International Journal of Current Engineering and Technology
E-ISSN 2277 – 4106, P-ISSN 2347 - 5161

Available at http://inpressco.com/category/ijcet

Research Article

Biological Denitrification with Immobilized *Pseudomonas Syringae* on Granular Activated Carbon using three Phase Draft Tube Spouted Bed Reactor

Keshava Joshi[A*] Reshma Rajan[B], G. Srinikethan[B] and M. B. Saidutta[B]

[A]Department of Chemical Engineering, S. D. M. College of Engineering and Technology, Dharwad, India.
[B]Department of Chemical Engineering, National Institute of Technology Karnataka, Surathkal, India

Accepted 05 Sept 2014, Available online 01 Oct 2014, **Vol.4, No.5 (Oct 2014)**

Abstract

Nitrate is a common pollutant present in wastewater from many industries, sewage and leachate from municipal waste and this has become a major concern in recent past because of its effect on life forms and also to the environment. Biological nitrification and denitrification is one of the most versatile processes of nitrate removal from wastewaters. Experiments were conducted to evaluate the performance of draft tube spouted bed reactor for denitrification process using Pseudomonas syringae, which was isolated from secondary clarifier of a fertilizer waste water treatment plant and immobilized on granular activated carbon (GAC). The effect of dilution rates on denitrification was investigated and it was found that there is decrease in percentage nitrate removal with increase in influent nitrate concentration as well as dilution rate. For the range of dilution rates studied, the reaction attained a steady state between 30 to 45h. The results indicate that, nitrate and carbon simultaneously can be removed effectively in draft tube spouted bed reactor and therefore, it may serve as a promising reactor in COD and nitrogen removal fields.

Keywords*: Pseudomonas syringae, Draft tube spouted bed reactor, Granular activated carbon Dilution rate and Nitrate removal.*

1. Introduction

Nitrogen in various forms is a major pollutant from many industries like fertilizers production, explosive manufacture, coking, refining, food processing, organic chemical manufacturing, alcohol production, starch industry, metallurgy and petrochemicals. In many parts of the world where ground water is being used as a main source of drinking water, nitrate concentration in groundwater has steadily been increasing over the years mainly due to the extensive use of chemical fertilizers in intensive agriculture as well as discharge of municipal and industrial wastes (Wang *et al*, 2009). This excess nitrate in drinking water causes adverse effects including depletion of dissolved oxygen (DO) in receiving waters, eutrophication, ammonia toxicity to aquatic life, and public health problems like "methemoglobinemia" cancer, birth defects, abortions, hypertension thyroid hypertrophyin infants (USEPA, 1993; Burghate and Ingole, 2013; Srinu *et al*, 2011). Conventional physico-chemical methods to remove nitrate from water are ion exchange, reverse osmosis and electro-dialysis. These processes are expensive and the concentrated waste brines require further treatment or their disposal is a problem (Sarina and David, 2004; Wang *et al*, 2009)

Biological nitrogen removal involves two successive processes, i.e. nitrification and denitrification. Biological nitrification and denitrification is one of the most economical processes of nitrogen removal from wastewaters (Han-Woong *et al*, 2002; Kyun *et al*, 2005; Youyuan *et al*, 2011). Denitrification is just one pathway in the nitrogen cycle. It is a biochemical reaction carried out by microorganisms, which transforms nitrates to nitrites and to the gaseous form nitrogen. This reaction couples the transport of electrons by the respiratory chain to energy production via oxidative phosphorylation.

$$2NO_3^{-(+5)} \geq 2NO_2^{-(+3)} \geq 2NO_{(g)}^{(+2)} \geq N_2O_{(g)}^{(+1)} \geq N_{2(g)}^{(0)}$$

Denitrification is accomplished by chemoheterotrophic bacteria that obtain energy from chemical reactions involving organic carbon material under anoxic conditions (i.e. when the dissolved oxygen conditions are very low but not necessarily zero). Recently, researchers have reported that aerobic denitrifying species isolated from canals, ponds, soils and activated sludge have the capacity to simultaneously utilize oxygen and nitrate as electron acceptors. These species are *Thiosphaera pantotropha* (*Paracoccus denitrificans*) (Su *et al*, 2001), *Alcaligenes faecalis* (Joo *et al*, 2005), *Citrobacter diversus* (Huang *et al*, 2001), *Pseudomonas stutzeri* (Su *et al*, 2001) and *Pseudomonas aeruginosa* (Chen *et al*, 2006), *Pseudomonas putida AD21* (Kim *et al*, 2008) etc. Aerobic denitrification have some advantages over anoxic denitrification. (i) aerobic denitrifiers can be easily controlled during the operation and (ii) it can accelerate

*Corresponding author: **Keshava Joshi**

nitrification and denitrification by directly utilizing nitrite-N and nitrate-N, as the denitrifying reactant. (iii) It can be operated simultaneously both nitrification and denitrification in the same reactor to reduce the construction cost and the operational complexity.

Various systems like rotating biological contactors (Gupta and Gupta, 2001), sequencing batch reactors (Lee *et al*, 2008) were investigated for removal of organic substances and nitrogen. Yamagiwa et al, (1995) studied simultaneous removal of organic carbon and nitrogen in a plunging jet bioreactor for domestic wastewater treatment. Jianapag *et al*, (2003) was the first to use airlift reactor for nitrate removal. Dhamole *et al*, (2009) have reported that, at a minimum aeration rate, if all the oxygen is utilized in the draft tube (riser), for carbon utilization where oxygen level is very high and anoxic conditions will prevail in the annular part of the reactor, which helps to utilization of nitrate.

1.1 Draft tube spouted bed contactor

Mathur and Gishler in 1955 developed a spouted bed technique for handling course size particles for drying of agricultural grains. Presently this contactor has been applied in wide varieties of operation. Spouted bed contactor has the following well defined regions. Inlet zone where influent stream mixes with gas and enters into the lower conical section of reactor. Riser is centrally located up flow region from where air, solids and liquid moves upward. It contains a central draft tube supported by two guides each at top and bottom of the column to hold the draft tube in its position (Toumi *et al*, 2008; Dhabade *et al*, 2009).

The insertion of a draft tube in a conventional spouted bed mitigates the limitations of the spouting for improving gas-solid and liquid contact. Several advantages of using a draft tube in a conventional spouted bed are (Buchanan and Wilson, 1965; Muir *et al*, 1990; Konduri *et al*, 1995; Ishikura *et al*, 2003): greater flexibility in the operation; lower gas flow and pressure drop; solids of any size or nature may be treated; narrower residence time distribution; better control of solid circulation; avoids maximum spoutable bed height. Consequently, solid circulation may be controlled by changing independently column diameter, stagnant bed height or particle diameter. Among the disadvantages the following are worth mentioning: lower mixing degree; complexity of design; risk of tube blockage; lower contact between gas and solids.

In the present study, performance of draft tube spouted bed reactor for denitrification process using *Pseudomonas syringae*, which was isolated from secondary clarifier of a fertilizer waste water treatment plant and immobilized on granular activated carbon (GAC) for varying dilution rates and influent nitrate concentration was investigated.

2. Materials and Method

2.1 Microorganism

A strain of microorganism *Pseudomonas syringae* was used for all experiments. It was isolated from wastewater treatment plant of a nearby fertilizer industry (Joshi *et al*, 2014). The strains were periodically sub cultured once in 15 days on nutrient agar slants and were stored at 4^{o}C

2.2 Growth media composition

Media composition for growth of microorganism used is as follows.

NH_4Cl-0.3g/L, KH_2PO_4-1.5g/L, $Na_2HPO_4.7H_2O$- 7.9g/L, KNO_3-2 g/L, $C_4H_40_4Na_2.6H_2O$-27g/L
$MgSO_4.7H_2O$ Solution (5 mL/L): 20g/L

Trace element solution (5mL/L):

EDTA-50.0 g/L; $ZnSO_4$-2.2 g/L; $CaCl_2$-5.5 g/L; $MnCl_2.4H_2O$-5.06 g/L; $FeSO_4.7H_2O$-5.0 g/L; $(NH_4)_6Mo_7O_{24}.4H_2O$-1.1 g/L; $CuSO_4.5H_2O$-1.57 g/L and $CoCl_2.6H_2O$-1.61 g/L.

All the reagents used were of analytical grade.

2.3 Analytical Methods

Samples were collected from the draft tube spouted bed reactor at regular intervals and tested for various characteristics. The culture samples were centrifuged at 10,000 rpm for 10 min (REMI, India) and supernatant was used for nitrate and nitrite. Nitrate and nitrite were analyzed using standard method by UV spectrophotometer. Attached biomass was analyzed by weight difference before and after washing of solids with 0.25N NaOH soluion and drying it at 105^{o}C for 24 h. Biofilm thickness was mesured using scanning electron micoscope (SEM).

2.4 Reactor and experimental details

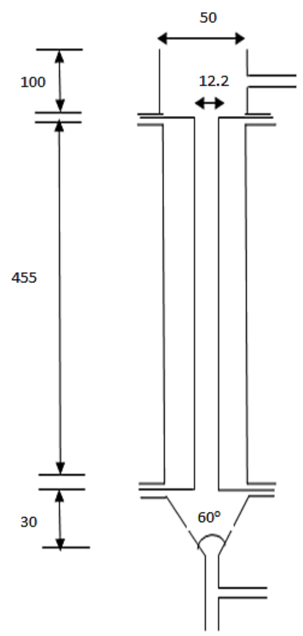


Fig.1 (a): Schematic diagram of Draft tube Spouted Bed Bioreactor

The experimental setup of draft tube spouted bed reactor consisted of a cylindrical column with an inner diameter 50 mm and outer diameter 60 mm as shown in Fig 1(a).

The draft tube had an of inner diameter 12.2 mm, outer diameter 16 mm and height of 455 mm. Volume of the reactor is 1.2 L. The cylindrical column was provided with a conical bottom made of brass where the angle of the cone was 60^o. Synthetic media of different nitrate concentrations maintaining carbon to nitrogen ratio 3:1 in distilled, reverse osmosis processed water with all the other nutrients as indicated in Section 2.2, were pumped using a peristaltic pump as shown in Fig. 1 (b). In all runs, the reactor was loaded with 40g granular activated carbon immobilized with *P. syringae*. Compressed air was introduced through filter in controlled rate just to spout the solids in the draft tube. Effluent samples were collected at appropriate time intervals and centrifuged at 10,000 rpm for 10 minutes. Supernatant was analyzed for the nitrate and nitrite and residue was analyzed for biomass. Reaction was carried till the steady state is attained. Then, contents were emptied and attached biomass and biofilm thickness were measured.

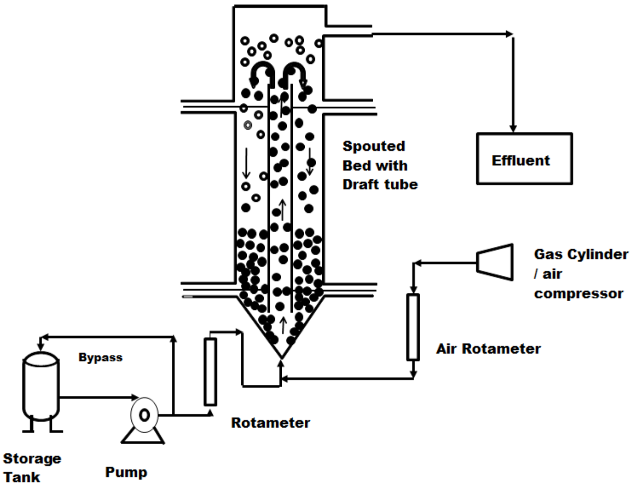
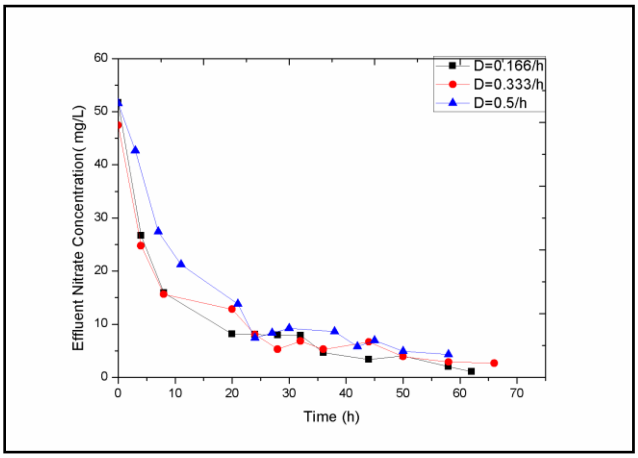
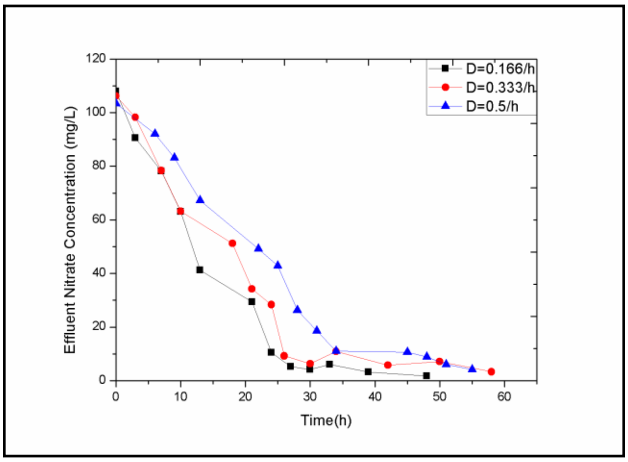


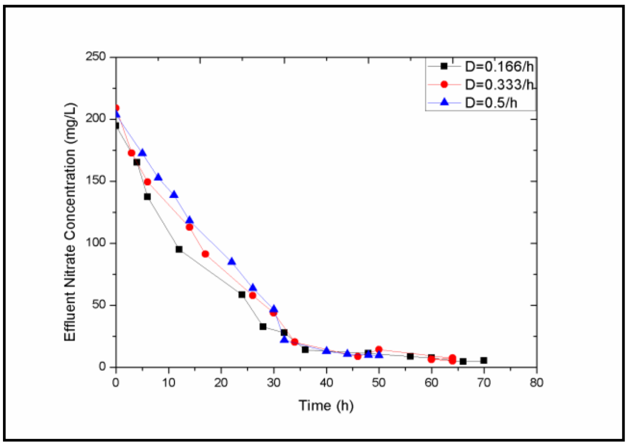
Fig.1 (b): Experimental setup of Draft Tube Spouted Bed Bioreactor

3. Results and Discussion

3.1 Effect of dilution rate on percentage nitrate removal during startup of the reactor







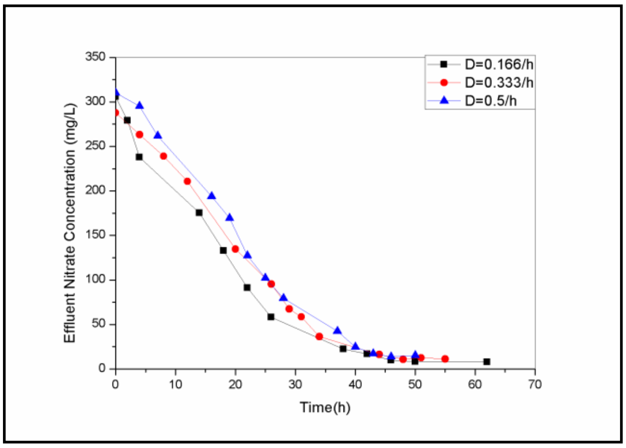


Fig.2: Effect of dilution rate on effluent nitrate concentration during start up for different influent nitrate concentration (a):50 mg/L (b): 100mg/ L (c): 200 mg/L (d): 300 mg/L

The effect of dilution rate on nitrate removal was studied for various inlet nitrate concentration in the effluent. Fig. 2 (a),(b), (c) and (d) presents the effect of dilution rate on nitrate concentration during start up of the bioreactor for influent nitrate concentrations of 50, 100, 200 and 300 mg/L. It is evident from these figures that, there is a continues decrease in nitrate concentration till 30^{th} h and thereon removal rate becomes slow for influent nitrate concentrations of 50 and 100 mg/L. For influent nitrate concentration 200 and 300 mg/L there is a continuos decrease in nitrate concentration till 40th h and there after the nitrate concentration decreases as marginally.

The rate of denitrification during start up decreases with increase in dilution rate. It may be because of at higher dilution rate there will be higher velocity that leads to wash out of biomass, and also lesss retention time. The time taken to reach steady state has increased with increase in dilution rate for all influent nitrate concentrations. During initial stage, the adsorption of nitrate along with organic substrate onto GAC particle takes place and as the bacteria grows, the attached biomass increases and hence, biofilm thickness increases. Thicker and denser biofilm may offer more resistance to substrate mass transfer. It was presumed that the attached biomass consumes more of carbon in draft tube area where oxygen level is very high and nitrate is consumed in annular area where oxygen level is low. This concept was also explained in their experiments by Dhamole *et al*, (2008). Once the biofilm grows and becomes stable, substrate mass transfer would have increases and then effluent nitrate concentration reaches to a steady state value. Higher nitrate concentration feeds result in the formation of thicker and denser biofilm, which may offer more resistance to substrate mass diffusional transfer rate, which intern results in lower denitrification rates. Therefore, time taken to achieve steady state value has increased for higher nitrate concentration feeds.

3.2 Effect of dilution rates on percentage nitrate removal

The effect of dilution rates on percentage nitrate removal was studied for varoius dilution rates**.** Fig.3 presents the effect of influent nitrate concentration on percentage nitrate removal at different dilution rates of 0.166/h, 0.333/h, and 0.5/h by varying the influent nitrate concentration from 50 mg/L to 300 mg/L. The maximum steady state percentage nitrate removal was 98.45 percent for dilution rate of 0.166/h. At higher dilution rates 0.333/h and 0.5/h the percentage nitrate removal decreased to around 96 percent for 50 mg/L nitrate concentration in the feed. For 300 mg/L nitrate concentration feed, the maximum steady state percentage nitrate removal was 97 percent for dilution rate of 0.166/h. At higher dilution rate 0.5/h the percentage nitrate removal decreased to around 94 percent.

At a constant influent nitrate concentration, with increase in dilution rate the contact time between the liquid and solid phase reduces and this might have slowed down the biomass growth rate resulting in reduced attached biomass dry weight as observed in Fig.3. It is also observed that increase of dilution rate would have increased the attrition and shear section especially in disengagement section of the spouted bed (Wang *et al*, 2003), which might have caused the sloughing of attached biomass. This would have reduced the attached biomass dry weight and hence the decrease in percentage denitrification. The hydrodynamics of reactor substrate concentration and environmental factors etc affects the biofilm thickness (Toumi *et al*, 2008). Attrition and shear effect are major factors in the operation of spouted bed reactors. Increased dilution rate increases the liquid velocity resulting in shear and also cell wash out this might be the reason to decrease in percentage nitrate removal.

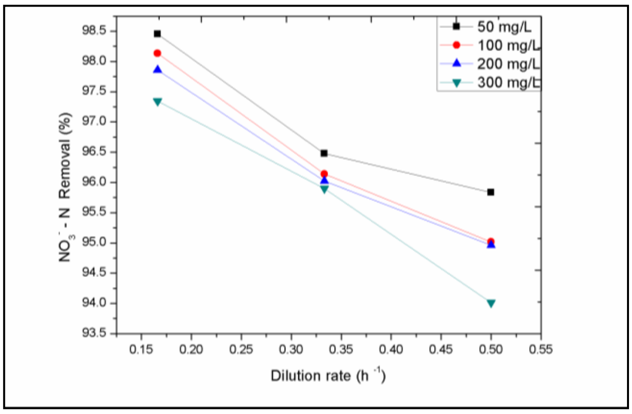


Fig.3: Effect of dilution rate at different influent nitrate concentration on percentage nitrate removal at steady state for GAC loading 40 gm

3.3 Effect of influent nitrate concentration on percentage nitrate removal

The effect of influent nitrate concentration on percentage nitrate removal was studied for varoius nitrate concentration feeds. Fig.4 presents the effect of influent nitrate concentration on percentage nitrate removal for influent nitrate concentrations of 50 mg/L, 100 mg/L, 200 mg/L and 300 mg/L at different dilution rates. It is evident form the Fig. 4 that percentage nitrate removal decreases with increase in influent nitrate concentration. For influent nitrate concentration of 50 mg/L the maximum steady state percentage nitrate removal achived was 98.45 percent and decreases to 94.01 percent for influnet nitrate concentration of 300 mg/L at 0.166/h dilution rate. It is observed that higher inlet nitrate concentration in feed would have resulted in forming more thicker and denser biofilm on solid particle. Biofilm consists of microbial cells and EPS mixture (Extra cellular polymeric substances) (Toumi *et al*, 2008; Shetty *et al*, 2008).

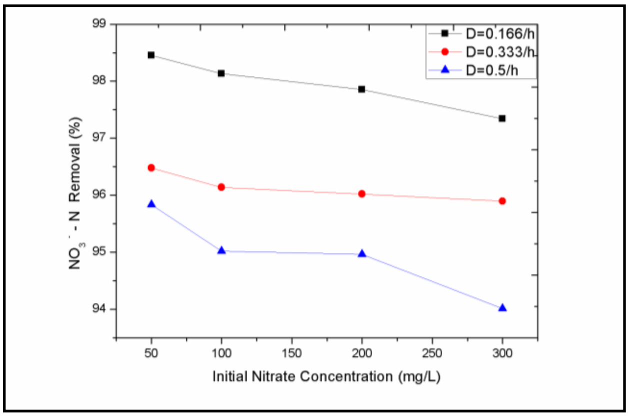


Fig.4: Effect of influent nitrate concentration at different dilution rates on percentage nitrate removal at steady state

As biofilm becomes thicker and denser then external mass transfer of substrate (nitrate in this case) in the film gets reduced through the film because of resistance. The dense and heterogeneous structure of biofilm may contain a fraction of biofilm that is inactive due to presence of EPS and dead cells (Dabhade *et al*, 2008). Such a dense biofilm offers more resistance to diffusional mass transfer. This could be the reason for slow and less nitrate removal observed for higher influent nitrate feeds. The percentage denitrification using different rectors and microorganisms by various researchers is presented in table.

3.4 Effect of dilution rate on attached biomass dry weight and biofilm thickness at steady state

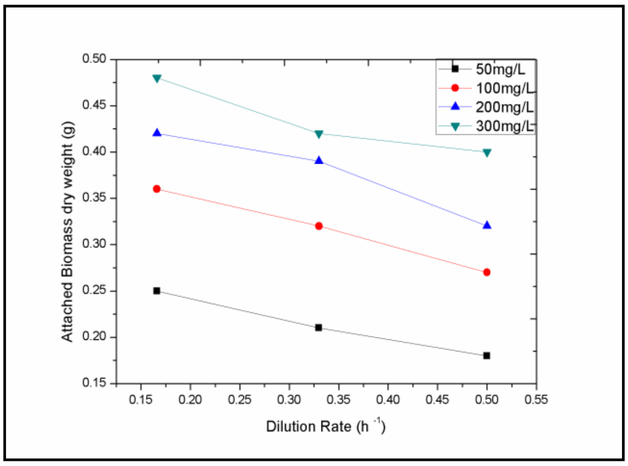


Fig. 5: Effect of Dilution rate on attached biomass for different infuent nitrate concentration at steady state

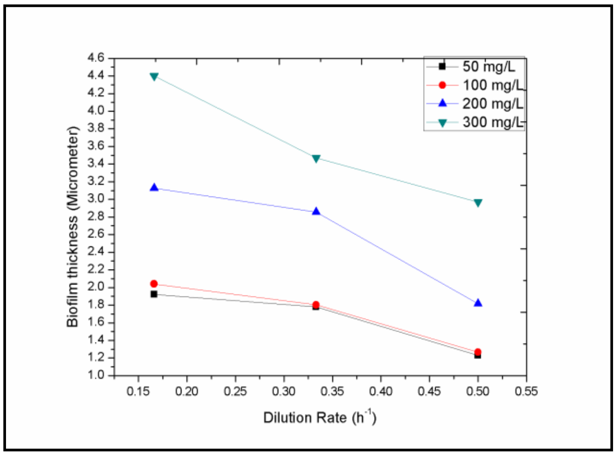


Fig. 6: Effect of dilution rate on biofilm thickness for different infuent nitrate concentration at steady state

The effect of dilution rate on biofilm thickness was studied for different infuent nitrate concentration at steady state. Fig. 5 and 6 presents the effect of dilution rate and influent nitrate concentrations on the attached biomass dry weight and biofilm thickness obtained at steady state are shown in respectively. It is evident from the figures that as dilution rate increases, attached biomass dry weight and biofilm thickness decreases for all influent nitrate concentration. With increase in dilution rate, the time of contact between the cells and substrate (residence time in the reactor) reduces and this would result in less cell growth. As the dilution rate increases, the flow velocity past the particle increases. This increased flow velocity might have resulted in higher erosion of the biomass from the biofilm surface. Reduced cell growth and increased cell detachment by erosion, would have resulted in reduction in attached biomass dry weight and biofilm thickness, with increased dilution rates.

This may probably be due to the structure or morphology of the biofilm formed, owing to the exposure of microorganisms to high substrate concentrations during start up. Structure and morphology of the biofilm depends on the conditions at which its grown.The becteria produced heterogeneous, dense and slimy biofilm on the GAC particle. (Fig 7). The extracellular polymeric substance (EPS) molecules are the exopolymers, which provide the forces responsible for cohesion of the biofilm and adhesion to the substratum. The stress and grwoth conditions affects the compostion of EPS (Schmitt *et al*, 1995). Biomass is believed to detach when the tensile forces caused by the external shear exceed the tensile strength of the EPS matrix that holds together the biofilm (Kwok *et al*, 1998; Ohashi and Harada, 1994). This could be the reason to reduction in biofilm thickness at higher dilution rates.

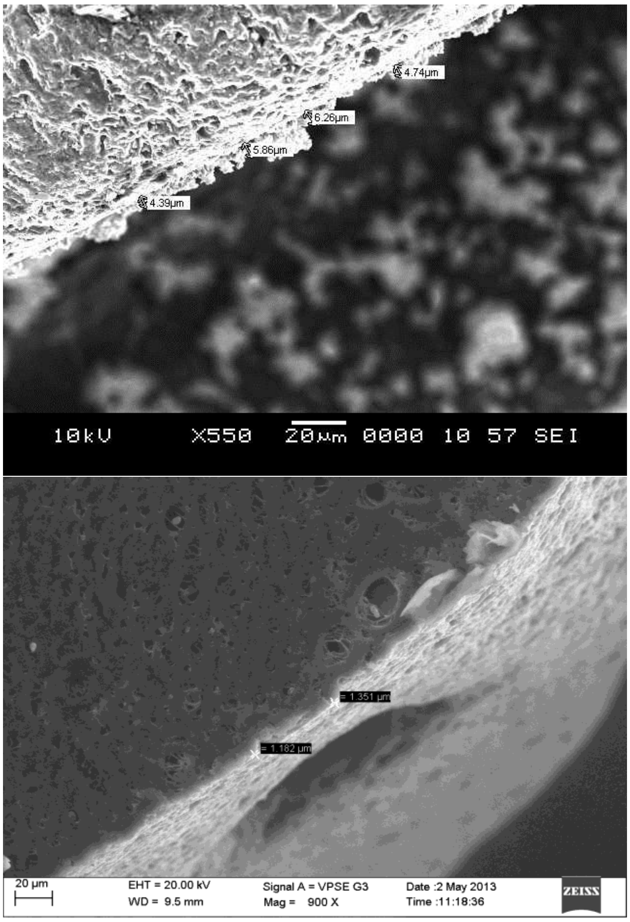


Fig.7: SEM Pictures of biofilm on Granular activated carbon

Conclusion

Present work demonstrates a novel application of draft tube spouted bed reactor for nitrate removal under aerobic conditions using *Pseudomonas syringae* which was isolated from secondary clarifier of fertilizer waste water treatment plant and immobilized on granular activated carbon (GAC) (Joshi et al., 2014). A 98% nitrate removal for 50 mg/L inlet nitrate concentration feed and 94% nitrate removal for 300 mg/L inlet nitrate concentration feed was observed for 0.166/h dilution rate. High rate of nitrate removal was observed in present study with controlled airflow rate in draft tube spouted bed reactor in its distinct conditions in different zones of the reactor. The feasibility of gas–liquid–solid three-phase flow draft tube spouted bed reactor for treating nitrate-nitrogen wastewater has been demonstrated in the present work. Therefore, the draft tube spouted bed reactor may serve as a promising reactor for removal of COD and nitrogen in industrial effluents.

Acknowledgement

The authors wish to thank, Department of Chemical Engineering, National Institute of Technology Surathkal, Karnataka for providing research facilities. One of the authors, Keshava Joshi is thankful to the Management and Principal S D M College of Engineering and Technology Dharwad for their support and encouragement.

References

Buchanan, R.H., Wilson, B. (1965), Fluid-Lift Solids Recirculator, *Mech. & Chem. Eng. Transactions*, 1(1), pp.117-124.

Burghate, S.P., Ingole N.W. (2013), Biodenitrification by Fluidized Bed Biofilm Reactor, *International Research Journal of Environment Sciences*, 2(12), pp.42-51.

Chen, F., Xia Q., Ju, L.K. (2006), Competition between oxygen and nitrate respirations in continuous culture of *Pseudomonas aeruginosa* performing aerobic denitrification, *Biotechnol. Bioeng.,* 93, pp.1069–1078

Dabhade, M.A., Saidutta, M.B., Murthy, D.V.R., (2009), Continuous phenol removal using *Nocardia hydrocarbonoxydans* in spouted bed contactor: Shock load study, *African Journal of Biotechnology*, 8 (4), pp.644-649.

Dabhade, M.A., (2008), Studies on Phenol Biodegradation in Spouted Bed Contactors. *PhD Thesis*.

Dhamole, P.B., Rashmi, R. N., Stanislaus, F., D'Souza, Lele, S.S., (2008), Denitrification of Highly Alkaline Nitrate Waste Using Adapted Sludge, *Appl Biochem Biotechnol*, 151, pp.433–440

Dhamole, P.B., Rashmi, N., Stanislaus Souza, F.D., (2009), Simultaneous removal of carbon and nitrate in an airlift bioreactor, *Bioresource Technology*, 100, pp.1082–1086.

Gupta, A.B., Gupta, S.K., (2001), Simultaneous carbon and nitrogen removal from high strength domestic wastewater in an aerobic RBC biofilm, *Water Res*., 35, pp.1714– 1722.

Huang, H.K., Tseng, S.K., (2001), Nitrate reduction by *Citrobacter diversus* under aerobic environment, *Appl. Microbiol Biotechnol*., 55, pp.90-94.

Han, W., Lee, S. Y., Lee, J.W., Park, J., Choi, E., Park Yong, K., (2002), Molecular Characterization of Microbial Community in Nitrate-removing Activated Sludge, *Journal of Microbiology Ecology*, 41, pp.85-84

Ishikura, T., Nagashima, H., Ide, M., (2003), Hydrodynamics of a Spouted Bed with a Porous Draft Tube Containing a Small Amount of Finer Particles, Powder Tehnol. 131, pp.56-65.

Jianping, W., Lei, P., Liping, D., Guozhu, M., (2003), The denitrification of nitrate contained wastewater in a gas–liquid–solid three phase flow airlift loop bioreactor, *Biochem. Biochem. Eng. J.,* 15, pp.153–157.

Joo, H.S., Hirai, M., Shoda, M., (2005), Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* No. 4, *J. Biosci. Bioeng.,* 100, pp. 184-191.

Joshi, K., Joseph, J., Srinikethan, G., Saidutta, M.B., (2014), Isolation and characterization of *Pseudomonas syringae* for nitrate removal under aerobic conditions, *J Biochem Tech*, 5(2), pp. 693-697

Kim, M., Jeong, S.Y., Yoon, S.J., Cho, S.J., Kim, Y.H., Kim, M.J., Ryu, E.Y., (2008), Aerobic denitrification of *Pseudomonas putida* AD-21 at different C/N ratios, *J Biosci Bioeng.*, 106(5), pp. 498-502.

Kim Joong, K., Park Kyoung, J., Cho Kyoung, S., Nam Soo, W., Park Tae, J., Bajpai, R., (2005), Aerobic Nitrification–Denitrification by Heterotrophic Bacillus Strains, *Journal of Bioresearch Technology*, 96, pp.1897-1906.

Kwok, W.K., Picioreanu, C., Ong, S.L., Loosdrecht, M., Heijnen, J.J., (1998), Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor, *Biotechnology and Bioengineering*, 58, pp. 400–407.

Konduri, R.K., Altwicker, E.R., Morgan, M.H., (1995), Atmospheric Spouted bed Combustion: the Role of Hydrodynamics in Emissions Generation and Control, *Can. J. Chem. Eng*., 73(5), pp.744-754.

Lee, J.K., Cho,i C.K., Lee, K.H., Yim, S.B., (2008), Mass balance of nitrogen and estimates of COD, nitrogen and phosphorous used in microbial synthesis as a of sludge retention time in a sequencing batch reactor system, *Bioresour. Technol.,* pp. 7786–7796.

Mathur, K.B., Gishler, P.E., (1955). A technique for contacting gases with coarse solid particles, *American Institute of Chemical Engineers, Journal*., 1, pp. 157-169.

Muir, J.R., Berruti, F., Behie, L.A., (1990), Solids Circulation in Spouted and Spout-Fluid Beds with Draft Tubes, *Chem. Eng. Commun*., 88, pp.153-171.

Ohashi, A., Harada, H., (1994), Adhesion strength of biofilm developed in an attached-growth reactor, *Water Sci. Technol*., 29(10 – 11), pp. 281 – 288.

Sarina, J., David, E., (2004), Drinking water Denitrification using a membrane bioreactor, *Water Research*., 38, pp. 3225–3232.

Su, J.J., Liu, B.Y., Liu, C.Y., (2001), Comparison of aerobic denitrification under high oxygen atmosphere by *Thiosphaera pantotropha* ATCC 35512 and *Pseudomonas stutzeri* SU2 newly isolated from the activated sludge of a piggery wastewater treatment system, *Journal Appl. Microbiol*., 90, pp.457-462.

Shetty, K.V., Kalifathulla, I., Srinikethan, G., (2007), Performance of pulsed plate bioreactor for biodegradation of phenol, *J. Hazard. Mater*., 140(2), pp. 346-352.

Srinu Naik, S., Pydi Setty, Y., (2011). Effect of Carbon Sources on Biological Denitrification of Wastewater by Immobilized *Pseudomonas Stutzeri* Bacteria in a Fluidized Bed BioReactor (FBBR). *2011 2nd International Conference on Chemical Engineering and Applications IPCBEE*, 23, pp. 114-118

Toumi, L.B., Fedailaine, M., Khedidja, A., (2008), Modeling Three-Phase Fluidized Bed Bioreactor for Wastewater Treatment, *International Journal of Chemical Engineering Reactor*, 6(1), pp. 1542-6580.

U.S.EPA, (1993), Nitrogen Control Manual, EPA-625/R-93/010; Office of Research and Development, Environmental Protection Agency, Washington, DC, USA.

Wang, Q., Feng, C., Zhao, Y., Hao, C., (2009), Denitrification of nitrate contaminated groundwater with a fiber-based biofilm reactor. *Bioresour. Technol*., 100, pp. 2223–2227.

Wang, D, Lin, J, Sun, R (2003), Optimum nitrogen rate for a high productive rice-wheat system and its impact on the ground water in the Taihu Lake area, *Acta Pedol Sin* 40, pp. 426–432

Yamagiwa, K., Oohira, Y., Ohkawa, A., (1995), Simultaneous removal of carbonaceous and nitrogenous pollutants by a plunging liquid jet bioreactor with cross flow filtration operated under intermittent aeration, *Bioresour. Technol*., 53, pp.57–62.

Youyuan S., Li Wei, Yao Chuang, Qin G., Szewczyk W., (2011), Influence of COD/N Ratios on Simultaneous Removal of C and N Compounds in Biological Wastewater Treatment in Sequencing Fed-Batch Reactor and Kinetic Analysis, *Journal of hazardous, Toxic, and Radioactive Waste , ASCE*, 15(1) , 42-47.

Schmidt I., Sliekers O., Schmid M., Bock E., Fuerst J., Kuenen J.G., Jetten M.S.M., Strous M., (2003), New concepts of microbial treatment processes for the nitrogen removal in wastewater, *FEMS Microbiol. Rev*. 27, 481–49