

# Denitrification of Wastewater Containing High Nitrate Using a Bioreactor System Packed by Microbial Cellulose

H. Godini, A. Rezaee, A. Jafari, and S. H. Mirhousaini

**Abstract**—A Laboratory-scale packed bed reactor with microbial cellulose as the biofilm carrier was used to investigate the denitrification of high-strength nitrate wastewater with specific emphasis on the effect the nitrogen loading rate and hydraulic retention time. Ethanol was added as a carbon source for denitrification. As a result of this investigation, it was found that up to 500 mg/l feed nitrate concentration the present system is able to produce an effluent with nitrate content below 10 ppm at 3 h hydraulic retention time. The highest observed denitrification rate was 4.57 kg NO<sub>3</sub>-N/ (m<sup>3</sup> .d) at a nitrate load of 5.64 kg NO<sub>3</sub>-N/(m<sup>3</sup> .d), and removal efficiencies higher than 90% were obtained for loads up to 4.2 kg NO<sub>3</sub>-N/(m<sup>3</sup> .d). A mass relation between COD consumed and NO<sub>3</sub>-N removed around 2.82 was observed. This continuous-flow bioreactor proved an efficient denitrification system with a relatively low retention time.

**Keywords**—Biological nitrate removal, Denitrification, Microbial cellulose, Packed-bed reactor.

## I. INTRODUCTION

NITRATE released into environment can create serious problems, such as eutrophication of rivers, deterioration of water quality and potential hazard to human health, because nitrate in the gastrointestinal tract can be reduced to nitrite ions. In addition, nitrate and nitrite have the potential to form N-nitrous compounds, which are potent carcinogens [1]-[3]. To address this problem, specific rules have been established globally. The European Community and the USA Environmental Protection Agency, set the 5.6 mg (NO<sub>3</sub>-N)/L and 10 mg (NO<sub>3</sub>-N)/L respectively [4]. This danger necessitates the removal of NO<sub>3</sub><sup>-</sup> from water reserves. Biological denitrification is an attractive treatment option, for the NO<sub>3</sub><sup>-</sup> is converted by the denitrifying bacteria to inert nitrogen gas and the waste product usually contains only biological solids. Biological removal of nitrate is widely used in the treatment of domestic and complex industrial wastewaters [5]-[8]. The denitrification could be achieved

either in the suspended or attached growth systems. Attached growth reactors are the favored bioreactors for denitrification because they may be made much more compact. The treatment of wastewater in packed bed bioreactors is attracting increasing interest with the application of a variety of carriers [9]-[12]. Several natural materials (agar, agarose, collagen, alginates and chitosan) and synthetic polymer materials (polyacrylamide, polyurethane, polyethylene glycol and polyvinyl alcohol) have been applied as media [13]. Among the various matrixes that are available, the Microbial cellulose (MC) had been chosen for its ease of use, low cost, low toxicity, high operational stability [14], biopolymer without lignin or hemicelluloses, high strength crystalline, light weight, selective porosity, and high surface-to-volume carrier capacity. The MC synthesized by *Acetobacter xylinum* is identical to that made by plants in respect to molecular structure. Because of these features there is an increasing interest in the development of new fields of application [14], [15]. The microbial cellulose media provides a continuously high cell concentration in the bioreactor. To ensure complete denitrification, an external carbon source is often used that serves as the electron donor and facilitates the denitrification process [16], [17]. The usage of ethanol is common not only in experimental pilot plants [18]-[20], but also in full-scale technologies [21], [22]. Results of study conducted by Saliling et al (2007) indicate that wood chips and with straw can used as alternative biofilter media for denitrification of wastewater with high nitrate concentrations [23]. In this study it is aimed to investigate performance of high nitrate removal in a microbial cellulose packed-attached growth biofilm reactor. These parameters are nitrate concentration in feed solution and feed solution flow rate. The microbial cellulose is known to be effective in holding organic substances in water streams. Thus by the use of microbial cellulose bed it is aimed to minimize the contamination of the product water by residual organics. The aim was to attain a constantly high denitrification activity and a minimal NO<sub>2</sub><sup>-</sup> concentration in the effluent with a low retention time.

## II. MATERIAL AND METHODS

### A. Microbial Cellulose Production

In this study *A. xylinum* (ATCC 23768) was used. It was grown in SH medium at 28°C under static culture conditions. Preinoculum for all experiments was prepared by transferring a single *A. xylinum* colony grown on SH agar into a 50 ml

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Erlenmeyer flask filled with liquid SH medium. After 5 days of cultivation at 28°C, the cellulose pellicle formed on the surface of the culture broth. Ten milliliters of the cell suspension was introduced into a 500 ml Erlenmeyer flask containing 100 ml of fresh SH medium. The culture was carried out statically for 72 h and the cell suspension derived from the synthesized cellulose pellicle was used as the inoculum for further cultures. The stationary cultures in Erlenmeyer flasks filled with different volumes of the medium lasted for 7 days. After cultivation, the cellulose sheets were removed and rinsed with distilled water and cleaned of bacterial and medium residues using 2% sodium dodecyl sulfate and 4% NaOH solutions in a boiling-water bath. The MC was cut into 5-10 mm pieces and used for cell immobilization, bioreactor media and carbon source.

#### *B. The Denitrifier Bacteria and Inoculation of Bioreactor*

The Consortium microorganisms with high denitrification efficiency were isolated from effluent petrochemical industry taken from Razi in Iran. This industry produces Nitrogen fertilizer and have high nitrate. To inoculate the biofilter media with bacteria, the bioreactor was first filled up with nitrate-rich media and isolated bacteria for 48 h. After the static period, the waste storage tank was filled with more wastewater from the same source and circulated through the reactors in a closed loop, returning to the storage tank. This recirculation was continued until there was an indication of a substantial decline of the nitrate–nitrogen concentration of the wastewater in the storage tank. During this acclimation period, the wastewater in the storage tank was amended with the addition of nitrate and ethanol to improve bacterial growth. After recirculating the wastewater for 3 days, feeding of the synthetic wastewater began at an influent  $\text{NO}_3^- + \text{NO}_2^-$ -N concentration of 100-700 mg/L. During this study, reactor was fed from a common source of synthetic wastewater.

#### *C. Synthetic Wastewater*

The synthetic wastewater was prepared using deionized water in addition to other chemicals. Potassium nitrate was added as the nitrogen source at a concentration of 100-700 mg  $\text{NO}_3^-$ -N/L. ethanol was added as the carbon source at a concentration of 300-2100 mg COD/L. The ratio of the nitrogen to COD was taken as 1:3 to keep the nitrogen as the limiting substrate. Trace mineral constituents essential to the bacterial growth added per liter were: 0.85 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25 mg  $\text{NaMoO}_4$ , 0.157 mg  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ , and 33 mg  $\text{NaHCO}_3$ . Sodium Sulfite and cobalt chloride were added at concentration of 20 and 0.55 mg/L, respectively, to reduce the oxygen concentration to below 0.5 mg/L to ensure anoxic conditions in the reactors. Monobasic and dibasic potassium phosphate was added as a buffer system.

#### *D. Bioreactor Operation*

To increase the biological denitrification efficiency, packed-bed reactor was applied with microbial cellulose beads. In the long-term operation test, the synthetic wastewater was fed following as; 100-700 mg/l of nitrate-N, 300-2100 mg/l of ethanol and the pH was adjusted to 7.2. The experimental set-up used in investigation was microbial

cellulose packed bioreactor, a Plexiglas column has been used as reactor followed by a 5 liter sedimentation tank. The ends of the PVC column were covered with plastic screens to hold the biofilter media. The total volume of the reactor up to the top level was 3500 ml, with height 70 cm, and diameter 8 cm. which only 50 cm portion was filled with microbial cellulose. The synthetic wastewater was fed from the bottom of the reactor and left it from its top. Ethanol was used as carbon source which was added into the solution in such a quantity to give a COD/N ratio of 3. A constant flow rate was applied, at which the average HRT of the influent referred to the total volume of the reactor was 1-3 h. The wastewater influent was fed to the bottom of the reactor through 0.635 cm (1/4 in.) clear vinyl tubing. Similarly, vinyl tubing was used to carry effluent away from the top of the reactors for disposal (e.g. this was a flow through system). The vinyl tubing was cleaned at least once every 2 weeks to minimize biofilm and solids buildup inside the influent and effluent lines. This maintenance procedure was implemented to minimize denitrification in the influent and effluent lines. The reactor was operated at 30 °C. Samples were taken from the bioreactor every 24 h and the  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , COD and alkalinity concentrations of the samples were determined to study the spatial separation of the  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction steps of the denitrification process. The temperature of synthetic wastewater was controlled to 30 °C in the controller.

#### *E. Analytical Methods*

Samples were collected at the influent and effluent ports. Liquid samples were centrifuged at 5 °C. Thus, obtained supernatant was used for nitrate and nitrite analysis. Samples were analyzed for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ -N, COD, and alkalinity using Standard Methods [24]. The pH was measured routinely throughout the trials.

### III. RESULTS AND DISCUSSION

Table I summarizes the different average influent and effluent concentrations, the corresponding percent reduction in  $\text{NO}_3^-$ -N concentrations, and denitrification rates under pseudo steady-state conditions. This study showed that the nitrate removal efficiency was 90-100 % at COD: $\text{NO}_3^-$ -N ratios of 3:1, with HRTs of 3 h. In this study a low nitrite was attained.

TABLE I  
THE DIFFERENT AVERAGE INFLUENT AND EFFLUENT CONCENTRATIONS, THE CORRESPONDING PERCENT REDUCTION IN NO<sub>3</sub>-N CONCENTRATIONS, AND DENITRIFICATION RATES (HRT= 3 h AND T= 30 °C)

Influent NO <sub>3</sub> -N concentration (mg/L)	Effluent NO <sub>3</sub> -N concentration (mg/L)	Percentage reduction (%)	Denitrification rates kg N/(m <sup>3</sup> .d)
99.98±4.76	0.95±0.35	98.06±1.36	0.78±0.08
201.27±5.86	0.596±0.25	99.70±0.2	1.610±.15
299.81±9.34	0.758±0.28	99.75±0.1	2.39±.123
399.541±10.84	5.63±1.29	98.59±0.12	3.15±.234
501.25±8.86	9.34±1.08	98.14±0.45	3.94±.245
599.63±10.89	69.68±3.89	88.38±0.89	4.23±.384
699.60±9.89	119.55±5.63	82.95±2.87	4.57±0.324

Dahab and Lee (1988) and Mohseni-Bandpi and Elliott (1999) reported that a nitrate removal efficiency of nearly 100% was achieved with HRTs of 9 and 8.8 h, respectively, using a bench-scale anoxic filter and the RBC system [25], [26].

Denitrification rates for the different NO<sub>3</sub>-N loading values are shown in Table I and Fig. 1. The highest observed denitrification rate was 4.57 kg NO<sub>3</sub>-N/(m<sup>3</sup> d) for a nitrate load of 5.64 kg NO<sub>3</sub>-N/(m<sup>3</sup> d). These values are comparative to those previously reported for high load studies [9 and 11]. They Reported NO<sub>3</sub>-N loadings for up-flow packed-bed postanoxic denitrification reactors are in the range from 3 to 3.98 kg NO<sub>3</sub>-N/(m<sup>3</sup> d) to achieve effluent NO<sub>3</sub>-N concentrations below 5.0 g/m<sup>3</sup>. Hirata et al. (2001) reported a maximum nitrogen volumetric rate of 0.24 kg NO<sub>3</sub>-N/(m<sup>3</sup> day) by using an anaerobic aerobic circulating bioreactor system to remove ammonia and nitrate from two- to five-fold diluted industrial wastewater discharged from metal recovery processes [27]. Denitrification rates increased when loading rates increased for reactor (Fig. 1), ranging from approximately 0.72 to 4.57 kg N/(m<sup>3</sup> d). As can be seen under low load conditions, the denitrification rate essentially equals the load, with removal efficiencies close to 100%. The critical nitrate load, that is, the lowest value that generates removal efficiencies lower than 100%, was about 3.5 kg NO<sub>3</sub>-N/(m<sup>3</sup> d).

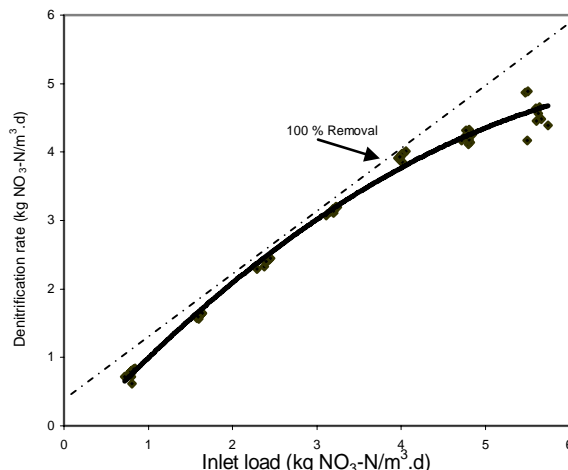


Fig. 1 Denitrification rate vs. NO<sub>3</sub>-N load of the synthetic wastewater (HRT= 3 h and T= 30 °C)

The reactor gave essentially the maximum daily denitrification rate of 4.57 kg nitrogen removed/m<sup>3</sup> media/day. Our calculated rates are in the high range of the rates reported by other researchers [28]-[34], for the other biological reactors. All studies referenced in the above focused on wastewater treatment with a variety of laboratory and pilot plant systems. This is the first paper to describe the use of microbial cellulose as a media and carbon source for nitrogen removal in a bioreactor system.

For the nitrite accumulation, maximum 45 mg/l of nitrite- N was accumulated in the reactor with 1 h retention time and 700 mg/l initial nitrate concentration (Fig. 2). However accumulated nitrite was decreased with increase of hydraulic retention time and decrease of nitrate loading rate.

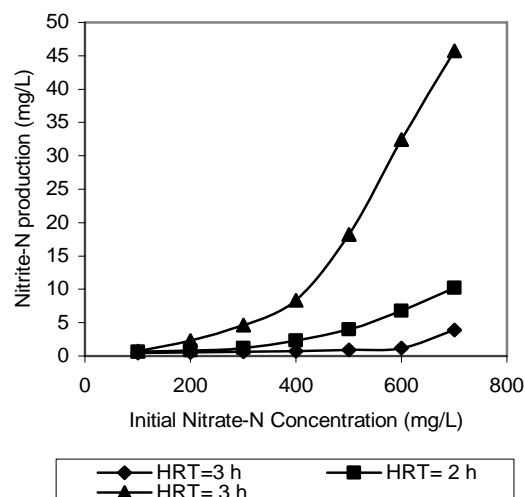


Fig. 2 Nitrite accumulation at different hydraulic retention time and initial nitrate concentration

There was a significant correlation with alkalinity gain and NO<sub>3</sub>-N reduced for bioreactor that shown in Fig. 3.

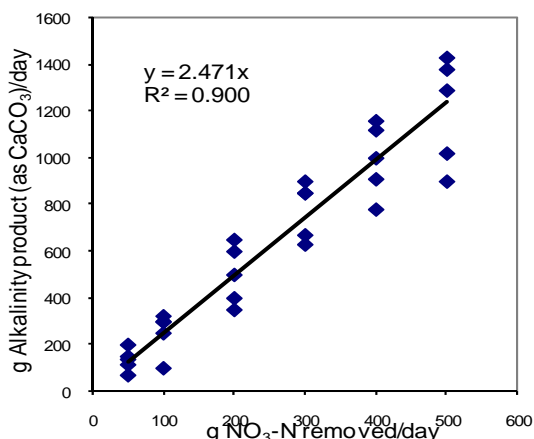


Fig. 3 Alkalinity gains of denitrification units supplemented with ethanol as carbon source

Alkalinity in the effluent increased with increasing nitrate loading rates. In all cases, the amount of alkalinity produced was related to amount of NO<sub>3</sub>-N removed. Alkalinity production averaged more than 2.5 mg CaCO<sub>3</sub>/mg NO<sub>3</sub>+NO<sub>2</sub>-N removed at reactor. This values was in the lower than of amount of removed which would be predicted from stoichiometry with ethanol being used as carbon source [35].

The denitrification process caused a pH rise that cannot be buffered by the alkalinity of the synthetic wastewater. This effect was more relevant as the inlet concentration increased; it has been reported that pH values between 7.0 and 8.0 have no significant effects on denitrification rate [36]. In this study high removals were even possible for pH above 9.0. Effluent pH readings were between 7.32 and 9.17 confirming alkalinity production.

Denitrification rate versus COD removal for reactor (HRT=3 h and T=50°C) showed at Table II. These data imply that the reactors were not carbon limited, and were receiving enough carbon to facilitate the denitrification process. Effluent COD concentrations are kept between 19 and 126 g/m<sup>3</sup> so the addition of ethanol should be adjusted in relation to the denitrification rate.

TABLE II  
AVERAGE INFLUENT COD, COD REMOVED AND COD REMOVED PER NITRATE-NITROGEN REDUCED IN EACH LOADING RATE (VALUE ± S.D.)

Influent COD Concentrations (mg/L)	COD removed (mg/L)	Residual COD (mg/L)	COD removed/per NO <sub>3</sub> -N reduced
300	281±8.35	19 ± 2.5	2.84 ± 0.2
600	560±21.8	40 ± 3.96	2.84 ± 0.16
900	846±19.78	54 ± 5.2	2.83 ± 0.12
1200	1128±35.47	72 ± 9.9	2.81 ± 0.13
1500	1410±33.41	90 ± 4.96	2.79 ± 0.16
1800	1692 ± 38.1	108 ± 4.6	2.79 ± 0.23
2100	1974 ± 31.9	126 ± 9.5	2.78 ± 0.17

USEPA [35] estimated that a COD/NO<sub>3</sub>-N ratio of 3.75 is required for denitrification with methanol as carbon source. At

this reactor requirement was below this stoichiometric estimate. The lower COD consumption per nitrate removed by this reactor may be attributed to the fact that microbial cellulose nature may have added some COD to the reaction, thus lessening the net COD requirement. Robertson et al. (2005) reported that at the early stages of use with their wood chip filters, the media leached carbonaceous COD (from tannic acid, etc.) out of the media [37]. The microbial cellulose in this study may have also leached some carbonaceous COD, but it was likely minor compared to the ethanol contribution.

#### IV. CONCLUSION

Denitrification performance of attached growth biofilm on microbial cellulose in a packed bed reactor system has been investigated as function of Nitrate concentration and others environmental factors. The denitrification reactor design used in this study was effective at significantly reducing nitrate concentrations within a relatively short timeframe. The spatial separation observed throughout the entire period of operation of the bioreactor is well represented by the average data. 90-100 % of the NO<sub>3</sub><sup>-</sup> content of the influent had already been reduced. The reduction of the NO<sub>3</sub><sup>-</sup> was followed by the accumulation of low NO<sub>2</sub><sup>-</sup>. The maximum NO<sub>2</sub><sup>-</sup>-N concentration at reactor was about 45 mg l<sup>-1</sup> at 1 h retention time, and the concentration progressively decreased with increase of hydraulic retention time and decrease of nitrate loadings. Conclusion derived from this work showed that up to 500 mg/L of feed solution nitrate-N content, the present system is able to produce an effluent with nitrate content below allowed limits. The study showed that Microbial cellulose was suitable supporting bacterial growth to provide biological denitrification and can be used as biofilter media.

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