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High Nitrate Removal in a Packed Bed Bioreactor Using Microbial Cellulose

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Abstract: An up-flow packed bed reactor has been operated to investigate the technical feasibility of biological nitrate removal applied to the synthetic solution. This study was evaluated Microbial Cellulose (MC) as a biopolymer carrier in a laboratory bioreactor. The effects of nitrate content and flow rate on the nitrate removal performance of a MC packed bed bioreactor have been investigated. Ethanol was used as a carbon source for biological denitrification. It was found that up to 500 mg L^{-1} 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_3-N/(m^3 day) at a nitrate load of 5.64 kg NO_3-N/(m^3 day). The removal efficiencies higher than 90% were obtained for loads up to 4.2 kg NO_3-N/(m^3 day). A mass relation between COD consumed and NO_3-N removed by 2.82 was observed. This continuous flow pilot bioreactor proved an efficient denitrification system with a relatively low retention time.

Key words: Nitrate, denitrification, microbial cellulose, packed bed, bioreactor

INTRODUCTION

Discharge of industrial wastewater is the cause of significant deterioration of the environment largely because of the presence of nutrients such as nitrate. Nitrate released into environment can create serious problems, such as eutrophication, 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 (Yang *et al*., 2007). To address this problems, specific rules have been established globally. The European Community and Environmental Protection Agency (EPA), set the 5.6 mg (NO_3-N)/L and 10 mg (NO_3-N)/L, respectively (Aslan and Cakici, 2007). Biological denitrification is an attractive treatment option, for the nitrate 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 (Sozen and Orhon, 1999; Dong and Tollner, 2003). 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 (Borregaard, 1997). 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 (Manohar and Karegoudar, 1998). Among the various matrixes that are available, the MC had been chosen for its ease of use, low cost, low toxicity, high operational stability (Son *et al*., 2003). Wood chips can used as alternative biofilter media for denitrification of waste water with high nitrate concentrations

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(Saliling *et al*., 2007). Results from studies by indicate that wheat straw can be used as biofilter media and as a carbon source for the denitrification of drinking water (Soares and Abeliovich, 1998; Aslan and Turkman, 2003). Also wheat straw was used to denitrify turbid and nitrogen-rich irrigation water (Lowengart *et al*., 1993). Similarly it has have demonstrated that wood chips can be used to as a biofilter media to treat runoff and irrigation water (Blowes *et al*., 1994). Sawdust, leaf compost, unprocessed grain seeds and wood mulch have evaluated as reactive barriers to the flow of nitrate-laden waters (Robertson *et al*., 2000). Commercially available wood-based biofilter media was reported to remove nitrate-N from a pretreated residential septic tank effluent (Robertson *et al*., 2005). During continuous operation, the effectiveness and the stability of attacked growth reactors depends on the structure of the biofilm formed (Alves *et al*., 2002). The concentration of the substrates in the influent affects the growth and the composition of the biofilm. The growth and the activity of the bacteria increase if the carbon source is available in excess, but this leads to a higher cell accumulation as well. Destruction of the biofilm by the high velocity of the liquid phase (shear stress) is also frequent (Chang *et al*., 1999). All these effects can be avoided by the application of a suitable media for denitrifying immobilization. The MC 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 (Van Rijn *et al*., 2006; Foglar *et al*., 2005). Methanol, ethanol and acetic acid are generally used as additional or individual carbon sources in these processes because they are easily available and give a high denitrification rate. The usage of ethanol is common not only in experimental pilot plants (Fuchs *et al*., 1997), but also in full-scale technologies (Hallin *et al*., 1996). Here we report high nitrate removal from synthetic solution using a consortium of bacteria immobilized onto MC packed-attached growth biofilm reactor.

MATERIALS AND METHODS

Microbial Cellulose Production

In this study *Acetobacter xylinum* (ATCC 23768) was used. It was grown in SH medium at 28°C under static culture conditions. The SH medium was composed of 2% (W/V) glucose, 0.5% (W/V) yeast extract, 0.5% peptone, 0.27% (W/V) Na_2HPO_4 and 0.115% (W/V) citric acid. Preinoculum for all experiments was prepared by transferring a single *A. xylinum* colony grown on SH agar into a 50 mL 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.

The Denitrifier Bacteria and Inoculation of Bioreactor

The consortium microorganisms with high denitrification efficiency were isolated from effluent petrochemical industry. Denitrifier bacteria inoculated at MC. 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 $NO_3 + NO_2 - N$ concentration of 100-700 mg L^{-1}. During this study, reactor was fed from a common source of synthetic wastewater.

Bioreactor Operation

To increase the biological denitrification efficiency, packed-bed reactor was applied with MC beads. In the long-term operation test, the synthetic solution was fed following as; 100-700 mg L^{-1} of nitrate-N, 300-2100 mg L^{-1} of ethanol and the pH was adjusted to 7.2. The experimental set up used in investigation was MC packed bioreactor, a Plexiglas column has been used as reactor followed by a 5 L sedimentation tank. The ends of the 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 MC. The dimensions of the MC particles were approximately 5-10 mm on a side and 5-7 mm in thickness. The total empty volume of the packed section of the reactor V_T was 2.5 L again measured experimentally. The contaminated water prepared superficially (feed solution) 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 3.0. Mixing in the feed solution tank was provided by nitrogen gas distributor fixed on the bottom of the tank. An adjustable speed peristaltic pump was used for pumping the feed solution into the reactor. The specific surface area of the packed material was 603 m^2 m^{-3}. The MC beads functioning as the active volume of the reactor. 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. A peristaltic pump was used to load the nitrate and ethanol stock solutions in appropriate proportions into the main influent flow. The nonsterilized influent solution applied at the bottom of the reactor was supplemented. The wastewater influent was fed to the bottom of the reactor through 0.635 cm 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 nitrate, nitrite, COD and alkalinity concentrations of the samples were determined to study the spatial separation of the nitrate and nitrite reduction steps of the denitrification process. The temperature of synthetic wastewater was controlled to 30°C in the controller. Synthetic solution was continuously fed by pump to the packed-bed reactor bottom and nitrogen gas purged into the controller to form the anaerobic condition. The experiments were performed in total darkness for achieving the condition suitable for natural denitrification. Denitrification occurs mainly in the dark, because the metabolic activity of the aquatic macrophyta and induce in light high and in dark low oxygen concentration (Eriksson and Weisner, 1999). The influent syntactic solution was prepared to simulate typical high nitrate waste with ethanol and $NaNO_3$ as carbon source and nitrate source respectively.

Calculation of Denitrification Rate

The eliminated kg nitrate-N by the bioreactor was calculated as the following equation:

$$\text{Denitrification rate (g } NO_3 - N/L.d) = \frac{[NO_3 - N]_{input} - [NO_3 - N]_{output} \times R}{V}$$

where, R is the wastewater flow rate, $[NO_3 - N]_{input}$ and $[NO_3 - N]_{output}$ are the influent and effluent NO_3 concentrations (g NO_3–N L^{-1}), respectively and V is the reactor volume.

Analytical Methods

Samples were collected at the influent and effluent ports. Liquid samples were centrifuged at 5°C and 12,000 g. Thus, obtained supernatant was used for nitrate and nitrite analysis. Samples were analyzed for nitrate, nitrite, COD and alkalinity using Standard Methods (APHA, AWW, WPCF, 2005). The pH was measured routinely throughout the trials. The specific surface area of the MC was determined using the multiple BET method (Micromeritics, Gemini) with nitrogen gas as the adsorbate.

RESULTS AND DISCUSSION

Denitrification Activity

In this study, the input water to the reactors was deoxygenated. The effect of nitrate load was studied by changing the influent nitrate concentration step by step from about 100 to 700 g NO_3-N m^{-3} (Fig. 1). The nitrate levels in the effluent were high for the first 2 days and gradually decreased until day 3 when then nitrate reached levels below 20 g NO_3-N m^{-3}. This period corresponds to the biomass acclimatization period and the biofilm's growth around the packed material. From day 3, nitrate removal efficiencies stayed in values higher than 90% (Fig. 2), but from day 65 that influent

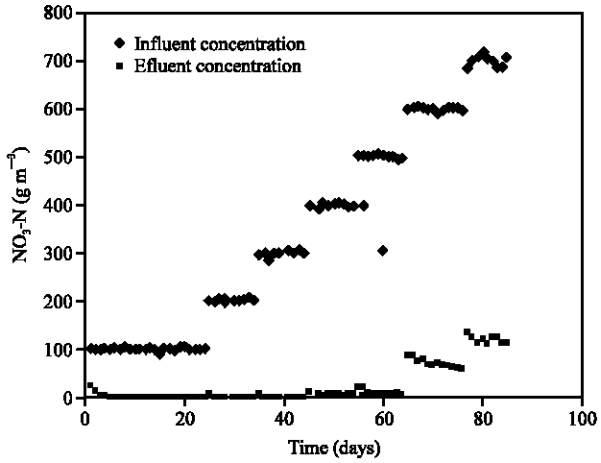

Fig. 1: Influent and effluent of NO_3-N concentrations at different loading rates

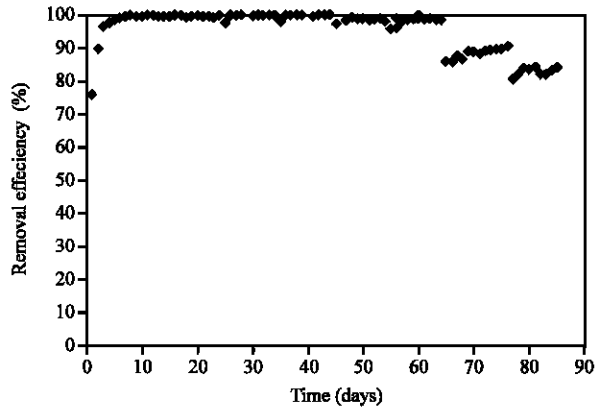

Fig. 2: Nitrate removal efficiencies at different nitrate loading rates

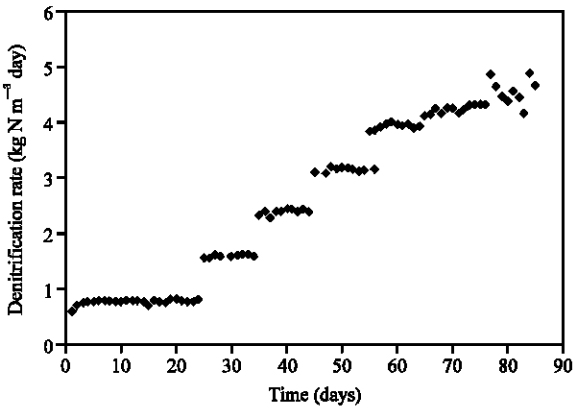

Fig. 3: Denitrification rates for the different NO_3-N loadings

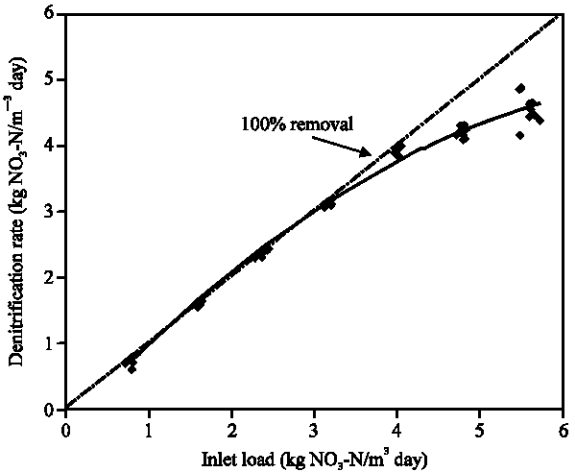

Fig. 4: Denitrification rate vs. NO_3-N load of the synthetic solution

nitrate concentration reach to 600 mg L^{-1} and above, removal efficiency decreased below 90% with deterioration in the quality effluent. Mohseni-Bandpi *et al*. (1999) reported that a nitrate removal efficiency of nearly 100% was achieved with HRTs of 9 h, using a bench-scale anoxic filter. With our bioreactor, the nitrate removal efficiency was 90-100 at ethanol-C:NO_3^--N ratios of 3:1, with HRTs of 3 h. In a low nitrite efflux was attained. Denitrification rates for the different NO_3-N loading values are shown in Fig. 3. The highest observed denitrification rate was 4.57 kg NO_3-N/(m^3 day) for a nitrate load of 5.64 kg NO_3-N/(m^3 day). These values are comparative to those previously reported for high load studies (Borregaard, 1997). They reported NO_3-N loadings for up-flow packed-bed postanoxic denitrification reactors are in the range from 3 to 3.98 kg NO_3-N/(m^3 day) to achieve effluent NO_3-N concentrations below 5.0 g m^{-3}. Denitrification rates were calculated for each sampling date (Fig. 3). Denitrification rates increased when loading rates increased for reactor, ranging from approximately 0.72 to 4.57 kg N/(m^3 day). Denitrification rates versus the nitrate load are presented in Fig. 4. 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_3-N/(m^3 day). The highest observed denitrification

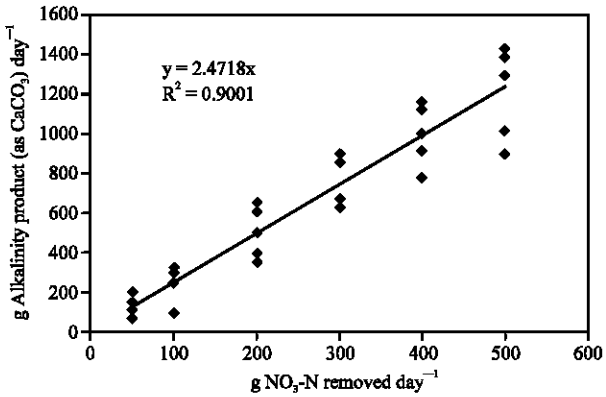

Fig. 5: Alkalinity gains of denitrification units supplemented with ethanol as carbon source

rate was 4.57 kg NO_3-N/(m^3 day) at a nitrate load of 5.64 kg NO_3-N/(m^3 day). Hirata *et al*. (2001) was reported a maximum nitrogen volumetric rate of 0.24 kg NO_3-N/(m^3 day) 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. Typical volumetric NO_3-N loadings for up-flow denitrification filters are in the range from 3.0 to 4.0 kg NO_3-N/(m^3 day) to achieve effluent concentrations below 5.0 g NO_3-N m^{-3}. As expected, the kg NO_3-N removed m^{-3} day is greatest at the most elevated system nitrate concentrations and decreases as system concentrations decrease (Fig. 3, 4). The reactor gave essentially the maximum daily denitrification rate of 4.57 kg nitrogen removed/m^3 media/day. All studies referenced in the above focused on waste water treatment with a variety of laboratory and pilot plant systems. This is the first paper to describe the use of MC as a media and carbon source for nitrogen removal in a bioreactor system. The denitrification rates from this study in the range of approximately 0.72 kg N/(m^3 day) to almost 4.57 kg N/(m^3 day) for the denitrification column was higher than of the range of volumetric denitrification rates of 0.036-4 kg N/(m^3 day) for various denitrifying reactors reported by van Rijn *et al*. (2006).

Nitrite Formation

For the nitrite accumulation, maximum 45 mg L^{-1} of nitrite-N was accumulated in the reactor with 1 h retention time and 700 mg L^{-1} initial nitrate concentration. However, accumulated nitrite was decreased with increase of hydraulic retention time and decrease of nitrate loading rate.

Alkalinity and pH

There was a significant correlation with alkalinity gain and NO_3-N reduced for bioreactor that shown in Fig. 5. Alkalinity in the effluent increased with increasing nitrate loading rates. In all cases, the amount of alkalinity produced was related to amount of NO_3-N removed. Alkalinity production averaged more than 2.5 mg $CaCO_3$ mg^{-1} NO_3 + NO_2-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 (USEPA, 1993). 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. 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.

Table 1: Average influent COD, COD removal and COD removal per nitrate-nitrogen reduced in each loading rate

Influent COD concentrations (mg L^{-1})	COD removed (mg L^{-1})	Residual COD (mg L^{-1})	COD removed/per NO_3-N reduced
300	281±8.35	19±2.50	2.84±0.20
600	560±21.8	40±3.96	2.84±0.16
900	846±19.78	54±5.20	2.83±0.12
1200	1128±35.47	72±9.90	2.81±0.13
1500	1410±33.41	90±4.96	2.79±0.16
1800	1692±38.1	108±4.60	2.79±0.23
2100	1974±31.9	126±9.50	2.78±0.17

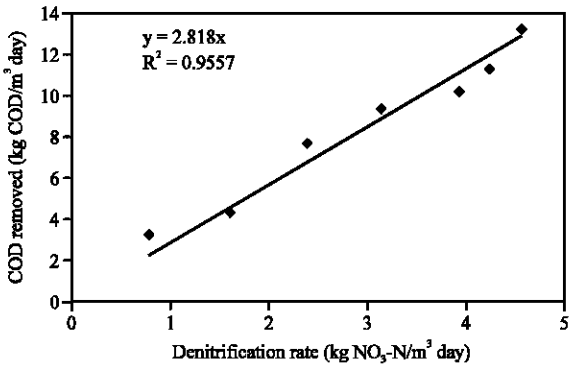

Fig. 6: Denitrification rate versus COD removal in the rector

COD Availability

COD measurements are used to quantify the mass of potential carbon available to fuel the denitrification process. The COD in the outflow of reactor was measured and showed that reactor contained significantly elevated COD concentrations. denitrification rate versus COD removal for rector showed at Fig. 6 and Table 1. 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^{-3}, only for higher flow rates, with removal efficiencies lower than 90%, effluent COD concentrations reached values greater than 150 g m^{-3}, so the addition of ethanol should be adjusted in relation to the denitrification rate. COD removal can be correlated with nitrate reduction to determine the appropriate amount of carbon (COD) needed to fuel denitrification. A deficiency of carbon can result in low nitrate removal and high nitrite production. USEPA (1993) estimated that a COD/NO_3-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 MC 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. The MC in this study may have also leached some carbonaceous COD, but it was likely minor compared to the ethanol contribution.

Effluent Quality

Mean effluent concentrations of ammonia-nitrogen (mg L^{-1}), nitrate-nitrogen (mg L^{-1}) and Alkalinity production/nitrate-nitrogen reduction (mg as $CaCO_3$ mg^{-1} N) are 0.04±0.02, 5.36±0.60 and 2.5, respectively.

CONCLUSION

Denitrification performance of attached growth biofilm on MC in a packed bed reactor system has been investigated as function of nitrate concentration and flow rate. The denitrification reactor design used in this study was effective at significantly reducing nitrate concentrations within a relatively short time frame. 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_3^- content of the influent had already been reduced. The reduction of the NO_3^- was followed by the accumulation of low NO_2^-. The maximum NO_2^--N concentration at reactor was about 45 mg L^{-1} 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^{-1} of feed solution nitrate-N content, the present system is able to produce a effluent with a nitrate content below allowed limits. The study showed that MC was suitable supporting bacterial growth to provide biological denitrification. MC can be used as biofilter media.

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