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Salt Inhibition Effects on Simultaneous Heterotrophic/ Autotrophic Denitrification of High Nitrate Wastewater

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ABSTRACT: Denitrification of high- nitrate high- salinity wastewater is difficult due to plasmolysis and inactivation of denitrifiers at high salinity conditions. In this study, the effects of salinity and empty bed contact time (EBCT) on simultaneous heterotrophic and sulfur based autotrophic denitrification of synthetic wastewater were evaluated in an up flow packed bed reactor .The reactor was filled with granular elemental sulfur particles with diameters of 2.8-5.6 mm and porosity of 40%. The initial culture was prepared from sludge of Shahrak-e- ghods domestic wastewater treatment plant. The influent nitrate concentration and EBCT were 600 mg NO3-N/lit and 16 h respectively. First, the stoichiometric fraction of nitrate removed by heterotrophic denitrification (with methanol as organic carbon source) supplied enough alkalinity to compensate the autotrophic alkalinity consumption, was determined 60%. Then, salt concentration was gradually increased with NaCl from 0% in the feed. The Process kept high nitrate removal efficiency (>99%) even at 3.5 % NaCl. During these changes the alkalinity variations were insignificant which showed the microbial population ratio of acclimated autotrophic to heterotrophic denitrifiers had no any significant changes with NaCl concentrations up to 3.5% in the feed. At 4 and 5% NaCl, the efficiency drastically decreased to 78% and 48%, respectively. Similar behavior was also observed for methanol removal efficiency, effluent turbidity as an indirect determinant of biological mass and sulfate production. The effects of flow rates on denitrification of synthetic high nitrate high salinity wastewater with 3.5 %NaCl under mixotrophic condition were also investigated by increasing the flow rate from 7.06 lit/day to 70.6 lit/day with corresponding EBCT 20 to 2 h. Denitrification efficiency was close to 100% at EBCT of 20 to 8 hr, but decreased to 79% and 39% when the EBCT was 4 and 2 h, respectively. The decrease in effluent sulfate concentration (as an indicator for autotrophic denitrification) and the increase in effluent alkalinity (as an indicator for heterotrophic denitrification) and pH at EBCT of 4 and 2 h were considerable correspondingly. These results imply that the population ratio of autotrophic to heterotrophic denitrifiers depends on EBCT.

Key words: Autotrophic, Heterotrophic, Denitrification, Saline wastewater, Nitrate, Sulfur, Packed bed reactor

INTRODUCTION

Increasing nitrate concentration of surface and groundwater resources in different countries is considered a common environmental problem threatening the water quality all around the world (Rabalis, 2002 ; Smith 2003).Various kinds of wastewaters have received ample attention to conserve environment (Mehrdadi *et al*., 2007; Yoochatchaval *et al.*, 2008). In Iran, due to Increasing agricultural and industrial activities and consequently excessive land discharge of treated and untreated wastewaters,nitrate concentrations in groundwater resources in many of urban and rural areas like Tehran, Mashhad and Isfahan have exceeded the acceptable limits (Torabian *et al.*, 2002). Causing eutrophication, nitrate has adverse effects on water ecosystems.

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Furthermore, high concentration of this contaminant in drinking water may result in health risks like methemoglobinemia in infants and children (Wolfe & Patz, 2002), childhood diabetes (Parslow *et al.*,1997) and formation of carcinogenic compounds like nitrosamines (Nash, 1993).

Among different physical, chemical and biological nitrate removal processes, biological denitrification is considered as an efficient method (Bathlor & Lawrence, 1978; Flere & Zhang, 1999). A wide range of heterotrophic and autotrophic bacteria may use nitrate as a terminal electron acceptor. Hetrotrophs use organic matters while autotrophs use inorganic compounds like molecular hydrogen, sulfur, sulfide, thiosulfate and Fe^{++} as electron donors. However, both groups may encounter some deficiencies in nitrate removal. Heterotrophic denitrification may have the problems like need for excess organic matter in the case of wastewaters containing low C/N ratio , need for additional treatment for remained organic matter removal, excess sludge generation and relevant costs (Zhang & Lamp, 1999) and nitrite accumulation (Francic & Mankin, 1977), while autotrophic denitrification may encounter problems like alkalinity consumption in high-nitrate low – alkalinity wastewater, high sulfate production and low rate nitrate removal.

During recent years, simultaneous heterotrophic/autotrophic denitrification has been studied increasingly for nitrate removal from high nitrate wastewater with low organic matter due to its high treatment performance and cost-effectiveness. Gommers et al. (1988) showed that use of nitrate as an electron acceptor may result in simultaneous sulfide and acetate removal from synthetic wastewater. Sulfide, acetate and nitrate were efficiently removed in a fludized bed reactor.Yamamoto et al. (2000) proved symbiosis of heterotrophic and autotrophic as well as sulfate reducing bacteria in fixed bed reactors. The effect of organics on sulfur- utilizing autotrophic denitrification was investigated under batch and continuous conditions. The results showed that controlled increase of organic matter not only has no inhibitive effects on the process, but also may be considered useful regarding reduction in sulfate production and alkalinity consumption, due to the existence of heterotrophs (Oh *et al.*, 2002, Kim *et al.*, 2002).An heterotrophic / autotrophic denitrification approach supported by cotton (as insoluble carbon source) and zero valent iron (for reducing dissolved oxygen and supply of required H^2) was studied and the results showed that the hybrid process is more efficient in comparison with heterotrophic ones (Rocca *et al.*, 2006). Kim et al. (2004) showed that sequential heterotrophic /autotrophic denitrification has more advantages than simultaneous heterotrophic autotrophic denitrification.

Denitrification of saline wastewater like spent brine from regeneration of exhausted nitrate removal ion exchange resins, wastewater generated in industries like metal finishing, explosives, fertilizers, nuclear etc., may encounter some difficulties due to adverse effects of salt on microbial flora. High salt concentrations (>1% salt) cause plasmolysis and /or loss of activity of cells (kargi and Dincer, 1998). Until recently there have been few studies on denitrification of saline wastewaters and microbial analyses (Van der Hoek *et al.*, 1987, Clifford & Liu, 1993, Byung-Uk Bae *et al.*, 2002, Glass & Silversein, 1998, Gu *et al.*, 2004, Yoshie *et al.*, 2001, 2006).

Considering the advantages of simultaneous heterotrophic/autotrophic denitrification of wastewaters containing high nitrate with low organic matter and alkalinity, it was felt worthwhile to study the effects of salinity on this process. The objectives of this study were, therefore, to examine the inhibitive effects of salt concentrations on nitrate removal, sulfate production, alkalinity and COD consumption.

MATERIALS & METHODS

A two-wall cylindrical column reactor was constructed of Plexiglas with an inner diameter of 10 cm, height of 1 m and a volume of 7.85 lit. (Fig.1).The sulfur particles used were circular in shape and had diameters ranging from 2.8 to 5.6 mm and a porosity of 40%. Sampling ports were installed every 25 cm along the column. The flow of water with constant temperature through the two-wall cylindrical column assured the temperature fixation of reactor contents.A schematic diagram of the units is shown in Fig. 1. The reactor was fed continuously in the up-flow mode using adjustable peristaltic pump. All experiments were conducted at 25±2° C.

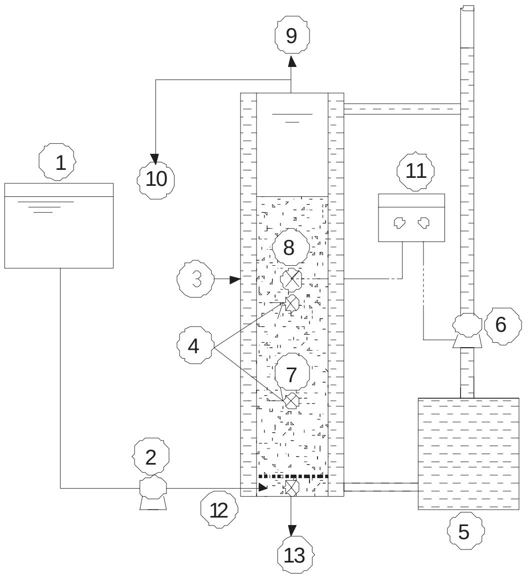

Fig. 1. Continuous-flow, laboratory-scale packed-bed reactor system

1- feed tank 2-feed pump 3-reactor column 4-sampling ports 5-water tank 6-water pump 7-reactor bed 8-temperature meter 9-gas exit 10-wastewater outlet 11-temperature controller 12-feed inlet 13-sludge disposal

To obtain a suitable culture of sulfur-utilizing denitrifiers for inoculating the sulfur-packed bed columns, anaerobic sludge taken from Shahrak-e-ghods municipal wastewater treatment plant was acclimated by synthetic feed in a master culture reactor. The reactor had a liquid volume of 1.5 liter and a sulfur particle volume of 750 ml. To enable the denitrifying bacteria to accumulate and form a biofilm on the sulfur particles, 600 ml of the solution of the culture reactor was wasted and replaced with 600 ml fresh feed once every 3 days. The feed to the culture reactor contained 2 g/L KNO_3, 2 g/L K_2HPO_4, 5g/L $Na_2S_2O_3$, 1 g/L $NaHCO_3$, 0.5 g/L NH_4Cl, 0.5 g/L MgCl, 6 H_2O and 0.01 $FeSO_4$,7 H_2O (Kim *et al.*, 2002).

After sufficient bacterial growth, the sulfur-packed column was inoculated with the one liter reactor culture and aerobic return sludge from the municipal sewage treatment plant. The rest of the reactor volume was filled with the feed. After 24 hours the sulfur-packed column was operated continuously at an empty bed contact time (EBCT) 24 hours. In order to introduce sulfur as the only electron donor, sodium thiosulfate concentration in the feed was gradually decreased 5% and 10% once every two days during the first and the second two weeks, respectively. After one month, suitable development of microorganisms on the solid media was achieved. Then the concentration of nitrate in the influent synthetic wastewater (with the same components of culture reactor feed without thiosulfate) increased gradually to a final value of 600±20 mg/L as NO_3^- –N. The influent alkalinity was finally 870±30 mg/L as CaCO3, which was far less than the alkalinity requirement for sulfur-based autotrophic denitrification. In the next step methanol was gradually added to the feed. In methanol concentration of 741 mg/L (equivalent to 60% of its stoichiometric requirement in heterotrophic denitrification process without considering bacterial growth) the autotrophs required alkalinity was supplied by heterotrophs and there was no need for additional alkalinity.

In order to monitor the inhibitive effect of salt on performance of the reactor in the above mixotrophic condition, NaCl with different concentrations (0.0-5%) was added to the feed. During these changes, the EBCT was fixed at 16 h. Furthermore, the nitrate removal efficiency was monitored in constant salt (3.5%) and influent nitrate concentrations at different EBCTs. During the experiments, influent and effluent samples were collected through the sampling ports and were kept at 4 °C before being tested.For each sample, NO_3^- –N, NO_2^- –N, and SO_4^{-2} after filtration through a 0.45-µm membrane filter were determined by ion chromatography (761, Metrohm). pH values were monitored using a standard digital pH meter (691 Metrohm), and COD was measured using a spectrophotometer (HACH DR2000). Influent and effluent alkalinity and turbidity were measured according to standard methods (APHA, 1992).

RESULTS & DISCUSSION

Fig. 2, 3 and 4 show the profile of nitrate concentration, sulfate production and COD versus reactor height for different concentrations of NaCl respectively. In all cases the rates of nitrate and COD removal and sulfate production were high in the first sampling port (below25 cm) followed by relatively low rates in the upper height parts of reactor column(50 and 75 cm). Excess cell growth in lower part of the reactor due to high concentration of nitrate and methanol in the feed was predictable. The range of turbidity values (as

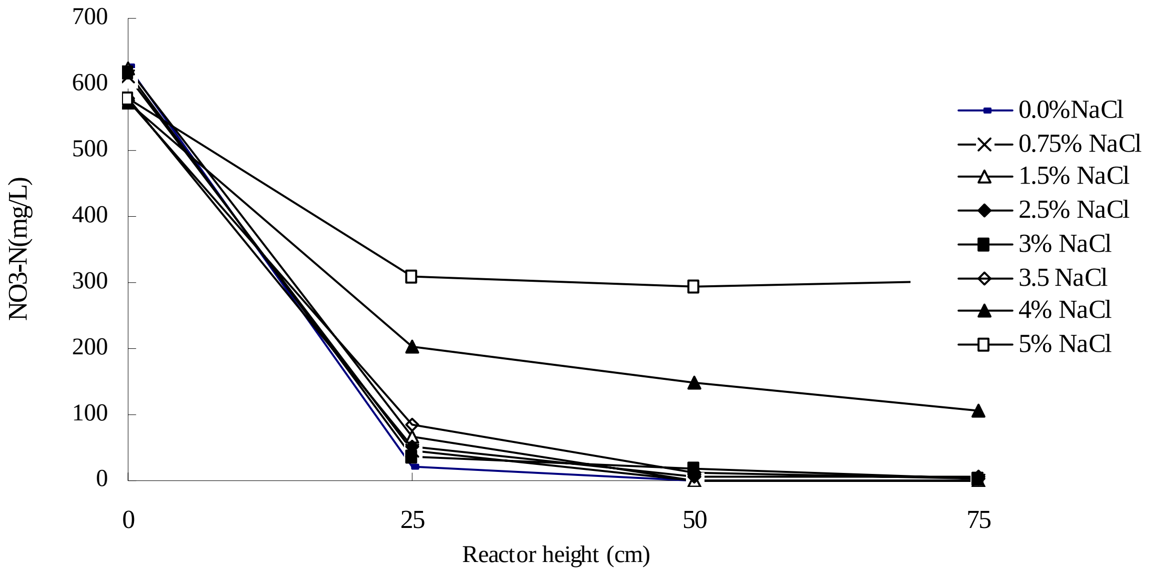

Fig. 2. NO3- N concentration profile along the height of the reactor at different concentrations of influent NaCl

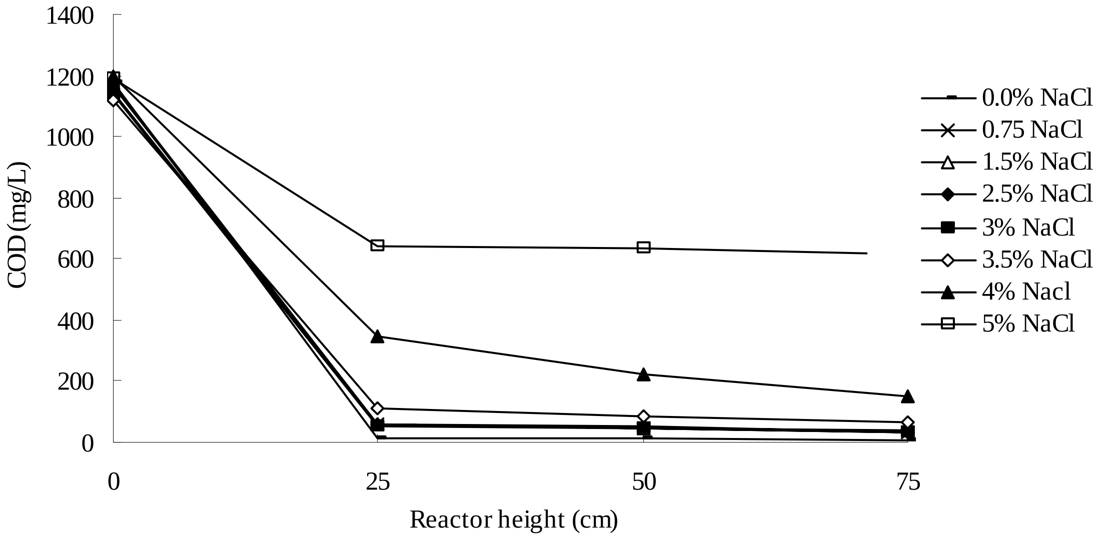

Fig. 3. COD concentration profile along the height of the reactor at different concentrations of influent NaCl

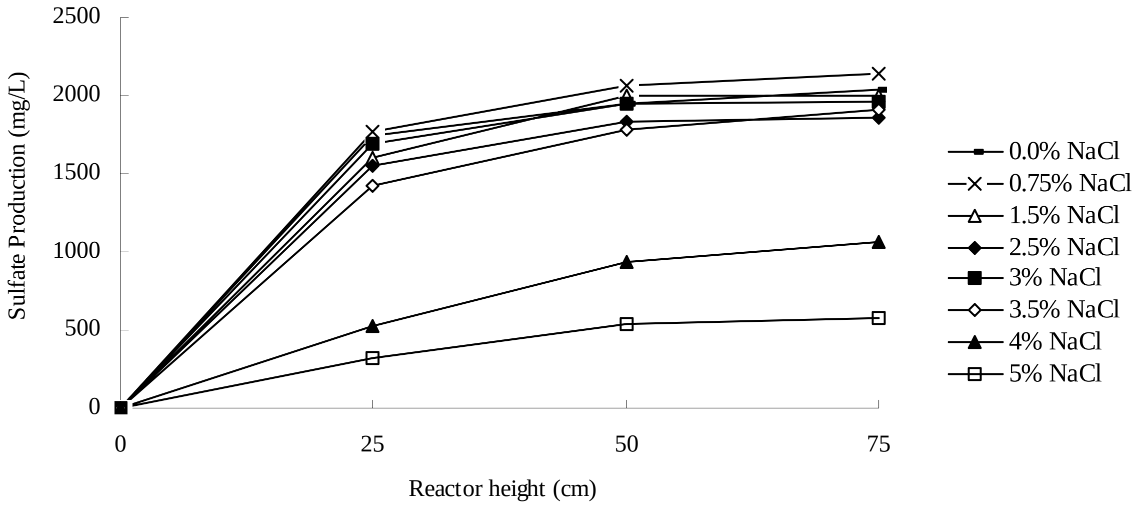

Fig. 4. Sulfate production profile along the height of the reactor at different concentrations of influent NaCl

indirect determinant of cell mass) in the first, second and the effluent sampling ports for different conditions were (16.8-54.7 NTU), (14.4-47.1NTU) and (8-38.1 NTU) respectively. As shown in Fig.2 and 3,by gradually increasing the NaCl concentration, the efficiency of nitrate and COD removal kept high even at 3.5% NaCl (>99 and 94% respectively) as well as sulfate production, with 1.4 kg NO_3-N/(m^3-day) maximum volumetric loading rate and EBCT 10.7 h (based on 50cm from the bottom of the reactor.During the experiments alkalinity variations were insignificant. Hence the microbial population ratio of autotrophic to heterotrophic denitrifiers appeared to be unaffected by salt concentration up to 3.5%. At 4 and 5% NaCl the efficiency of nitrate removal drastically decreased to 78 and 48% corresponding to COD removal efficiency (87 and 49%) and sulfate production (Fig. 4). Also effluent turbidity decreased significantly (lower limits of mentioned turbidity values).

Van der Hoek et al. (1987) found that 2.5% NaCl only slightly inhibited heterotrophic denitrification Kristensen and Jepsen (1991) studied heterotrophic denitrification in the presence of chloride ion, finding successful denitrification at 3% NaCl. Osaka et al. (2004) demonstrated that the type of organic carbon source had a strong effect on the activity of nitrate removal and bacterial populations in a saline environment. They showed methanol –fed process resulted in the drastic reduction of nitrate removal at 4% NaCl while acetate-fed process kept a high performance of nitrate removal even at 10%. Removal of high nitrate concentration in saline wastewater with 3.3% NaCl by autotrophic denitrification was investigated in a sulfur-limestone column reactor (Gu *et al*., 2004). More than 97.5% nitrate was removed after 14.3 h in the reactor. According to these studies the inhibitive concentration of NaCl for drastic reduction of nitrate removal by heterotrophs or autotrophs was about 4% which was nearly the same as our study in the mixotrophic conditions.The effects of flow rates on denitrification of synthetic high salinity wastewater with 3.5 %NaCl under mixotrophic conditions was investigated by increasing the flow rate from 7.06 lit/day to 70.6 lit/day with corresponding EBCT 20 to 2 h. Fig. 5. shows the variations of effluent nitrate, COD and sulfate production during the operation. Denitrification efficiency was close to 100% at EBCT of 20, 16, 12 and 8 h but decreased to 79 and 39% when the EBCT was 4 and 2 h respectively. A maximum volumetric loading rate 1.90 kg NO_3—N/(m3-day) achieved the maximum denitrification efficiency (>99%) at EBCT 8 h . On the other hand more than 97% nitrate removal efficiency could be achieved at EBCT 5.34 h and maximum volumetric loading rate 2.86 Kg NO_3 N/(m3-day), based on 50 cm from the bottom of the reactor.Few researches have evaluated the effects of hydraulic conditions for simultaneous heterotrophic and sulfur based autotrophic denitrification. Lee et al. (2001) determined EBCT and volumetric loading rate as 6.76 h and 2.84 Kg NO_3 N/(m3-day) at a flow rate 2 lit/day, considering the required volume for 100% nitrate

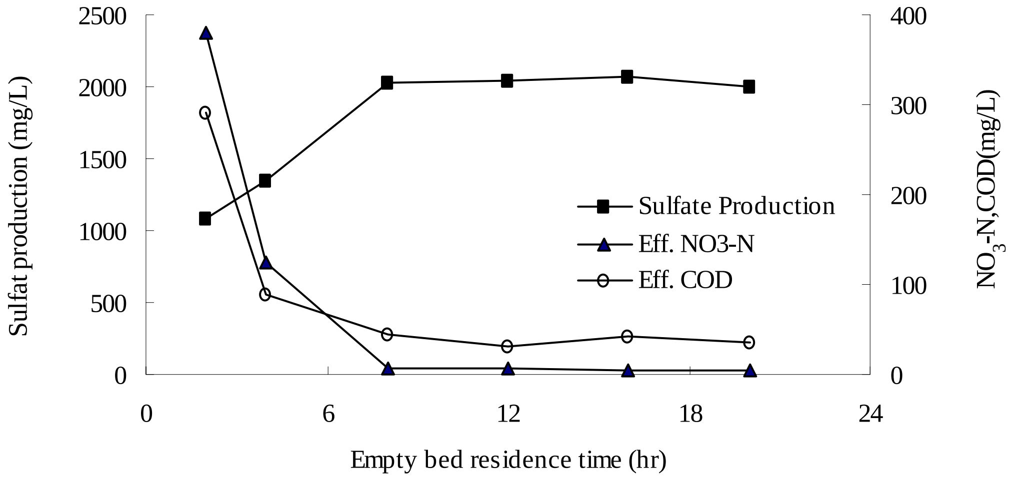

Fig. 5. Variations of effluent NO_3-N, COD and sulfate production at different EBCTs

removal efficiency (based on first sampling port). According to Kim et al.(2002),100% denitrification efficiency could be achieved at EBCT 14 h and loading rate 1.2 Kg NO_3 N/(m^3/day). Maximum volumetric nitrate loading rate achieved maximal efficiency (>97%) was determined 1.4Kg NO_3 N/(m^3/day) and 1.92 Kg NO_3 N/(m^3/day) at EBCT 7.5 h, under mixotrophic condition, with a lower than stoichiometric and sufficient methanol concentrations for heterotrophic denitrification (Oh *et al.*, 2001). Although there is a significant similarity between our results and Lee's report implies the independent performance of mixotrophic denitrification process under high salinity conditions, the difference between the all above the results may be acceptable due to composition of feed, microbial population, size and shape the sulfur particles, temperature, etc. The drastic decrease in nitrate removal efficiency at EBCTs 4 and 2 h might have been caused by relatively low contact time been denitrifiers and nitrate and slow dissolution of sulfur as a limiting factor (Koenig and Liu, 2001).

According to Fig. 3. the effluent COD was less than 45 mg/L at EBCT 20 to 8 h .When the EBCT decreased to 4 and 2 h , the effluent COD was 88 and 292 mg/L,respectively with corresponding removal efficiency 92.6 and 75.6%. The increase in effluent COD indicated that heterotrophic denitrification with added methanol was not completed in the reactor. Sulfate production decreased from 2000 mg/L at EBCT 20 h to 1341 and 1080 mg/L at EBCT 4 and 2 h respectively. Also, drastic reduction of sulfate production at EBCT 4 (critical point) and 2 h showed undesirable performance of autotrophic denitrification in the reactor.

Fig. 6. shows the variations of effluent pH and alkalinity versus EBCT. As flow rate increased (EBCT decreased) from 7.06 to 70.6 lit/day, the effluent pH and alkalinity increased from 6.9 to 8.1 and 831 to 1388 mg/L, respectively. The increase in effluent pH and alkalinity at EBCT 4 and 2 h was considerable and corresponded to the decrease of sulfate production. In fact the alkalinity produced by heterotrophic denitrifiers was not completely utilized by autotrophic denitrifiers. Hence the microbial population ratio of autotrophic to heterotrophic denitrifiers may be affected by EBCT. This behavior can be explained by half order kinetic constants. Koenig and Liu (2001) reported that the half order kinetic constants of sulfur utilizing autotrophic denitrification are approximately one order of magnitude lower than those for heterotrophic denitrification and it has a slower growth rate compared to heterotrophic denitrification and hence with slower rate of biomass build up for the same nitrate volumetric loading rate. During the operations, in spite of high influent nitrate, insignificant concentration of nitrite (as an

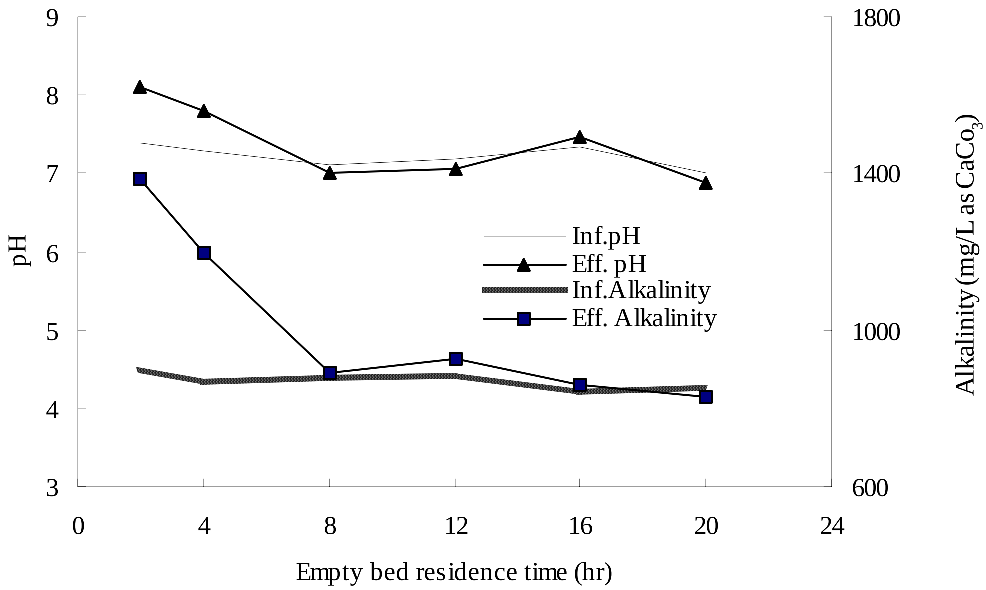

Fig. 6. Variations of pH and alkalinity at different EBCTs

intermediate product) was recorded along the media bed. The maximum concentration of effluent nitrite was 1.1 mg/Lit as NO_2-N. Thus accumulation of nitrite in the reactor had no adverse effect on performance of the reactor.

CONCLUSION

This study proved the feasibility of nitrate removal from high-nitrate high- saline synthetic wastewater using simultaneous autotrophic and heterotrophic denitrification in an up-flow sulfur packed bed reactor. Methanol was added as carbon source for heterotrophic denitrifiers. The influent nitrate concentration and empty bed contact time were 600 mg NO3-N/lit and 16 h, respectively. Salt inhibition effects on the performance of the reactor were investigated by gradual increasing NaCl to the feed. High nitrate and COD removal efficiency (99.2% and 94% respectively) were achieved up to 3.5% NaCl. It was concluded that gradual acclimation of biofilm to high nitrate and salt concentrations in a step-wise manner developed a consortium capable of treating high nitrate wastewater in a saline environment. Since the variations of alkalinity and pH during the operation were insignificant, it was concluded that the population ratio of acclimated autotrophic to heterotrophic denitrifiers may not be affected by salt concentration up to 3.5%. At higher concentrations of salt (4% and 5% NaCl) percent nitrate and COD removal efficiencies and sulfate production decreased drastically because of plasmolysis and loss of activities of both denitrifying bacterial groups.

Based on the results of this study a volumetric loading rate between 1.9 kg NO_3–N/(m3-day) with corresponding EBCT 8 h would achieve the maximum denitrification efficiency (>99%) at 3.5% NaCl in the feed . Nitrate removal efficiency was lower for the higher wastewater flow rate (EBCT 4 and 2 h). The decrease in effluent sulfate concentration (as an indicator for sulfur based autotrophic denitrification) and the increase in effluent alkalinity (as an indicator for heterotrophic denitrification) and pH at EBCT of 4 and 2 h was significant correspondingly. These results showed that the mixotrophic denitrification performance of saline wastewater in the sulfur packed bed reactor may be affected by EBCT. Overall, the simultaneous sulfur based autotrophic and heterotrophic denitrification was an effective and reliable process that could have application to nitrate removal from high –nitrate high-saline wastewater.

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REFERENCES

APHA, AWWA and WEF , (1992). Standard Methods for the Examination of Water and Wastewater.18th ed.,American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D. C.

Baea, B. U., Jung, Y. H., Han, W. W. and Shin, H. S. (2002). Improved brine recycling during nitrate removal using ion exchange. Water Res., **36**, 3330-3340.

Batchelor, B. and Lawrence, A. W. (1978). Autotrophic denitrification using elemental sulfur. J. WPCF, **50**, 1986-2001.

Clifford, D.A. and Liu, X. (1993). Biological denitrification of spent regenerant brine using a sequencing batch reactor. Water Res., **27 (9)**, 1477-1484.

Francis, C. and Mankin, J. (1977). High nitrate denitrification in continuous flow stirred reactors. Water Res., **11**, 289-294

Flere, J. M. and Zhang, T. C. (1999). Nitrate removal with sulfur-limestone autotrophic denitrification processes. J. Envir. Eng., **8 (125)**, 721-729.

Glass, CH. and Silverstein, J., (1999). Denitrification of high nitrate, high salinity wastewater. Water Res., **33 (1),** 223-229.

Gommers, Peter J. F., Buleveld, W. and Kuenen, J. Gus. (1988). Simultaneous sulfide and acetate oxidation in a denitrifying fluidized bed reactor –I. Start-up and reactor performance. Water Res., **22 (9)**, 1075-1083.

Gu, J. D., Qiu, W., Koenig, A. and Fan, Y. (2004). Removal of high NO3–concentrations in saline water through autotrophic denitrification by the bacterium Thiobacillus denitrificans strain MP. Water Sci. Technol., **49 (5-6)**, 105-112.

Kargi, F. and Dincer, A. R. (1998). Saline wastewater treatment by halophile supplemented activated sludge culture in an aerated rotating biodisc contactor. Enzyme and Microbial Technology, **22**, 427-433.

Koenig, A. and Liu, L. H. (2001).Kinetic model of Autotrophic Denitrification in sulfur packed bed reactors. Water Res., **35 (8)**, 1969-1978.

Kim, S., Jung, H., Kim, K. S. and Kim, I. S. (2004) .Treatment of High Nitrate-Containing Wastewaters by Sequential Heterotrophic and Autotrophic Denitrification. J. Envir. Eng., **130 (12)**, 1475-1480.

Kim, I. S., Oh, S. E., Bum, M. S., Lee, J. L. and Lee, S. T. (2002). Monitoring the denitrification of wastewater containing high concentrations of nitrate with methanol in a Sulfur packed reactor. Appl. Microbiol. Biotechnol., **59**, 91-96.

Kristensen, H. and Jepsen, S., (1991). Biological denitrifcation of wastewater from wet lime-gypsum flue gas desulphurization plants. Water Sci. Technol., **23 (4-6)**, 691-700.

Mehrdadi, N., Joshi, S. G., Nasrabadi, T. and Hoveidi, H. (2007). Application of solar energy drying of sludge from pharmaceutical industrial wastewater and probable reuse. Int. J. Environ. Res., **1(1),** 42-48.

Nash, L. (1993). Water quality and health; A guide to the worlds fresh water resources. Oxford University Press, New York, 25-39.

Oh, S. E., Bum, X. B., Zubair, A. and Kim, I. S. (2002). Nitrate removal by simultaneous sulfur utilizing autotrophic and heterotrophic denitrification under different organics and alkalinity conditions; batch experiments. Water Sci. Technol., **47 (1)**, 237-244.

Oh, S.E, Yoo, Y.B., Young, J.C. and Kim, I.S., (2001). Effect of organics on sulfur utilizing autotrophic denitrification under mixotrophic conditions. Journal of Biotechnology, **92,** 1–8.

Osaka, T., Shirotani, K., Yoshie, S. and Tsuneda, S. (2008). Effects of carbon source on denitrification efficiency and microbial community structure in a saline wastewater treatment process. Water Res., **42**, 3709-3 7 18.

Parslow. R. C, Mckinne, P. A, Law, G. R., Staines, A., William S. G. and Bodansky, H. J. (1997). Incidence of childhood diabetes mellitus in youk shire, northern England, is associated with nitrate in drinking water, an ecological analysis. Diabelologia, **40 (5)**, 550-560.

Rabalais, N., (2002).Nitrogen in aquatic ecocystems. Ambio. **31**, 102-112.

Rocca, C. D., Belgiorno, V. and Meric, S. (2006). An heterotrophic/autotrophic denitrification (HAD) approach for nitrate removal from drinking water. Process Biochemistry, **41**, 1022-1028.

Smith, V. H., Tilman, G., and Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut., **100**,179-196.

Torabian, A., Aminzadeh, B. and Hashemi, S. H. (2002). A Study of nitrate in Tehran ground water and a method of removal. Int. J. Environ. Stud., **57**, 725-734.

Van der Hoek J. P., Paul, J. M. and Abraham K. (1987). Combined ion -exchange/biological denitrification for nitrate removal from ground water under different process conditions. Water Res., **22 (6)**, 679-84.

Wolfe, H. and Patz, J. A. (2002). Reactive nitrogen and human health: acute and long-term implication. Ambio., **31**, 120-125.

Yamamoto Ikemoto, R., Komori, T., Nomura, M., Ide, Y. and Matsukami, T. (2000). Nitrogen removal from hydroponic culture wastewater by autotrophic denitrification using thiosulfate. Water Sci. Technol., **42 (3)**, 369-376.

Yoshie, S., Makino, H., .Hirosawa H., Shirotani, K., Tsuneda S. and Hirata, A. (2006). Molecular analysis of halophilic bacterial community for high-rate denitrification of saline industrial wastewater. Appl. Microbiol. Biotechnol., **72**, 182-189.

Yoshie, S., Noda, N., Miyano, T., Tesuneda, S., Hirata, A. and Inamori, Y. (2001). Microbial community analysis in the denitrification process of saline-wastewater by denaturing gradient gel electrophoresis of PCR-Amplified 16S rDNA and the cultivation method. J. of Bioscience and Bioengineering., **92 (4)**, 346-353.

Yoochatchaval, W.; Ohashi, A; Harada, H.; Yamaguchi, T. & Syutsubo, K. (2008). Characteristics of granular sludge in an EGSB reactor for treating low strength wastewater. Int. J. Environ. Res., **2(4),** 319-328.

Zhang, T. C. and Lamp, D. G. (1999). Sulfur-Limestone autotrophic denitrification process for treatment of nitrate-contaminated water: batch experiments. Water Res., **33**, 599-660.