Chapter 3 ~ Previous Research Conducted with respect to Nitrogen Removal on the Esholt Primary Facultative WSP

3.1 Introduction

Full details of the design, construction, fabrication and initial start-up of the pilot-scale primary facultative WSP used for this research, are given in Abis (2002). In summary, the ponds are located within the grounds of the Esholt Wastewater Treatment Works in Bradford part of Yorkshire Water plc. Three pilot-scale primary facultative ponds, codenamed the Red pond, Green pond, and the Blue pond and operating in parallel, were initially built on the site and commissioned in July 2000. In 2002, two pilot-scale maturation ponds and a reed bed were added. Further additions included three rock filters, constructed and commissioned in August 2003. It became evident that the two maturation ponds were underloaded and so in 2004 one of the maturation ponds was divided, to create two equal-sized maturation ponds operated in series.

Each primary facultative pond was filled with humus tank effluent and fed with preliminary treated raw sewage (having undergone screening and grit removal). This was pumped from the adjacent works inlet channel by individual Watson Marlow model 604 S/R Washdown peristaltic pumps (Watson Marlow Bredel Inc., Wilmington) with internal 12-mm Marprene tubing. The screened sewage was delivered to the ponds through 15.9-mm bore, 3-mm wall, reinforced Nalgene 980 PVC tubing (Abis, 2002). Attached to the end of this pipe, which extended into the screened wastewater channel, 6-mm mesh hose tail strainers were modified and connected by jubilee clips. This was to prevent further debris from the screened wastewater, being pumped from, and thus blocking, the small internal bore of the tubing which would terminate the flow to the system.

3.2 Ammonia nitrogen removal from the primary facultative WSP operating under normal conditions

Abis (2002) tested the three facultative ponds within a loading range of 63 to 169 kg BOD/ha day over varying seasonal conditions, and found the most appropriate surface BOD loading for the UK climatic conditions to be 80 kg/ha day. During the time of this research, although the loading rates were varied across the ponds, the hydraulic retention times were not, and remained similar for each of the three ponds.

Abis (2002) succinctly documents ammonia-nitrogen removal from the Esholt WSP in her PhD thesis during the experimental study period from July 2000 to March 2002. Ammonia-nitrogen was the only nitrogen fraction measured throughout this study from pond influent, in-pond column and pond effluent samples. Visible fluctuations in the data were observable for influent ammonia concentrations − for example, these were typically higher in the summer months than in the winter months. The seasonal influence was also found to have implications within the ponds, as ammonia removal efficiencies were higher in the summer periods than winter periods in the second year of pond operation, whereas no visible effect of ammonia change with respect to seasonality was observed in the first year. This was attributed to the pond establishing a regime after the start-up period, the lack of an established sludge layer, and duckweed infestations which swamped the pond surface – conditions completely opposite to those occurring in the second year (Abis, 2002). As is presented in much of the literature reviewed in Chapter Two, Abis (2002) also found a strong correlation between an increased surface ammonia removal (g/m^2 d), pH and temperature values measured from the surface of the ponds. The data collected also provided evidence that facultative conditions are required for the removal of ammonia (Abis and Mara, 2003).

A relationship was also observed between ammonia removal and the chlorophyll-*a* concentration: when the former increased, so did the latter. The data suggested a fit with the Pano and Middlebrooks (1982) model for ammonia removal. In practice, the summer ammonia results obtained from the Esholt ponds during the first year of pond operation were much lower values than the values obtained from the Pano and Middlebrooks (1982) model; however, the winter results did strongly correlate with the model. In the second year, both summer and winter data sets agreed closely with the model values, although the summer results were still lower than the predicted values. It must be noted that the rate of ammonia volatilization was not actually measured by Abis (2002). It can therefore only be speculated from her research that ammonia removal by volatilization may have been a possible route for overall nitrogen removal from the system.

3.3 The effect of hydraulic retention time on ammonia nitrogen removal from the primary facultative WSP

Initial research conducted by Abis (2002) suggested that there was a relationship between hydraulic retention time (θ) and ammonia concentration in the pond effluent in the summer months only. During this time, the hydraulic retention time was not manually controlled; natural variations in hydraulic retention time occurred principally through the strength of the raw influent sewage feed, according to the relationship between strength of influent wastewater, influent flow rate and pond volume, all of which are parameters synonymous with pond loading and hydraulic retention time. Influent ammonia concentrations typically varied with seasonal changes; influent concentrations were observably higher in the summer than in the winter. A graphical trend showed that in the summer months ammonia removal generally lessened as the retention time increased (Abis, 2002), but that the effect of hydraulic retention time bore no significance in winter months. It must be observed that at this time hydraulic retention time and pond surface loading had not been isolated and investigated as separate experimental variables.

In a second research phase, which spanned from April 2003 to March 2004, all three primary facultative ponds were loaded with a fixed influent BOD of 80 kg/ha d, but the hydraulic retention times were varied over θ values between 20 and 60 d (Abis and Mara, 2005). The alteration of hydraulic retention time was achieved by diluting the influent BOD stream, by mixing the sewage feed with the introduction of a fresh water flow (further details are given in section 4.2.1 of this thesis). Results from this study revealed that there was no loss of pond performance with respect to ammonia removal, even when θ = 20 d. It was noted that there was no seasonal variability between influent ammonia concentrations during this 11-month period. An average of around 50% ammonia removal from the ponds was achieved in summer and winter conditions (Abis and Mara, 2005), although it is important to stress that both DO and chlorophyll-*a* values were also higher during this year in comparison with the first two years of pond operation; both these parameters have direct affects on ammonia removal within a WSP system.

3.4 Ammonia volatilization

Preliminary ammonia volatilization research was undertaken on one of primary facultative ponds over a 13-week period in the spring, and on one maturation pond, over an 8-week period in the summer of 2004 on the Esholt pilot scale WSP by Epworth (2004). A Perspex chamber measuring 33 × 34 × 48cm with a surface area of 0.1122 m^2 was constructed and suspended over each pond in turn (Epworth, 2004), following a similar methodology devised by Peu *et al.* (1999) and Zimmo *et al.* (2003). The completed design, including the various modifications made throughout the fabrication and testing process, is depicted in Figure 3.1. A peristaltic pump was attached from a length of tubing which ran from inside the box to the pump house; this drew air through the box and pushed it through an ammonia absorbtion system, comprised of a Dreschler train of three conical flasks, each containing 100 ml of 2% boric acid (H_3BO_3), where ammonia was stripped from the air and retained within the boric acid.

The volatilization results gained from this study showed that only a minute proportion of ammonia was captured; the average volatilization rate measured over the 13-week spring period for the primary facultative pond was 0.019 g/d which equated to an average of 0.19% of the total nitrogen removed from the pond. Over the summer period for the maturation pond, the average amount of ammonia volatilized was 0.004 g/d, only an average of 0.18% of total nitrogen removed from the pond.

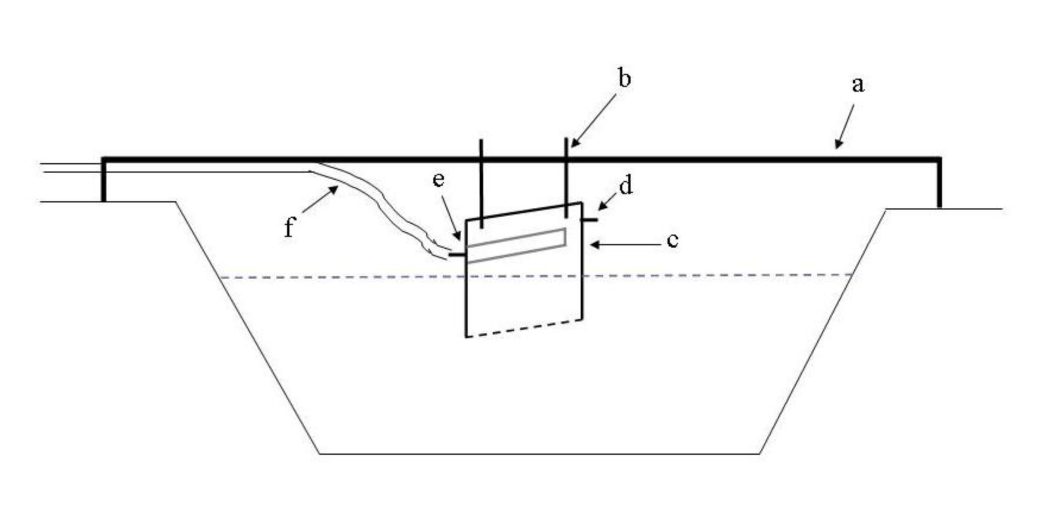

Figure 3.1: A cross sectional view of the final design of the ammonia volatilization capturing chamber showing modifications, where (a) is the steel suspension rig, (b) are the metal mounts for the chamber, (c) is the Perspex volatilization chamber, (d) is the air inlet, (e) is the condensation trap inside the chamber, attached to the chamber outlet, and (f) is the gas/condensation draw off tube from the chamber, to the pump and boric acid scrubbing solution.

When the data from the facultative and maturation pond effluents were compared to the values calculated by the Pano and Middlebrooks (1982) ammonia-stripping model, the Esholt data did not correlate well; only a very weak positive correlation was found between the actual and model values. In almost all cases, both ponds experienced less ammonia volatilization than the model predicted (Epworth, 2004).

From the results gained and commented upon by Epworth (2004), no definitive answer could be established regarding the exact nature of nitrogen removal mechanisms and pathways at work within the Esholt ponds. It was suggested that ammonia removal within the facultative and maturation ponds occurred by algal assimilation, followed by algal die-off and sedimentation, as well as through simultaneous nitrification and denitrification processes.

3.5 Nitrification

The results obtained by Abis (2002) suggest that nitrification can be discounted as an active and major participatory pathway in the removal, or rather the conversion, of ammonia within the facultative ponds. Data obtained for the study period showed that nitrite concentrations were less than 0.01 mg N/l, and nitrate concentrations were consistently less than 0.7 mg N/l in the pond effluents (Abis, 2002). The results from the microbiological methods detailed in section 4.7 in this thesis will reveal an affirmative presence or absence of nitrifying bacteria, enablling a valid conclusion to be reached; they will also reveal if nitrification processes are being masked by denitrification or other biochemical workings. Abis (2002) concluded that the data gained, supported the hypothesis that algal uptake and/or ammonia volatilization were the principal pathways for ammonia removal within the ponds.

Once again, the data collected by Epworth (2004) provide further evidence to suggest the oxidation of ammonia, via nitrification, was absent. During the 13-week study period for the primary facultative pond, 7 pond influent and 7 pond effluent samples were analysed for nitrite and nitrate. The maximum value obtained for influent nitrite was 0.061 mg nitrite-N/l, and 0.068 mg nitrite-N/l in the pond effluent. No nitrate-N was detectable in either the pond influent or effluent samples analysed.

3.6 Ammonia removal by β-subdivision ammonia-oxidising bacteria

Kartal (2002) undertook a molecular microbiological study of the Esholt WSP to determine if β-subdivision ammonia-oxidising bacteria were present in the water columns of the ponds. The three facultative and the two maturation ponds were sampled and analysed. During the study period, the Green pond was operating with a surface BOD loading of 80 kg/ha d and a retention time of 81 d; and the Blue pond was loaded at 100 kg/ha d with a retention time of 60 d. These long retention times may have allowed for the formation of ammonia-oxidising bacterial communities to develop. Chemical data collected did not detect nitrite and nitrate in pond influent and effluent samples.

Pond column samples were analysed and two primers used to target Eubacterial 16S rDNA; then two more primers were used to amplify the region specific to ß-proteobacterial ammonia-oxidizing bacteria (Kartal, 2002).

A number of bands from various DGGE gels were excised, PCR amplified and, once purified, run again in a DGGE gel. The cleanest bands yielded from this process were selected and sequenced and matched in FASTA (a DNA sequencing and alignment database); this returned results of organisms similar to those found on the database.

The sequences revealed that the bacteria found within the Esholt PFP's were similar to β-proteobacteria, but none of them was similar to the β-subdivision ammonia-oxidizing bacteria. Phylogenetic analysis further revealed that no β-subdivision ammonia-oxidizing bacteria were found within the water columns of any of the Esholt WSP; therefore the oxidation of ammonia did not occur via this possible in-pond route for nitrogen transformation and, ultimately, removal. It is possible that the techniques used to assess the ammonia-oxidisers from the β-proteobacteria were not sensitive enough (Kartal, 2002) and therefore did not pick up any β-subdivision ammonia-oxidizers present. A number of other options could have been tried − one being, if time had been available, to use a different range of primers to target the bacteria. It must also be mentioned that two different stains were used during the DGGE process: one was SYBR Green I and the other a silver staining technique. It is not possible to excise bands from the silver-stained gel process for sequencing, which is a drawback, as various bands, which were not visible on the SYBR Green I stained gel, were detectable under the silver stained gel. If it had been possible to excise these bands, β-subdivision ammonia-oxidizing bacteria might have been detected.