CHAPTER 5
SUMMARY AND CONCLUSION

The major findings and conclusions in the order of their conduct are summarized below:

- Physio-chemical characterisation of aquaculture wastewater from shrimp aquaculture farms revealed that almost all observed parameters such as Nitrate, COD, BOD, Ammonia and total phosphate, exceeded the prescribed limits.

- The Chemical oxygen demand (COD) of the aquaculture wastewater ranged from 314 – 354 mg/L, while the Biochemical oxygen demand (BOD) ranged from 210 – 230 mg/L. The ammonia levels in the aquaculture wastewater were observed to be in the range of 8.6 – 11 mg/L. Ammonia together with organic nitrogen measured as total kjeldahl nitrogen (TKN) was observed to be in the range of 15-27 mg/L. The total phosphate observed in the wastewater was in the range of 15-18 mg/L. The nitrate and nitrite levels were in the range of 27-32 mg/L and 17-20 mg/L respectively. The significance of these observations is that aquaculture wastewater discharged in large volumes possesses a large potential for eutrophication affecting aquatic life in receiving water bodies. This warrants the need for the shift in production strategies to indoor systems and justifies the need
for the development of integrated aerobic anaerobic processes, particularly for the treatment and recirculation of nitrate laden aquaculture wastewater.

- Preliminary nutrient removal studies were carried out to determine feasibility of using coconut coir fibre as support medium in 1.5 L upflow anaerobic packed bed column bioreactors. Sand was used for comparison as inert support medium in a parallel bioreactor. During the 3 week study, influent nitrate from synthetic wastewater ranged from 10 mg/L to 74 mg/L. Maximum percentage removal was 69 % in reactor packed with coconut coir fibre and 55 % in reactor packed with sand. For further studies, use of sand as inert medium was discontinued as it caused clogging during operation. Synthetic "Fujino spirals" was used in its place for further studies. Also, carbon source was changed from Acetic acid to Methanol.

- The experiment was scaled up in a 3.5 L upflow anaerobic packed bed column bioreactor with flow rate of 6 L/d and HRT of 6.72 h. Carbon source used was Methanol, and contributed the required amount of COD. After startup, initial concentration of nitrate fed to reactors was 60 mg/L. After 10 weeks wherein the removal of nitrate reached 98 %, the loading rate was further increased to 120 mg/L, studied for a total period of 28 weeks at which stage, steady performance and maximum removal was obtained.
Nitrate nitrogen levels were significantly reduced. At OLR 60 mg/L nitrate loading, maximum removal recorded in both column reactors reached up to 97%. The average removal percentages were 86% and 80% respectively for column reactor packed with coconut coir and Fujino spirals as support medium. At 120 mg/L, nitrate removal was not significant till 13 weeks, reducing to only up to 80 mg/L. The reason was attributed to insufficient carbon source. On subsequent increase of carbon source from an initial amount of 0.5 ml/L to 1.5 ml of Methanol/L, nitrate removal became pronounced from the 16th week and reached a maximum of 99% removal on the 28th week. The average values taken from the 16th to the 28th week in terms of nitrate removal efficiency for column packed with coconut coir and Fujino spirals were 76% and 74% respectively.

COD removal rates reflected the trend of denitrification. At 60 g/L nitrate loading, it took an average time of 4 weeks for the COD removal to become stabilized to about 71% reduction. The following weeks showed COD removal in the range of 82% to 87%. The maximum COD removal recorded in column with coconut coir as medium was 87% and with the column packed with Fujino spirals as support, it was 82%. The average removal percentages were 75% and 61% respectively. At OLR 120 mg/L nitrate, the maximum COD removal recorded in column with coconut coir was 81%, and the maximum COD removal recorded in column with Fujino spirals as support was 72%. The average removal rates were 74% and 72% respectively.
- Phosphate removal was not expected in the higher range. At 60 mg/L Nitrate concentration, the maximum phosphate removal recorded in column with coconut coir as support was 54% and with the column packed with Fujino spirals as support, it was 55%. The average removal were 49% and 47% respectively. At 120 mg/L, the maximum phosphate removal recorded in column with coconut coir was 60%, and the maximum phosphate removal recorded in column with Fujino spirals as medium was 63%. The average removal rate for both was 47% respectively.

- Physical and biochemical tests were conducted on coconut coir and Fujino spirals (where applicable) after 28 weeks of nutrient removal studies.

- During testing of tensile strength/breaking elongation, 6.35 N of energy was required to break a raw coconut coir fibre while only the energy required to break a fibre after 28 weeks of exposure to reactor conditions was 3.78 N. The retention of residual tensile strength after prolonged exposure is a useful indicator of the physical durability of coconut coir.

- Scanning electron microscopy carried out for both support medium at their raw/plain state and after 28 weeks showed significant attachment of biofilm. Changes in the percentage compound weight of elements on the surface of biofilm were also carried out by Energy dispersive X-ray analysis that showed a clear increase in certain elements, characteristic of microbial activity.
Biochemical tests carried out on coconut coir fibres showed good retention of moisture of upto 0.6 %, an increase of 0.53 % from an initial of 0.07 % in the raw fibre. The fibre had an initial solids content of 99.93 % which remained largely unaffected and ash analysis showed the added contribution of biomass as it increased from 0.97 % in raw fibre to about 6.64 % in the fibre post-nutrient removal studies.

The most important results which imply a demonstration of the structural integrity is the amount of total percentage lignin (acid soluble and acid insoluble) which reduced to 45 % from an initial of 61 % in the raw coconut coir fibre during the course of the study.

Molecular identification of denitrifying microorganisms from genomic DNA extracted from respective sampling locations of upflow anaerobic packed bed bioreactors was carried out. PCR amplification done to genomic DNA pooled from various regions of the upflow anaerobic packed bed columns showed positive results against specific primers for nirK, nirS (nitrite reductase genes) and nosZ (nitrous oxide reductase genes). Direct sequencing of the elutes from the positive bands showed greater than 90 % similarity to several prominent denitrifying species namely *Alcaligenes xylosoxidans*, *Paracoccus* sp., *Nitrospira* sp., *Ochrobacterium* sp., *Halomonas denitrificans* strain DSM 18045, *Cupriavidus* sp. R-31544, *Ralstonia eutropha*, *Comamonas denitrificans*.

Coconut coir fibre as support medium was chosen for further studies in a laboratory scale anaerobic baffled reactor. The maximum COD removal obtained in coconut coir packed ABR was 96 % while the control ABR only obtained 65 % of
removal. The maximum TKN removal obtained in coconut coir packed ABR was 95 % while the control ABR only obtained 61 % of removal. On an average, the removal was 57 % in coconut packed ABR and 38 % in control ABR. The maximum percentage ammonia removal obtained in coconut coir packed ABR was 97 % while the control ABR obtained 95 % of removal. On an average, the ammonia removal was 82 % in coconut packed ABR and 72 % in control ABR.

- Post-anaerobic treatment studies using aeration by cascading method was used and showed a resumption of dissolved oxygen in the effluent from ABR by up to 7.8 mg/L.

- During the nitrate removal studies at both 60 mg/L and 120 mg/L of influent nitrate in the synthetic wastewater, the performance of the column packed with coconut coir has been consistent and marginally better than Fujino spirals. This is a significant observation and has been attributed to the organic nature of coconut coir, which could have added some COD, thus lessening the net carbon requirement. The presence of micronutrients such as Ca, K, Mg, Na, etc., in a bacterial support medium could also have served as an influencing factor for denitrification, which is independent of the nutrients obtained from the wastewater going into the reactor system. Such advantages cannot be observed in synthetic medium though they may offer non-degradability and increased surface area. This is a perceived advantage of the consistent and stable performance of Coconut coir fibre in the present study.
The molecular identification of denitrifiers by PCR amplification and subsequent identification has important implications, particularly with regards to the positive detection of nirK and nirS genes within the population. The presence of this gene is indicative of the possibility of anammox bacteria within the population and the possibility of dissimilatory nitrate reduction (DNRA) processes. The latter possibility also supports the hypothesis of residual ammonia observed in the ABR and the nitrogenous content measured as TKN in the upflow anaerobic packed bed columns.

The work done in this thesis clearly outlines the significant comparibility of a commonly available material (coconut coir fibre) for implementation, in place of conventionally used expensive synthetic support medium. The findings of this thesis have led to a conceptualization of an integrated anaerobic aerobic process for the treatment of nitrate laden aquaculture wastewater, based on concrete experimental results. The relevance and corresponding relations drawn between the findings of this thesis and the principles of environmental sustainability are expected to be an important reference point for transition from traditionally practiced outdoor aquaculture to the widespread implementation of intensive indoor aquaculture with mandatory recirculating systems. A scoresheet model for evaluation has been presented to this effect.

Future studies radiating from this thesis work should address several of the shortcomings encountered during this work, when taken up for implementation. Much of the experiments
have been conducted in carefully controlled laboratory conditions and their comparative performance can be evaluated in the field scale, where the conditions are dynamic.

- In this study, the adsorptive properties and leaching potential of the bacterial support medium (coconut coir fibre) were unaccounted. It should be considered important in future studies to have an approximate estimation of the role of adsorption and leachability of the naturally derived bacterial support medium in order to optimally reduce the amount of carbon source actually required to achieve complete denitrification.

- The conceptual model presented in this thesis shall be significantly benefited by the inclusion of a phosphorus removal process which could not be effectively removed by the anaerobic process in this study. The conceptual model would also be benefited by proper incorporation of alternate sources of energy for required pumping and transfer operations during treatment and recirculation. It can be suggested from the review of literature done in this thesis that seaweed culture be incorporated as an additional treatment. This can also give an additional economic incentive for aquaculture wastewater treatment.