

9. GENERAL DISCUSSION

Feasible transformation pathways and removal mechanisms for nitrogen control in domestic wastewater treatment by WSP could include: (a) ammonia volatilisation, (b) biological nitrogen uptake, (c) nitrification, (d) denitrification, and (e) sedimentation of dead biomass and accumulation on sludge layer (Craggs, 2005). However, nitrogen removal in WSP systems has been mainly attributed to ammonia volatilisation and sedimentation of organic nitrogen (Pearson, 2005). Unfortunately current evidence is far from being able to determine which mechanism(s) dominate(s) nitrogen removal in WSP and most importantly, under which operational and environmental conditions. This chapter summaries new evidence found as part of the research work undertaken, which will contribute to improve our understanding about the fate of nitrogen compounds in WSP.

9.1 Ammonia Volatilisation in WSP: A Paradigm Shift

Before this work, ammonia volatilisation had generally been reported as the main nitrogen removal mechanism in WSP, or in many cases when a nitrogen mass balance did not coincide because of nitrogen losses, it was assumed that nitrogen was lost to the atmosphere as ammonia gas (e.g., Hemes and Mason, 1968). This hypothesis was based on two observations: (a) in-pond pH values can reach high values (>9, even >10), so increasing the proportion of the total ammonia present as the un-ionized form or free ammonia (NH₃), and (b) in-pond temperatures can also be high, so improving the mass transfer rate of free ammonia to the atmosphere. One of the most widely accepted models for ammonia removal in WSP was developed by Pano and Middlebrooks (1982) under the same two hypotheses.

Krefx *et al.* (1958) published a short note on the possibility of removing ammonia from sewage effluents by raising the pH. They were working on sewage works effluent and found that ammonium disappeared from solutions of the order of 20 to 50 mg N/l within hours when these solutions were buffered to a pH of over 8.4; whilst at pH values of 4.5 to 6.5, there was no appreciable disappearance after a week (Harrison *et al.*, 1960). Indeed, the chemical equilibrium between ammonium and free ammonia makes ammonia

volatilisation a feasible mechanism for ammonium removal in alkaline waters (see Table 2.2).

Stratton (1968) determined ammonia losses in an open channel ($0.15 \times 6.00 \times 0.03$ m) where synthetic river water was circulating along a closed loop system (i.e., 48 litres of distilled water enriched with ammonium sulphate (20 mg NH_4^+ -N/l) and potassium phosphate buffer). Mass transfer coefficients for ammonia volatilisation rates were calculated under different pH values (7.5, 8.0, 8.5, 9.0 and 9.5) and water temperatures (15, 20, 25 and 30°C). Results showed that on average, 50 percent of ammonium was removed in 14 hours (at pH = 8.5 and T = 20°C). Subsequently, Stratton (1968) reported a model to predict ammonia mass transfer coefficients and concluded that considering the fact that algae are capable of increasing pH in surface water bodies (up to 10), it could be expected large ammonia losses to the atmosphere in shallow streams with growth of algae and water plants.

In order to confirm that hypothesis, Stratton (1969) carried out outdoor experiments subjected to favourable natural conditions for ammonia volatilisation (i.e., summer conditions). Firstly a set of four large battery jars (6 litres each) containing synthetic lake water (i.e., deionised water enriched with ammonium nitrogen (20 mg NH_4^+ -N/l) and 1M phosphate buffer to pH 7.5, 8.0, 8.5, 9.0 and 9.5, separately) were placed outdoors; ammonia nitrogen determinations were made every 48 hours for about 21 days. Results were in agreement with previous work with synthetic river water (Stratton, 1968) and high ammonia volatilisation rates were reported (e.g., ~2,153 g N/ha d at pH=8.5, T= 19.6°C and ammonia concentration of 10 mg NH_4^+ -N/l). No evidence of biological activity during both experiments with synthetic alkaline waters was reported.

Secondly, Stratton (1969) made direct measurements of gaseous ammonia nitrogen liberated through the surface of two small reservoirs (~2 ha each) by using a floating cell and acid trap (i.e., similar principles but different design than the one developed in this research work; see chapter 6). Site 1 was Elfin Forest Lake (Escondido, CA) and site 2 was a pond receiving the effluent from a wastewater treatment plant (Santee, CA). Tests were conducted for a period of one hour and sampling points were selected based on the presence of high algal activity to guarantee high pH values.

The measured rates of ammonia losses in site 1 and 2 were considerably below the predicted volatilisation rates from outdoor experiments with synthetic lake water (approximately 14% and 23% of the predicted value, respectively). For instance, the average ammonia volatilisation rate for site 1 was 513 g N/ha d ($9.5 < \text{pH} < 9.8$; $28 <$

$T < 29^{\circ}\text{C}$); that figure did not consider diurnal pH variations and hence, the average daily ammonia volatilisation rate would be expected to be considerably lower. Nevertheless, it was estimated that ammonia volatilisation would contribute to remove only ~5.8 percent of the total daily influent ammonia entering Elfin Forest Lake.

Stratton's work (1968, 1969) gave a clear panorama about the possibility of removing ammonia from alkaline waters. Therefore, ammonia volatilisation could be considered the main mechanism for ammonium removal when waters are buffered to pH values higher than 8.5, but and most importantly, very low ammonia volatilisation rates should be expected in alkaline waters with high algal activity such as in-pond WSP waters.

Paradoxically, the model to predict ammonium removal in WSP reported by Pano and Middlebrooks (1982) was based on Stratton's work with synthetic river and lake waters (Stratton 1968; 1969), and therefore ammonia volatilisation was assumed as the main nitrogen removal mechanism. The resulting equation reported by Pano and Middlebrooks (1982) is actually a simple first-order equation for a completely mixed reactor with three key parameters: pH, water temperature and hydraulic loading rate (retention time). Their model was calibrated against monthly mean ammonium concentrations from pond influent and effluent, and although many authors have reported good results for predicting ammonia removal in facultative and maturation ponds by using Pano and Middlebrooks' model (e.g., Silva *et al.*, 1995; Soares *et al.*, 1996), it does not provide any further information on the nature of the nitrogen removal mechanisms involved.

In fact, any agreement of field results with values predicted by Pano and Middlebrooks' equation only shows that ammonia removal has a statistically significant relationship with pH, water temperature and hydraulic loading rate, which is very common to most biochemical reactions that involve nitrogen (e.g., biological nitrogen uptake) as they will respond strongly to these three factors as well as ammonia volatilisation does (Reed, 1985).

Models based on ammonia mass transfer from water pond to the atmosphere have been used to calculate theoretical ammonia volatilisation rates (e.g., Rockne and Brezonik, 2006); however, none of these models had been calibrated or validated by means of direct measurements of ex-pond ammonia volatilisation rates in situ. The prediction of ammonia mass transfer coefficients based on the chemical equilibrium of ammonium-ammonia (e.g., Stratton's equation in Ferrara and Avci, 1982; and Zimmo *et al.*, 2003) does not include the influence of strong interferences on free ammonia concentration (e.g., phytoplantonic activity). Therefore, it is only to be expected that theoretical models for

ammonia volatilisation rates would not be able to predict ammonia removal in maturation ponds.

In this research project, mean ammonia volatilisation rates measured from alkaline synthetic water in the laboratory were 2,517 g NH₃-N/ha, whilst ammonia volatilisation rates measured on site in the pond M1 ranged from <1 to 27 g NH₃-N /ha d in summer and from <0.4 to 2.0 g NH₃-N/ha d in winter; these figures were much lower than the calculated theoretical values (107–812 g NH₃-N /ha d in summer) (see chapter 6). For these reasons, models based on mass transfer coefficients for the ideal water–ammonia–air system overestimate ammonia losses to the atmosphere, simply because they ignore the fact that the water in a WSP is a very complex matrix where ammonia is involved in more than one transformation pathway simultaneously. High pH and water temperature values should not necessarily favour ammonia volatilisation over alternative mechanisms such as algal uptake. Moreover, results from tracer experiments with ¹⁵N stable isotopes reported any ¹⁵N enrichment in the small fraction of ammonia nitrogen liberated from the tested maturation pond (see chapter 8).

In this research work, algal uptake was clearly identified as the major mechanism for ammonium removal. In agreement with the hypothesis formulated by Pearson *et al.* (1988), as an increasing pond water temperature increases phytoplanktonic activity and consequently, in-pond algal biomass would take up and remove ammonium at a faster rate than expected via ammonia stripping. The increment of pH in WSP is a consequence of algal activity and it makes a small contribution to ammonia volatilisation as ammonia concentration drops due to algal uptake.

9.2 Biological Nitrogen Uptake and Sedimentation of Dead Algal Biomass

WSP systems are based on biochemical processes occurring naturally in water bodies; therefore, it would be expected that mechanisms controlling nitrogen transformations and removal in natural environments would also play an important role in WSP systems. The results of ¹⁵N tracer experiments in an undisturbed natural stream showed that biological nitrogen uptake and retention of nitrogen in fine benthic organic material may be highly efficient as it represented approximately 32 percent of the spiked ¹⁵N-labelled ammonium (Askemas *et al.*, 2004). Zhang *et al.* (2008) studied the dynamics of nitrogen in a large shallow eutrophic lake in China, where a great amount of industrial wastewater and domestic sewage were discharged into the lake; they reported positive correlation between ammonium fluxes and algae biomass and chlorophyll-*a* concentrations and

concluded that higher fluxes of ammonium may support a higher biomass of the phytoplankton and consequently, a higher algal nitrogen uptake rate.

Algal nitrogen uptake and the subsequent sedimentation of the biologically incorporated organic nitrogen were early reported as the principal mechanism for total nitrogen removal in WSP by Ferrara and Avci (1982). In fact, maturation ponds are mainly designed to provide favourable conditions for algal growth (i.e., shallow ponds, low organic loadings and long retention times) and it would be expected that algal activity plays an important role in all algae-induced processes – for example, faecal bacteria removal and nitrogen transformation and removal.

In fact, the results reported in this research work (see Chapter 7) showed that algal uptake of inorganic nitrogen species and further sedimentation of dead algal biomass is clearly one of the major mechanisms controlling ammonium and total nitrogen removal in WSP, particularly when environmental and operational conditions are favourable for algal growth. The increment of chlorophyll-*a* in the maturation pond effluents under study undoubtedly indicated an increment of VSS, whilst a detailed analysis of collected data indicated that the nature of the organic nitrogen fraction in VSS from the maturation pond effluents (M1 and M2) was mainly algal biomass and consequently, that revealed the occurrence of biological (algal) nitrogen uptake. Sedimentation rates of organic nitrogen (dead algal biomass) were also in agreement with total nitrogen removal rates under summer conditions.

Considering that mean nitrogen content in VSS from M1 and M2 effluents was 9.8 and 10.0 percent respectively, it could be assumed that the nitrogen content in ‘fresh’ dead algal biomass is about the same figure; however, once dead algal biomass reaches the bottom of the pond, anaerobic digestion of pond sediments partially recycles ammonium nitrogen to the water column and the nitrogen content in sediment and sludge samples was consistently lower. During the experimental timeframe, nearly 49 percent of the total settled organic nitrogen was recycled to the water column in the M1 pond, whilst 61 percent was recycled in the M2 pond. Therefore, it is to be expected that net total nitrogen removal rates by sedimentation of organic nitrogen would be lower than those achieved by other mechanisms leading permanent nitrogen removal (e.g., nitrification-denitrification), as most of the ammonium nitrogen removed by algal uptake would be washed out as suspended solids in the pond effluent

It is well known that suspended solids and BOD concentrations may rise in the effluent of conventional WSP systems, mainly because of carbon fixation during algal growth, and

therefore the final effluent may not meet its discharge consent. However, promoting algal growth is the very foundation of wastewater treatment by WSP. For that reason, upgrading technologies have been evaluated in order to control suspended solids leaving WSP in the final effluent (Middlebrooks *et al.*, 2005). Suspended solids removal from WSP effluents would be also beneficial for upgrading nitrogen removal, as most of the ammonium removed by algal uptake is incorporated in cell tissue. Results reported in this research work show for instance that the total nitrogen removal in maturation ponds M1 and M2 could be enhanced (by up to 82%) by removing algal biomass from the corresponding pond effluent.

9.3 Nitrification-Denitrification: The 'X' Factor in WSP

In view of the low nitrate and nitrite accumulation in WSP, it had been suggested that nitrification is not likely to be performed in maturation ponds despite prevalent in-pond aerobic conditions. However, this does not prove that nitrification does not proceed in WSP, but it may suggest that nitrification could be an intermediate step if it does occur (Reed, 1985). Although simultaneous denitrification would help to explain the apparently absence of nitrification in WSP, the concurrence of an anaerobic (or anoxic) environment for nitrate reduction and an aerobic environment for ammonia oxidation was described as unlikely to happen in WSP systems by Reed (1985).

Ferrara and Avci (1982) also did not consider nitrification-denitrification as an important mechanism for permanent nitrogen removal in WSP. Their conclusions were based on: (a) the concentration of nitrifiers in the upper zone of maturation ponds was not sufficient for significant oxidation of ammonia; (b) the availability of aerobic surface area to facilitate attachment and growth constituted a difficult barrier to be overcome by nitrifying microorganisms; and consequently (c) in-pond nitrate production would be expected to be very low. Although they considered that denitrification in the sediment zone could be a feasible mechanism for nitrogen removal, the progress of the denitrification process would be restrained by in-pond nitrate availability.

Finally, Ferrara and Avci (1982) stated that denitrification could make a major contribution to nitrogen removal in WSP only if nitrate was supplied to the pond via the influent. Somiya and Fujii (1984) reported denitrification in a pilot-scale WSP system (4 ponds in series: $7.00 \times 1.00 \times 0.75$ m, each) fed with a nitrified secondary effluent ($7.66 \text{ mg NO}_3^- \text{-N/l}$). In that case, denitrification did perform very well and become the main

removal mechanism as it accounted for 100 percent of the total nitrogen removal (nitrate removal = 2.26 kg N/ha d; total nitrogen removal = 2.24 kg N/ha d).

Nowadays, it has been demonstrated not only that nitrifiers can grow in WSP (up to 10^7 organisms/ml in in-pond water samples; Morrison, 1984), but also that high concentrations of nitrite and/or nitrate (up to 6 mg N/l) can be found in pond effluents which usually correspond with high ammonium nitrogen removals (Hurse and Connor, 1999). Tracer experiments with ^{15}N -labelled nitrogen compounds carried out in this research project showed that oxidised forms of nitrogen (nitrite and nitrate) were highly enriched with ^{15}N both in summer and winter, even though there was no nitrite or nitrate accumulation in the maturation pond under study (see chapter 8). Such evidence helps to show that nitrification does occur in WSP, despite low nitrite and nitrate concentrations in the pond effluent.

Nitrate and nitrite concentrations do not increase in pond effluents because nitrification may be masked by simultaneous biochemical reactions such as biological nitrate uptake and/or denitrification; therefore, nitrification can definitely be considered as an intermediate step in nitrogen transformation and removal in WSP. A net accumulation of oxidised nitrogen species (nitrite and nitrate) could be expected in the pond effluent, if environmental and operation conditions in WSP system were less favourable for algal growth and for the performance of the denitrification process.

Such conditions could be found in the final treatment units of a WSP system with a large number of ponds connected in series. The final ponds would receive lower ammonium and biodegradable organic matter (soluble BOD) loadings – ammonium is important for algal growth, whilst soluble BOD is essential as an organic carbon source for denitrification. In fact, a study case reported by Lai and Lam (1997) could help to consolidate this hypothesis: they found a net increment of nitrate and nitrite concentrations from samples collected in the effluent of the final ponds of a WSP system with eight ponds in series (Melbourne, Australia) during late autumn and winter.

Tracer experiments with ^{15}N -labelled ammonium in summer 2005 (sections 8.2.1) showed that nitrification was mainly masked by simultaneous biological nitrate uptake. That is based on the following observations: (a) $^{15}\text{NH}_4^+$ was mostly transformed into suspended organic nitrogen due to high algal activity, which dropped ammonia concentration to values as low as 0.6 mg $\text{NH}_4^+\text{-N/l}$ and kept high in-pond dissolved oxygen levels; (b) ^{15}N -labelled ammonium was also oxidised simultaneously to nitrite and nitrate; (c) there was no evidence of ^{15}N enrichment in gases leaving the maturation

pond to the atmosphere, so no ammonia volatilisation or denitrification was significantly performed, (d) a cumulative ^{15}N mass balance showed that the tracer was completely recovered after the experimental timeframe ($3 \times \theta_0$); and (e) there was no nitrate and/or nitrite accumulation in the pond effluent. Therefore, denitrification was unlikely to be performed and oxidised nitrogen species (nitrate and nitrite) may supply nitrogen requirements for algal growth as ammonium concentration was very low.

On the other hand, tracer experiments with ^{15}N -labelled ammonium and nitrite in winter 2006/2007 (see section 8.3) showed that nitrification was masked by simultaneous denitrification. This is based on the following observations: (a) ^{15}N -labelled ammonium was oxidised to nitrate and nitrite; (b) a cumulative ^{15}N mass balance showed that the tracers ($^{15}\text{NH}_4^+$ and $^{15}\text{NO}_2^-$) were not completely recovered after a $3 \times \theta_0$ time period (90% and 57% recovery, respectively); (c) gases leaving the maturation pond to the atmosphere were clearly enriched with ^{15}N both during the $^{15}\text{NH}_4^+$ spike (from 16.17 to 35.77 $\delta^{15}\text{N},\text{‰}$) and the $^{15}\text{NO}_2^-$ spike (from 10.05 to 52.79 $\delta^{15}\text{N},\text{‰}$), when compared with the ^{15}N ex-pond gases baseline (from -42.40 to -31.10 $\delta^{15}\text{N},\text{‰}$); and (d) there was a slight nitrate accumulation in the pond effluent. Therefore, simultaneous nitrification-denitrification may be responsible for most of the nitrogen permanently removed from the maturation pond M1, during winter experiments with ^{15}N stable isotopes.

Molecular microbiology analyses carried out on samples from the maturation pond M1 revealed the presence of ammonia-oxidising bacteria (AOB), which confirms that the maturation WSP under study has the capability to perform ammonia oxidation to nitrite and possibly, denitrification in anoxic and low-oxygen environments. On the other hand, metanotrophs (e.g., *Methylocystis* sp., *Methylosinus* sp.) were present in samples collected in winter which reveals a new feasible mechanism for nitrogen transformation on WSP, as they can also oxidize ammonia to nitrite, nitrate and nitrous oxide (Knowles, 2005). At this point, it is important to mention that metanotrophs require methane as carbon source and although it is believed by some authors that methanogenesis does not at low temperatures ($<10^\circ\text{C}$), it has been found that methanogens consistently exposed to such conditions maintain their activity (Pearson, 2005; Juanico *et al.*, 2000).

Results of PCR amplification targeting *nirS* and *nirK* genes revealed the presence of denitrifiers in M1 pond, not only in samples collected from the pond sidewall and sludge layer as expected, but also in water column samples. Therefore denitrification supported either by AOB or methanotrophs in WSP may be counted as a feasible mechanism for permanent nitrogen removal both in summer and winter, but its relative supremacy over

other nitrogen removal mechanisms (e.g., biological uptake) would depend upon phytoplanktonic activity.

Nitrification-denitrification is a feasible mechanism for nitrogen removal in WSP and, although many researchers have reported that it is the main mechanism for permanent nitrogen removal (e.g., Somiya and Fujii, 1984; Lai and Lam, 1997; Zimmo *et al.*, 2003; Picot *et al.*, 2005; Strang and Wareham, 2005; Picot *et al.*, 2007), hardly any evidence regarding to nitrogen transformation pathways dominating nitrification and denitrification has been reported before, apart from that described by Picot *et al.* (2007) who detected N₂O emissions in gas samples collected from maturation ponds in France. Thus the results reported in this research work which included ¹⁵N tracer experiments and molecular microbiology analyses are new evidence to support nitrification-denitrification being one of the two major mechanisms for permanent nitrogen removal in WSP, along with sedimentation of dead algal biomass after biological nitrogen uptake.

9.4 Are Maturation Ponds Suitable for Nitrogen Control in Wastewater Treatment?

Maturation ponds are wastewater treatment units which are mainly designed to reduce the number of pathogenic organisms (faecal coliform bacteria and helminth eggs) from secondary effluents. The size and number of these ponds working in series are normally determined by the required microbiological quality of the final effluent. In a WSP system, they act as tertiary treatment and are fed with the effluent from a facultative pond. Moreover, maturation ponds in operation have reported effective pathogen removal (*E. coli* <1000 cfu/100ml in a secondary maturation pond effluent) and low maintenance and operation costs (Mara, 2004).

They also provide a significant contribution to improve secondary effluent quality in terms of organic matter, suspended solids and nutrients prior to reuse or discharge into surface water bodies (Mara and Pearson, 1986, 1998; Pearson *et al.*, 1987c; Mara *et al.*, 1992). However, there are currently no models to design maturation ponds to achieve specific ammonium or total nitrogen discharge consents, apart from the model proposed by Pano and Middlebrooks (1982) for the prediction of ammonium removal in WSP.

Comparative cost data suggest that WSP systems should be an attractive option for small and rural communities (<2,000 p.e.), yet perceptions relating to land costs, climate and effluent quality have limited their application in the UK. Wastewater treatment plants serving populations of less than 2000 p.e. account for 78 percent of all treatment works in

2000 in England and Wales (Defra, 2002). Although small treatment works treat only 4 percent of the wastewater produced, they present the greatest risk of non-compliance with effluent quality requirements, particularly taking into account the fact that discharge permits more restrictive than those in the EU Urban Waste Water Treatment Directive (UWWTD) (CEC, 1991) are generally imposed when final effluents are discharged to small water courses (Griffin and Pamplin, 1998).

According to the results reported in chapter 5, maturation ponds M1 and M2 performed well in terms of *E. coli* removal (up to 3 log units) and therefore the FC count in the final effluent was always below the level required for unrestricted irrigation and aquacultural reuse (<1000 cfu/100 ml; WHO, 2006). M2 effluent also met the UWWTD requirements of ≤ 25 mg filtered BOD/l and ≤ 150 mg SS/l in the final effluent of WSP systems. Unfortunately the performance of maturation ponds is strongly dependant on weather conditions and hence there was a clear seasonally variation in the quality of M2 effluent. Therefore, conventional maturation ponds in the UK would not contribute substantially to improve the quality of facultative pond effluents, as M2 effluent would not be able to meet much stricter standards such as a “10/15/5” standard (BOD/SS/ NH_4^+) or even a “40/60” standard (BOD/SS), which are normally specified as 95-percentile values rather than mean values.

Therefore, conventional maturation ponds should not be included on WSP systems for domestic wastewater treatment in the UK, particularly when emerging technologies for nutrient control are available to upgrade facultative pond effluents (e.g., aerated rock filters, blast furnace slag filters), which perform consistently better over the year and require less surface area than maturation ponds. Recent research work in the UK has demonstrated that aerated rock filters (ARF) at pilot-scale outperform maturation ponds in terms of BOD, SS and ammonium removals, and that they are able to achieve discharge consents set by the UK environmental regulators even during cold winter months (Johnson *et al.*, 2007). Moreover, ARF could be operated in series with blast furnace slag (BFS) filters for phosphorus removal; this combination would be the perfect marriage to achieve a comprehensive nutrient control with low-cost technologies (Camargo Valero *et al.*, 2007).

In warm and hot climates, maturation ponds are clearly a suitable technology for ammonium and total nitrogen removal; however, the design of maturation ponds should be updated in order to achieve removal targets by improving in-pond phytoplanktonic activity. Considering that algal nitrogen uptake would be the dominant nitrogen removal mechanism under warmer weather conditions, enhanced maturation ponds should be

designed, equipped and operated to facilitate algal growth and the removal of suspended solids from the final effluent. That approach could also be beneficial to maturation ponds operating at high altitudes in Andean countries like Colombia, Ecuador, Peru and Bolivia, where a long photoperiod (12 hours) and a strong solar radiation are appropriate for algal growth despite low water and air temperatures.

Engineers designing maturation ponds would require the development of new models to predict algal biomass concentrations and algal activity, which are not available yet. Such models would maximise algal nitrogen uptake and other algae-induced removals (e.g., faecal coliforms). The removal of suspended solids from the final effluent is a must, considering that the bulk of nitrogen would appear as suspended organic nitrogen in the pond effluent; therefore, solids removal units should be incorporated into WSP systems, especially when taking into account the potential to harvest wastewater-grown algae for further economical exploitation (e.g., the production of biofuel, green fertilizer, animal food, etc.). Solids removal units should be selected in order to be in agreement with further use of wastewater-harvested algae (e.g., suspended air flotation systems).