO_2, NH_3, pH, DOC and bacterial count of the inoculated cellulose-degarding bacteria.

Paolo De Filippis et. al., [7] studied three different carbon sources (sucrose, acetic acid and methanol) that were used to treat high nitrate wastewater in a laboratory scale ASR. An inoculum was grown in a growth medium and transferred to water with 3 g NO_3/ L and C/N 3. Analyzes was done for pH, TOC, NO_3, NO_2 and DO. The study proved methanol to be the best carbon source among the three.

Jyothi. J. Madam [5] conducted a study to treat eutrophicated water under both aerobic and anoxic condition, with Pseudomonas species. Both groundwater and soil sample was taken for experiment and done using both synthetic and natural sample. 1% starch was used as representative carbon source for treatment. Nitrate reducing bacteria was isolated and identified from the natural sample. Analysis was done for nitrate, nitrite and phosphate. The study showed a nutrient removal efficiency of 94.5% for nitrate and 55% for phosphate, in natural sample. In synthetic sample Pseudomonas in presence of 1% starch has a higher nitrate removal of 98% in anoxic condition.

1.2 Need for Study

The need of the study gains its importance from the adverse effects that are caused due to the presence of nutrients in excess. Eutrophication and nitrate leaching have caused due to the presence of human excreta, sewage disposal, cattle seepage, fertilizer and explosive industries effluent, municipal waste and other industrial effluents in the natural water bodies and also in soil. It causes progressive silting, colouring of water, reduced transparency, oxygen depletion in deeper layer, greater biomass with appearance of species indicative of eutrophication. In addition to these, there are significant health concerns associated with waterborne disease outbreaks arising from inadequate wastewater treatment and agricultural discharges. When large quantities of nitrate are consumed by infants, their skin appears to have a blue tint due to lack of oxygen, a condition called methemoglobinemia or "blue baby syndrome" and also cause cancers, birth defects, abortions, hypertension thyroid hypertrophy and is also potentially harmful to animals causing abdominal pains, muscular weakness or poor coordination and brown or chocolate coloured blood [8]. One of the major factors affecting denitrification is the presence of adequate carbon source. In water containing limited carbon content, external sources are provided in the form of methanol, ethanol, acetic acid, etc. This study focuses on utilising cheap sources of carbon as packed bed that can also be used as a biofilm support. The bacteria strain used is of one of the most common species of denitrifiers and hence this study is done with a view of making denitrification easy and economical.

The objective of the study is to compare the denitrification capacity of three different carbon sources, namely wood, paddy straw and newspaper.

2. Materials and Methods

2.1 Experimental Setup

Three reactors each of 5 L capacity was used for this study. The reactor made of acrylic is of dimension 10 cm X 10 cm X 50 cm height. Since the experimental setup is done in anoxic condition, the top portion is covered by a lid. Three rubber tubes are provided, one each at top and bottom for wastewater inlet and outlet, and one in lid for gas collection if required.

The three reactors were packed with wood chips, paddy straw and newspaper, respectively. They can be filled either according to the weight or height of the media required. In this experiment, all the three media were filled to a compressed height of 20 cm.

Figure 1: Packed bed reactor

2.2 Bacterial Culture preparation

The bacterial culture selected for inoculating the synthetic medium is Pseudomonas aeruginosa. The genera Pseudomonas has been long identified as one of the most important group of dentrifiers present naturally. The micro-organism culture bought from Microbial Culture Centre (MCC), Pune was sub-cultured on nutrient broth. The standard composition of the broth was prepared by mixing the components in 50 mL distilled water. The inoculated broth was then placed in the incubator for 24 hrs for its growth.

The optical density and plate count of the inoculum was analyzed for microbial growth rate and viable count. Optical density of the inoculum was measured using a Double beam UV-VIS Spectrophotometer at an absorbance of 610 nm. It is based on the light absorbed by a suspension of bacterial cells of a solution and is used for comparison purpose.

Figure 2: Inoculated sample and blank used for optical density.

The optical density is insufficient to find out the bacterial count. Hence a plate count test for the bacteria was done by inoculating from the sample used for testing optical density. The Psuedomonas agar broth was obtained by mixing 13.6 mg/L of the powder in distilled water. 0.1 mL sample was transferred into the plate and mixed with the agar. This is then left for 24-48 hrs and then measured under a light microscopic.

2.3 Synthetic Wastewater Preparation

Nitrate containing synthetic wastewater was prepared for the experiment study. The composition of the wastewater prepared is as follows [9]:

Table 1: Composition of synthetic wastewater

Reagents	Quantity (mg/L)
KNO_3	361
NH_4Cl	20
$MgSO_4.6H_2O$	10
$FeSO_4.7H_2O$	0.2
$NaHCO_3$	1000
KH_2PO_4	50
Trace metal solution (mL/L)	25
Ethanol (mL/L)	1

The reagent KNO_3 provided the required nitrate for our study. A value of approximately 50 mg/L NO_3-N was selected as the initial nitrate concentration and the ratio of nitrate to phosphate was taken about 5. $NaHCO_3$ provided the required alkalinity for the process. A solution of trace metals was prepared in de-ionized water to include all the trace elements that would be present in a wastewater sample. The reagents in mentioned quantity were mixed in one litre distilled water. The synthetically prepared wastewater is then used for batch flow study, to compare the efficiency of the three carbon sources. A small amount of ethanol was added to provide an external carbon source.

Table 2: Composition of trace metal solution

Reagents	Quantity(mg/L)
EDTA	500
$CaCl_2$	55.4
$CuSO_4.5H_2O$	15.7
$CoCl_2.6H_2O$	16.1
$MnCl_2.4H_2O$	50.6
$ZnSO_4.H_2O$	220
$FeSO_4.7H_2O$	49.9

2.4 Characteristic Study of Synthetic Wastewater

The synthetic wastewater prepared during both the stages will be analysed for the following characteristics. Nitrate-nitrogen was analyzed using a Single Beam UV-VIS Spectrophotometer at an absorbance of 220 nm. The analysis was done after calibration using intermediate nitrate solution. Phosphate was calculated by using UV-VIS Spectrophotometer at an absorbance of 540 nm. pH, temperature and dissolved oxygen was measured using Water Analyzer 371. BOD and COD measurement is done according to the Standard Methods.

2.5 Preparation and Study of Carbon Media

The carbon media were prepared before filling up the reactor. Wood for making chips were obtained from a saw mill and reduced to size 8-50 mm side and 2-15 mm thickness. Paddy straw that was used to fill the reactor was cut to a length of 20-35 mm. Newspaper used as media was cut into small pieces of 4-8 mm size. Pretreatment was found unnecessary as studied from earlier works, and hence they were filled after preparing them to the required size. The characteristic study of the media was also performed to analyse the media changes and efficiency due to the denitrification process.

The three carbon media was used for batch flow denitrification study using the synthetic wastewater. The performance and efficiency of all the three carbon sources will also be compared in the study.

3. Results and Discussion

3.1 Characteristics of Synthetic Wastewater

The synthetic wastewater was tested and the following characteristics were obtained.

Table 3: Characteristics of synthetic wastewater

Parameters	Quantity (mg/L)
pH	7.79
Temperature	32.6
DO	3.5
BOD	80
COD	124
COD/ NO_3-N	2.52
NO_3-N	49.16
NO_2-N	Not detected
PO_4-P	10.98

The level of nitrate will be continuously analyzed for about 4 weeks. The analysis showed that the nitrate content was almost similar to the value required. The COD/ NO_3-N is low, but it will be enhanced by the addition of the external carbon source.

Dissolved oxygen is within the required limit, which can be from 0 to 4.5 mg/L. pH and temperature is found to be within the range and suitable for denitrification.

COD/ NO_3-N values for the three media were calculated after the completion of the experiment. The value obtained for wood, paddy straw and newspaper is 0.369, 1.661 and 18.135, respectively. This shows that there is a high enrichment of COD due to the addition of newspaper as external carbon source or may be due to the low value of nitrate. Similarly BOD was also found to increase, with a value of 270, 250 and 180 mg/L respectively.

3.2 Optical Density and Plate Count

The value of optical density for the inoculum was found to be 1.157. It shows the absorbance of the culture, and hence more the value, the growth will be more. There is no standard value for optical density and it varies depending on the species and the value obtained here showed a highly turbid culture. From the plate count a high value of growth of 70 CFU/mL was obtained. Optical density and cell count is proportional to each other.

3.3 Characterisation of Porous Material

The characteristic study of the three materials was done to study the subjective measure of degradation of the media due to denitrification. All the media were filled to a compressed height of 20 cm. Changes in height and mass have been observed and recorded during the course of this study. Porosity of the reactor media was determined before the denitrification test.

Table 4: Characteristics of media

Characteristics	Wood		Straw		Newspaper	
	initial	final	initial	final	initial	final
Mass (g)	296	820	72	525	246	1430
Height (cm)	20	26	20	28	20	30
C/N	7.33	4.31	8.44	1.73	6.79	11.83
Porosity	0.3		0.4		0.5	

The mass of all the three media was found to increase, with straw and newspaper by a value of more than 80%. The increase is due to the absorbance of water by the media and shows the degradation of the media. The initial height at which the media was filled was 20 cm. On filling 2L water the height of media was increased and this was used for the calculation of porosity. Newspaper was found to have maximum porosity among the three.

C/N ratio calculation was calculated by analysing total carbon and total nitrogen. Expect for newspaper, C/N ratio was found to decrease due to the enrichment of nutrients. Low C/N favors biodegradation and the least value was obtained for paddy straw. This shows that irrespective of the denitrification rate, the media degrades.

Visual observations of the media degradation were also noticed. Of the three media, paddy straw exhibited more signs of physical change. The spent straw was darker in colour. Fungal growth was noticed in both the organic media (wood and paddy straw). Newspaper had the least degradation along with providing the best media for denitrification.

3.4 Denitrification of Synthetic Wastewater

In order to study denitrification efficiency of the three media, the reactors have been filled with 2L synthetic wastewater as composed using Table 1 and 2. 20 mL of the acclimatized sample is also added to the reactor for the microbial action. The following figures show the various nitrate concentration obtained in a time period of 4 weeks. For most of the experimental period, the nitrate concentration in the effluent from reactor with newspaper was found to be lower than the other two media.

At the beginning of the experiment, the nitrate content in the reactor with newspaper was found to decrease. But a sudden increase in the nitrate content was noticed in the other two reactors. Within 5 days, the nitrate content started to decrease in the reactor with straw, while in the reactor with wood, decrease of nitrate was after a further increase. The nitrate content was observed to be double than the straw. Once it started to decrease, it has been a gradual process in both the reactors. All this while nitrate content in the reactor with newspaper was gradually decreasing with a slight increase after 17 days. The value was well below the initial nitrate concentration.

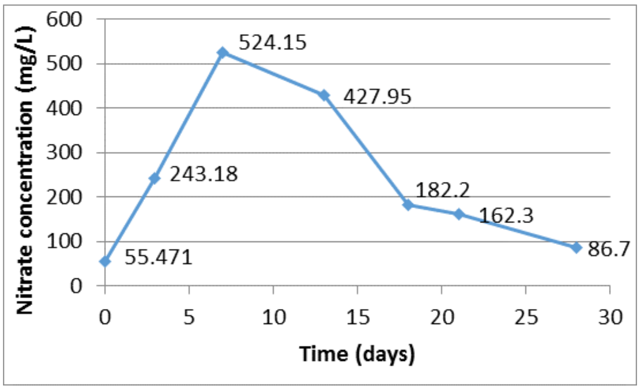

Figure 3: Nitrate concentration with wood as media

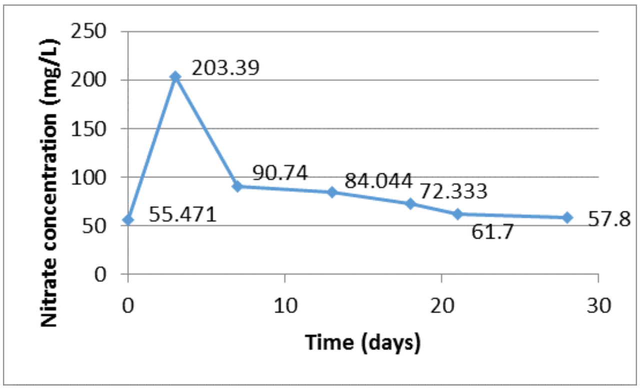

Figure 4: Nitrate concentration with paddy straw as media

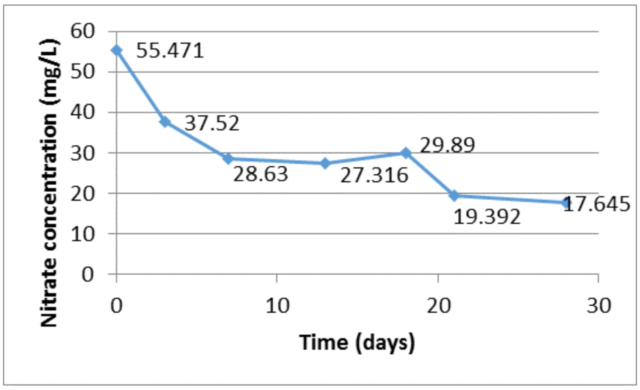

Figure 5: Nitrate concentration with newspaper as media

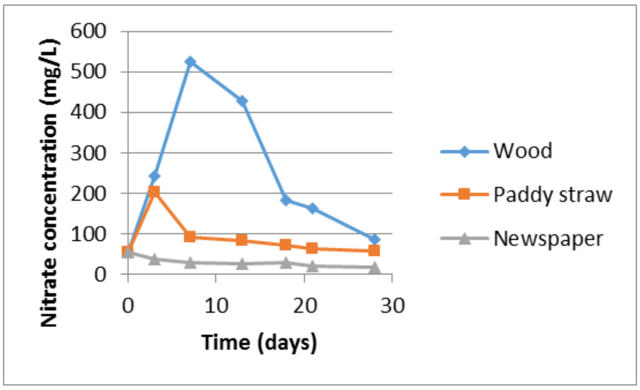

Figure 6: Comparison curve for the three media

4. Conclusion

It has already been established that biological methods of denitrification is the best compared to physical and chemical methods. The denitrification capacity among the three media was found to be maximum for newspaper. It ws found to provide a good bacterial support without getting biologically degraded. Moreover the effluent from this reactor does not require any further treatment. Hence newspaper can be effectively used for denitrification process of short duration and is a good method of reuse.

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