J Environ. Science & Engg. Vol. 57, No. 2, p. 131-136, April 2015

Studies on COD Removal in Upflow Anaerobic Attached Growth Bioreactor Treating Simulated Aquaculture Wastewater

VALSA REMONY MANOJ* AND NAMASIVAYAM VASUDEVAN

In the present study, the suitability of coconut coir fibre as a bacterial support medium was compared against a commercially available reticulated plastic support medium (fujino spirals) for nutrient removal. The suitability was studied using anaerobic packed bed columns using Methanol as exogenous carbon source. Simulated aquaculture wastewater was used. The most important evaluator on the extent of denitrification is the consumption of organic carbon measured as Chemical Oxygen Demand (COD). The average removal rates were 74% and 72% for coconut coir and fujino spirals respectively. The performance of the column packed with coconut coir has shown consistent and marginally better removal efficiency than fujino spirals.

Key words: *COD, upflow, bioreactor, aquaculture, wastewater*

Introduction

Farmed shrimp vide aquaculture is the most profitable commodity, and also the most polluting [1,2]. The aquaculture industry has been challenged to develop economically viable systems that not only produce species at high density but also must contend with limitations of location, water availability and environmental impact[3]. To maintain healthy ecosystem in aquaculture ponds, bio-remediation in zero exchange of water and integrated recirculating systems and treatment of wastes prior to discharge are the best eco-friendly practices[4]. Development of an effective, low-cost treatment is therefore imperative if aquaculture is to expand continually at the present rate[5].

The results presented here represent the observations on COD removal during studies in an upflow anaerobic packed bed column used to study the comparative efficiency of nitrate nitrogen removal from simulated aquaculture wastewater by two bacterial support media namely reticulated plastic medium (fujino spirals) and naturally available coconut coir. The major objective of this study was to determine whether coconut coir fibre could be utilized as effectively as commonly available synthetic bacterial support medium, for the removal of nutrients (particularly nitrate nitrogen) from aquaculture wastewater. The COD in this case was mainly contributed from methanol used as the exogenous carbon source. The most important evaluator on the extent of denitrification is the consumption of organic carbon measured by the Chemical Oxygen Demand (COD). carbon in this study was mainly sourced from the addition of methanol which is a C-1 carbon containing solvent.

Therefore, the principal purpose to provide a carbon source was for the removal of the said amount/ concentration of nitrate. However during the study, certain interesting observations with reference to the removal of COD were made, which are explained in this paper. Reduction of Nitrate nitrogen at two different Nitrate nitrogen loading rates (NLR-I and NLR II) by the process of biological denitrification was studied. Along with Nitrate nitrogen reduction, the reduction in Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN) and Dissolved Orthophosphate (PO_4) were also studied.

The Chemical Oxygen Demand (COD) of the aquaculture wastewater tested in this study ranged from 314 – 354 mg/L. There are several sources of COD for the aquaculture wastewater. The major types of COD are soluble and particulate COD. These can further be understood as fractions namely: (1) readily biodegradable soluble COD, (2) slowly biodegradable colloidal and particulate (enmeshed) COD, (3) non-biodegradable soluble COD, and (4) non-biodegradable

---

Center for Environmental Studies, Anna University Chennai, Chennai-600 025, India
*Corresponding author

colloidal and particulate COD[6]. In this study, the biodegradable and non-biodegradable COD was analyzed together using the open reflux method.

Materials and methods

Two independent acrylic cylindrical columns were used as the biofilters with a volume of 3.9 L each in the study. The size of the reactors was determined based on preliminary studies carried out using 1.5 L PVC cylindrical columns. The biofilters were each packed with coconut coir fibres (Reactor A) and fujino spirals (Reactor B). Prior to packing, the amount, physico-chemical characteristics, and nature of media were duly noted. Circular plastic displacer plates with uniform pores of 1-2 mm size arranged in circular fashion to contain the packed media were placed. Three sampling ports of 5 mm diameter were placed along the height of the reactors at 10 cm intervals. A common influent storage tank of 40 L capacity was placed above the reactors. A gas tube was connected to the top portion of the influent tank, which was in turn connected to a Nitrogen gas cylinder so as to remove dissolved oxygen by purging. By force of gravity, the flow into each reactor from the influent tank was regulated in an "upflow" mode by two independent gate valves at the base of each reactor. The top of each column contained a effluent collection port and gas collection port by water displacement method. A line diagram of the same is presented in Fig 1.

During startup, "real" aquaculture wastewater was cycled through the columns for an initial period until there was atleast 70% reduction in the effluent COD from the principal. The columns was fed with simulated aquaculture wastewater[7] at a constant flow rate of 6 L/d. The Hydraulic Residence Time (HRT) of the simulated wastewater in the bioreactors was 6.72 h. Two different Nitrate nitrogen loading rates (NLR) namely 60 mgl^{-1} and 120 mgl^{-1} Nitrate nitrogen were used to compare the two columns with different packing media. 60 NLR experiments were run for 10 weeks after startup period immediately followed by 120 NLR for a period of 28 weeks. Methanol was used as the carbon source equivalent to 1.5 grams of COD[8]. pH was maintained at a range of 7.5 to 8.0. Ambient temperature was at 28°C. Effluent samples were collected in cleanly labelled 1 L sampling bottles during operation, on a weekly basis for analysis. The analytical procedures were carried out according to APHA[9]. The daily generation of gas from the anaerobic activity in the columns was measured using a calibrated water displacement meter[10].

Results and discussion

COD removal

At NLR of 60 mg/L nitrate concentration, th maximum removal was observed for a period of 1 weeks. It took an average time of 4 weeks for th COD removal to become stabilized to about 71 reduction. The following weeks showed COD remova in the range of 82 % to 87 %. The maximum COI removal recorded in column with coconut coir a medium was 87 % and with the column packed wit fujino spirals as support, it was 82 %. The averag removal was 75 % and 61 % respectively. The result are indicated in Fig 2. At NLR of 120 mg/L nitrate the column stabilized by the 12th week where it reache 64 % COD removal in column with coconut coir a support. Interestingly, the column with fujino spiral as support medium showed stabilized values of up t 70 % as early as the 8th week. This condition wa unique to the higher nitrate loading rate of 120 mg/L and showed almost equal trends in COD removal This trend would indicate that it takes longer time fo the organic support medium to influence the nutrien removal than synthetic media at higher nitrate loadin rates. However, both columns showed a very paralle trend in the COD removal in the range of 79 % to 81 from the 18th week. The maximum COD remova recorded in column with coconut coir was 81 % (6 mg/L), and the maximum COD removal recorded i column with fujino spirals as support was 72 % (9 mg/L). The average removal rates were 74% and 72 respectively. The results are presented in Fig 3.

In both the NLR's, the performance of th column packed with coconut coir has shown consistenc and marginally better removal efficiency than fujin spirals. This could be attributed to the organic natur of coconut coir, which could have added some COD thus lessening the net carbon requirement. Such situation has been reported[11] where carbonaceous COI was leached out of the media into the reactor's reactio during early stages with wood chip filters.

The COD recorded in the effluent is an indicato of unutilized carbon source. If this were the case then far less amount of carbon source is required fo the column packed with coconut coir than the on packed with fujino spirals. Previous studies[12] have note that though there is a possibility of organic mediur leaching out carbonaceous COD, it is likely to b negligible compared to the methanol contribution. Thi factor is important to keep note at this juncture sinc he role of COD ie., carbon source is to transfer electrons from a reduced state to an oxidized electron acceptor, which is the Nitrate nitrogen ion (NO_3^-N). It s now important to understand that COD by itself can be divided into biodegradable COD and non-biodegradable COD. Biodegradable COD, that is readily soluble is quickly assimilated by the biomass, while he particulate and colloidal fractions of biodegradable COD must first be broken down by extracellular enzymes and are thus assimilated at much slower rates[13]. The dynamics of carbon utilization are also known to vary widely. An important observation in studies on columnar denitrification for aquaculture wastewater[14] reports that there can be instances of no exogenous carbon utilization as well as fairly long periods of up to 25 to 50 % utilization. The same study has also stated that effluent carbon increases when C: N ratio increases. This is an additional reason for the presence of residual COD in the effluent. Two studies[15,16] have made parallel observations/hypothesis hat the residual COD is mainly in the form of soluble microbial products (SMP), and is a result of substrate utilisation (UAP – Utilisation Associated Products) and biomass decay (BAP – Biomass Associated Products). An earlier observation[17] confirms that BAPs are the major contributor to effluent COD, where he has stated that the fraction of biomass decay products (high molecular weight fractions, e.g. cell walls and cell lysis products) would increase substantially as he substrate concentration decreases.

Whenever there is a deficiency in the biodegradable carbon within the column, there is the provision of extra carbon, albeit marginal, being leached from the organic packed medium (coconut coir). This carbon could also contribute to the biodegradable fraction of carbon engaged in the process of denitrification. The C/N ratio required for complete nitrate reduction to nitrogen gas by denitrifying bacteria depends on the nature of the carbon source and the bacterial species[18]. The type of denitrification where electrons and protons are derived by the denitrifiers for nitrate reduction to elemental nitrogen from organic compounds such as carbohydrates, organic alchohols (eg.methanol), amino acids, fatty acids is called heterotrophic denitrification[19].

COD / NO_3-N ratio

In order to draw an estimation of the bioreactor's capacity to utilize carbon for denitrification, it is important to draw a ratios of the COD consumed to the Nitrate nitrogen reduced. The COD / NO_3-N ratios for a stoichiometric reaction while using methanol as the carbon source is 2.95 – 3 mg COD/NO_3^--N[19]. In the present study, the influent COD/NO_3-N ratio for 60 NLR and 120 NLR was 4.3 and 2.8 respectively. As the level of nitrate reduction progressed primarily by the process of denitrification, the ratio of the COD/NO_3-N reached values closer to the theoretical stoichiometrical levels when methanol is used as a carbon source. Initially, the COD/NO_3-N ratio observed during 60 NLR was 4.6. At the 9th week when optimum nitrate reduction was observed in both upflow packed bed anaerobic column bioreactors, both the column bioreactors showed a reduction in the ratio. Column bioreactor with coconut coir as support medium reached a ratio of 4.1 , while column bioreactor with fujino spirals reached a ratio of 3.8 at their optimum Nitrate nitrogen removal (Fig 4). The marginally higher difference in the ratio obtained at the point of maximal nitrate reduction at 60 NLR in the column using coconut coir fibre as support medium; indicates a better utilization of COD for nitrate reduction.

At 120 NLR, the influent COD/NO_3-N ratio was more closer to the stoichiometric value namely 2.8. Until the 17th week, almost no nitrate removal could be observed in the column bioreactor with fujino spirals compared to the marginal removal observed in the column bioreactor using coconut coir as support medium. In the column bioreactor with coconut coir as medium, the ratio of COD consumed to the Nitrate reduced was higher at 5.4 and gradually reduced to 2.8. Both the support media namely coconut coir fibre and fujino spirals showed a ratio of 2.3 at the point when nitrate reduction was maximum ie., during the 28th week. The reduction in the COD/NO_3-N ratio to its optimal levels of 2.3 was obtained between the 17th and the 28th week. The results are shown in Fig 5.

In general, the COD/NO_3-N ratio and the nature of the carbon source would determine the nitrate reduction pathway. Excessive carbon addition may result in dissimilatory nitrate reduction to ammonia (DNRA). This might be the reason for the elevated levels of TKN (sum of ammonia and organic nitrogen) observed in the beginning of the column bioreactor study and its gradual decline as the carbon source was used more efficiently for nitrate reduction.

Removal of TSS and production of biogas

The TSS production, percentage COD reduction and relative gas production were closely monitored during the two nitrate loading rates of 60 and 120 mg/L. It is to be noted that TSS reduction in general was only 30 % to 40 % on an average. This is to be observed with some importance since the amount of readily digestable organic matter is considerably lower owing to the simulated nature of the wastewater. This is reflected in the lower production of gas which was also in the production range of only 30-40 %. The volume of gas produced (volumetric gas production) is theoretically expected to reach an average of 6 litres per day equivalent to the feeding rate of 6 L per day. However, the average volumetric production of gas measured was only about 2 L per day. The lower gas production however, did not reflect in the removal of nutrients, especially nitrate. The reason behind the lower gas production could therefore be considerably justified by the presence of non-biodegradable fraction of the COD and the retention of gas within the column reactor due to loss of sufficient pressure for the gas to exit the reactor. The relationship between percentage COD reduction and volumetric gas production during the studies with two nitrate loading rates (60 and 120 mg/L) has been shown in Fig 6 and Fig 7 respectively.

It is to be noted here that the production of gas with corresponding COD removal prior to the establishment of nitrate nitrogen reduction is not a result of denitrification process. Therefore, it is to be acknowledged here that several other pathways could have played a major role resulting in the reduction of COD and gas production. In case of COD removal at 60 mg/L loading rate, column with fujino spirals as support medium was able to remove a maximum amount of 82 % while coconut coir supported column was able to remove upto 87 %. On an average also, column with coconut coir as support medium showed slightly better performance than fujino spirals support medium with 69 % and 64 % respectively. At 120 mg/L nitrate loading rate, both coconut coir fibre and fujino spira supported columns reached a maximum removal about 81 %. The average percentage removal show a slightly higher removal in case of coconut coir bei 74 % than fujino spirals at 72 %. Towards the end each NLR (60 and 120), qualitative detection of t biogas constituents was carried out to determine t nature of gases generated. This demonstrated t presence of methane and hydrogen, both constitue of biogas mediated by anaerobic digestion. Quantitat measurements of methane generation or nitrog production by gas chromatography were not done.

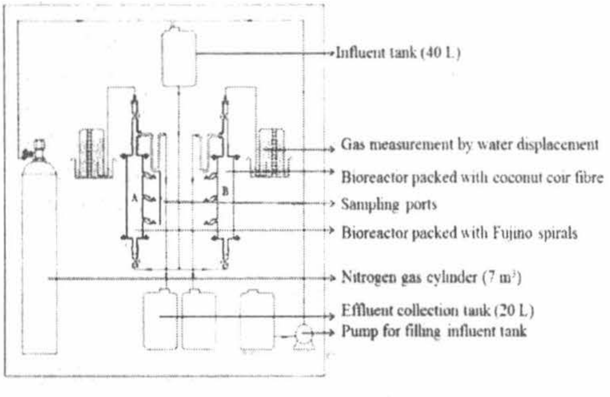

Fig. 1: Experimental set-up used in the study

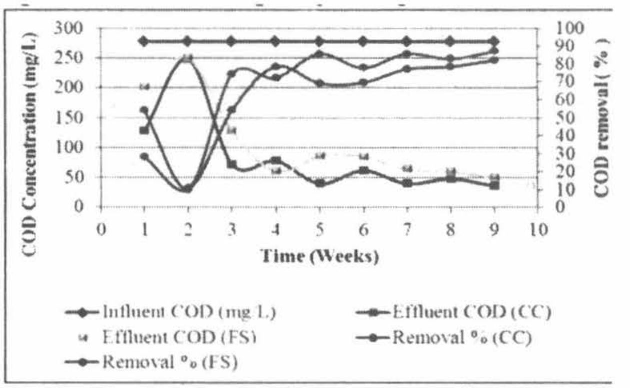

Fig 2: Removal of COD at 60 mg/L NO_3-N loading rate

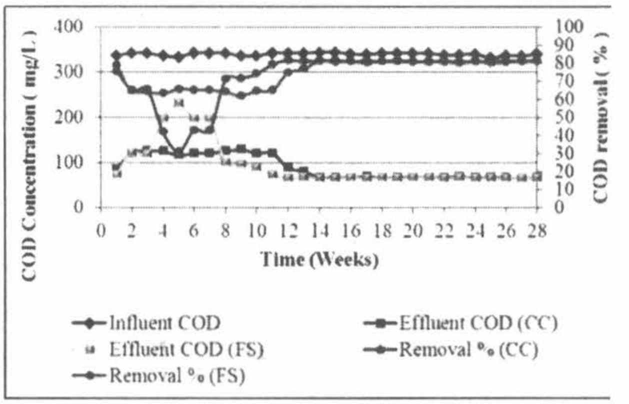

Fig 3: Removal of COD at 120 mg/L NO_3- loading rate

The presence of methane is contradictory sin methanogenesis is generally inhibited by the presen of nitrate, mainly in its reduced forms as NO_2 a others[21]. If this were the case, then the methanogene observed would have occurred after the nitrate exhausted or at a concentration low enough for t methanogens to grow. Such a scenario is doubtful i continuously operated bioreactor receiving consta influx of nitrate. However, literature does suggest t simultaneous denitrification and methanogenesis

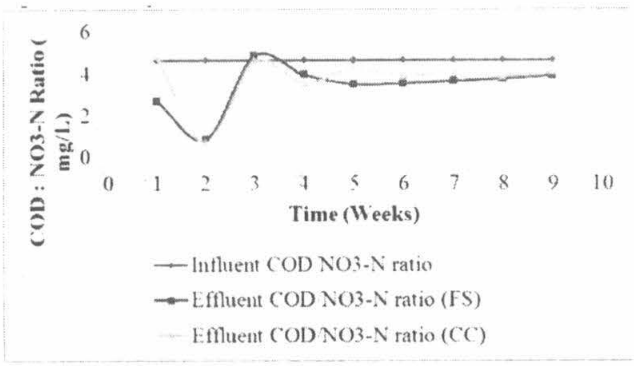

Fig. 4: COD/NO_3-N ratio at 60 NLR

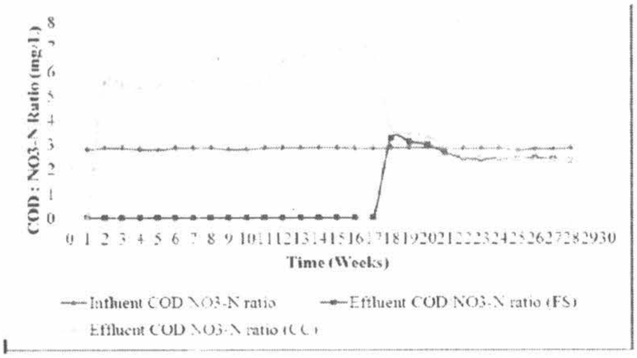

Fig. 5: COD/NO_3-N Ratio at 120 NLR

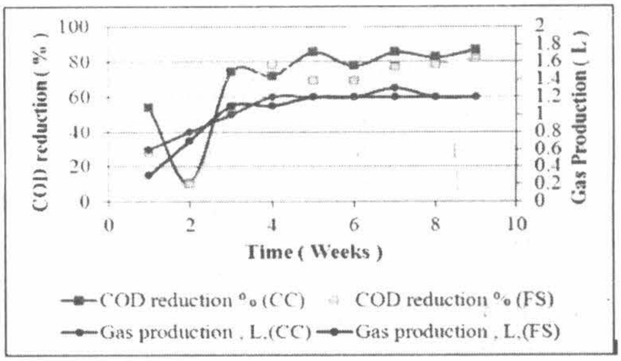

Fig. 6: Biogas production vs percentage COD reduction at 60 mg/L nitrate loading rate

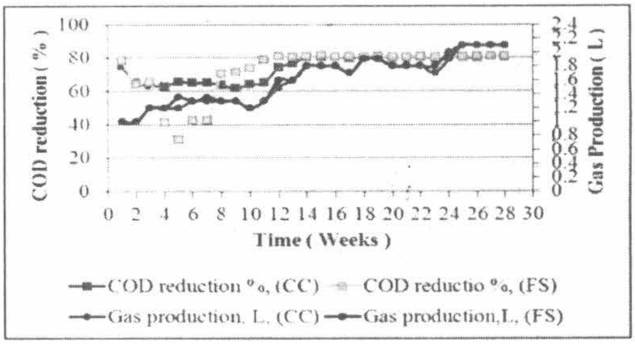

Fig. 7: Biogas production vs percentage COD reduction at 120 mg/L nitrate loading rate

possible. However, an earlier study[22] has reported the occurrence of simultaneous methanogenesis and denitrification in a methanol fed bioreactor anaerobically treating nitrate rich wastewater. The same study has suggested that "if methanogenic bacteria work together with denitrifying bacteria in a reactor, a low level of effluent organics, as well as nitrite or nitrate, can be achieved".

Conclusion

In the study on Nitrate nitrogen removal using upflow anaerobic packed bed columns, some interesting observations could be made on the COD removal. From the results obtained, residual COD could be attributed to the organic nature of the packing media or even attributable to simultaneous methanogenesis and denitrification. What is clear, is that while COD removal could be correlated to the removal of NO_3-N removal in the bioreactor system, it is always important to realize the role of other plausible factors that cause the reduction apart from the carbon utilisation process by denitrification.

References

1. R.L. Naylor, Goldburg R.J. and Primavera J.H. Effect of aquaculture on world fish supplied, *Nature*, **409 (29)**, (2000).

2. G.D. Treece, Shrimp farm effluents. In : Stickney R.R. and McVey J.P. (eds), Responsible Marine Aquaculture, CAB International, Wallingford, UK, (2002).

3. H.J. Schreier, N. Mirzoyan and K. Saito, Microbial diversity of biological filters in recirculating aquaculture systems, *Current Opinion in Biotechnology*, **21(3)**, 318-25 (2010).

4. Sudarno.U., Stephan Bathe, Josef Winter and Claudia Gallert, Nitrification in fixed-bed reactors treating saline wastewater, *Applied Microbiology and Biotechnology*, **85**, 2017-2030 (2010).

5. K.K. Krishnani, V. Kathiravan, M. Natarajan, M. Kailasam and S. M. Pillai , Diversity of Sulfur-Oxidizing Bacteria in Greenwater System of Coastal Aquaculture, *Applied Biochemistry and Biotechnology*, **162(5)**, (2010).

6. W.H. Zachritz and Jacquez, R.B., Treating intensive aquaculture recycled water with a constructed wetlands filter system, In: Moshiri, G.A. (Ed.), Constructed Wetlands for Water Quality Improvement. Lewis Publishers,Boca Raton, 609-613 (1993).

7. Pillay T.V.R., Aquaculture Development: Progress and Prospects, Fishing News Books,UK (1994).

8. Karima Morsi, Development of cost-effective denitrification system for aquaculture. Hamilton Sundstrand Summer Internship Program (HSSIP).29th August., 2002.

9. G. Tchobanoglous, F.L. Burton and H.D. Stensel, Wastewater Engineering: Treatment and Reuse, McGraw-Hill Professional, p.1848 (2003).

10. APHA, Standard Methods for Examination of Water & Wastewater (Standard Methods for the Examination of Water and Wastewater), (2001).

11. K.V. Lo, and P.H. Liao, Methane production from fermentation of winery waste, *Biomass*, 9, 19-27, (1986).

12. W.J.B. Saliling, P.W. Westermana and T. M. Losordo, Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations, *Aquacul. Engg*, 37, 222-233 (2007).

13. Metcalf and Eddy, Wastewater Engineering: Treatment and Reuse, Fourth edition, McGraw-Hills INDIA, ISBN: 0-07-041878-0 (2003).

14. W.L. Balderston and J M Sieburth, Nitrate removal in closed-system aquaculture by columnar denitrification, *Applied Environment* *Microbiology*, **32(6)**, 808-818 (1976)

15. Barber W.P. and Stuckey D.C., Nitrogen remov in a modified anaerobic baffled reactor (ABR): nitrification, *Water Research*, 34, 2423-243 (2000).

16. E Namkung. and B.E. Rittmann, Soluble microbi products (SMP) formation kinetics by biofilm *Water Research*, **20(6)**, 795-806 (1986).

17. Schiener P., S. Nachaiyasit and D.C. Stucke Production of soluble microbial products (SMP) i an anaerobic baffled reactor: compositio biodegradability, and the effect of proces parameters, *Environmental Technology*, **19**, 391 400 (1998).

18. Payne W.J., Reduction of nitrogenous oxides b microorganisms, *Bact. Rev.*, 37,409-452 (1973)

19. Rijn J.V., Y. Tal and H.J. Schreier, Denitrificatio in recirculating systems: Theory and application *Aquaculture Engineering*, **34(3)**,364-376 (2006

20. EPA Manual: Nitrogen Control. EPA/625/R-93 010 (1993)

21. Banihani Qais, Reyes Sierra- Alvarez, James. Field, Nitrate and nitrite inhibition methanogenesis during denitirification in granul biofilms and digested domestic sludge *Biodegradation*, **20**,801-812 (2009)

22. Hanaki Keisuke and Chongchin Polpraser Contribution of methanogenesis to denitrificatio with an upflow filter. *Journal of the Water Polluti* *Control Federation*. **61**,1604-1611 (1989)