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## **Chapter 7 ~ Conclusions and Recommendations**

## 7.1 Conclusions

A number of conclusions can be drawn from the research presented within this thesis. The key conclusions are summarised as follows:

- 1. The volatilization of ammonia accounted for essentially negligible permanent nitrogen removal from the PFP's studied. However, slightly more ammonia was volatilized from the pond during the winter months than the summer months.
- The physical characterization of the ponds revealed that both ponds were operating with an arbitrary flow regime and showed large degrees of hydraulic short-circuiting. The mean retention time (t<sup>-</sup>) of the Rhodamine WT dye in the system was shorter than the actual observed hydraulic retention time (θ)\*.
- 3. In both of the winter <sup>15</sup>N-spike tracer studies, the largest nitrogen fraction recovered was the unchanged <sup>15</sup>NH<sub>4</sub><sup>+</sup> fraction, which mimicked the passage of the Rhodamine WT dye exiting the pond system.
- 4. The stable isotope tracer studies revealed that the predominant nitrogen removal mechanisms and pathways in the Esholt PFP's was the uptake, and incorporation of <sup>15</sup>NH<sub>4</sub><sup>+</sup> into suspended organic-nitrogen, which was followed by a concomitant increase of <sup>15</sup>N soluble organic-nitrogen. Actual mass balances for all experiments reveal that the deposition of nitrogen through sedimentation played a key role in 'removing' nitrogen from the PFP's. The <sup>15</sup>N mass balances however, do not corroborate this.
- 5. In the winter experiments, influent  ${}^{15}\text{NH}_4^+$  rapidly underwent nitrification into  ${}^{15}\text{NO}_3^-$ , and was detectable at the PFP effluent point within the first two days after spike injection.
- 6. The incorporation of <sup>15</sup>N into cellular biomass, in these experiments, was less pronounced in the winter than in the summer.

<sup>\*</sup> As defined by equation A.4, in Appendix A.

7. PCR and DGGE molecular microbiological tools revealed that there was a large variety of nitrogen-utilising bacterial communities within all parts of the PFP. These included nitrifiers, denitrifiers and the novel anammox bacteria. It is highly possible that classical nitrification and denitrification processes occurred simultaneously within the PFP's, and can contribute to overall permanent nitrogen removal from the WSP system.

## 7.2 Recommendations for further work within the scope of this research

With respect to nitrogen removal processes within the ponds at Esholt, the following ideas presented here, if investigated, would provide an even greater understanding of processes acting within the system. The application of these findings would mainly enhance and provide more resolution of the data already collected and presented herein, but also contribute to and highlight any necessary steps which could be implemented by engineers to tangibly enhance the efficacy of pond design not only on an individual unit level, but also in the fabrication of whole pond systems.

There were many parameters, mechanisms and pathways which would have been very useful to investigate, but unfortunately time and circumstances did not allow. This work has without doubt produced many more questions than answers; therefore the recommendations for further research include:

- The comprehensive investigation of sludge feedback mechanisms, and interactions occurring at the sludge/water interface.
- 2) The analysis of hydrolytic processes occurring in the sludge layer, and the quantification of the recycling of nutrients from the sludge layer.
- 3) Formation of an algorithm to explain how nitrogen is removed with respect to the functions of pH, temperature and insolation (among other environmental and in-pond variables) for PFP within the UK.
- 4) More detailed and comprehensive study of nitrogen using microorganisms distributed throughout the pond using advanced molecular microbiological techniques to test for a wider range of speciation within individual groups.

- 5) The introduction of a labelled organic nitrogen spike to the pond, to isolate and succinctly examine the fate of organic nitrogen within the system.
- 6) The introduction of a labelled oxidised form of nitrogen, to determine if and how transformations of this fraction occur within PFP's.
- 7) A comprehensive gas sampling survey to reveal the quantities of  $N_2$  and  $NO_x$  leaving the surface of the pond in order to better refine the mass balance analysis approach towards total nitrogen removal.