NITROGEN TRANSFORMATION AND REMOVAL IN MATURATION PONDS: TRACER EXPERIMENTS WITH ^{15}N STABLE ISOTOPES IN THE UNITED KINGDOM IN SUMMER

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Abstract

The mechanisms and pathways by which nitrogen in its various forms is removed from waste stabilisation ponds (WSP) have been a subject of much debate. In order to improve current understanding of the dynamics of inorganic and organic nitrogen removal in WSP systems, a study using stable nitrogen isotopes (^{15}N) was undertaken on an experimental pilot-scale WSP system at Esholt Wastewater Treatment Works (Bradford, UK) in summer 2005 and 2006. A primary maturation pond was spiked with ^{15}N-labelled ammonia ($^{15}NH_4Cl$) and ^{15}N-labelled algae (*Chlorella vulgaris)* to track nitrogen transformations and removal associated with ammonia volatilization, nitrification, and algal uptake and its subsequent sedimentation and retention/hydrolysis in the sludge layer. Stable isotope analysis of $\delta^{15}N$, in conjunction with performance indicators, revealed that total nitrogen is poorly removed ($\leq$ 8%) but ammonium nitrogen removal is very good in summer (effluent concentration <2 mg NH_4^+-N/L). The nitrogen cycle in maturation ponds is dominated by biological uptake as ammonium nitrogen is rapidly transformed into algal biomass as suspended organic nitrogen which then either leaves in the pond effluent or is sedimented as dead cells. Ammonia removal by volatilization makes little or no contribution to nitrogen removal and biological nitrate uptake prevents classical nitrification. Thus algal uptake of ammonium and subsequent sedimentation and retention in the sludge layer, after partial ammonification of the algal organic nitrogen, appears to be the dominant mechanism for permanent nitrogen removal in maturation ponds during warm summer months in England.

Keywords ^{15}N stable isotopes; maturation pond; nitrogen removal; nitrogen transformation

Introduction

Eutrophication in aquatic ecosystems is a condition where high nutrient concentrations (nitrogen and phosphorus) stimulate excessive plant growth, creating conditions that deteriorate water quality and so may interfere with surface water uses (transportation, fishing, recreational, water supply, etc.) and the health and diversity of indigenous fish, plant and animal populations. Domestic wastewater discharge is one of the primary sources of nitrogen in watersheds with low agricultural activity and it may become a major environmental problem due to many of the existing domestic wastewater treatment plants not being equipped to control nutrients − only 5% of the total volume of wastewater receive tertiary treatment on global scale (Mulder, 2003). Accordingly, discharge consents for large wastewater treatment plants are being adjusted worldwide to achieve a greater degree of sustainable nutrient control and it is expected that, over the next few years, small domestic wastewater treatment plants will also be required to remove both nitrogen and phosphorus.

Waste stabilization ponds (WSP) are not normally considered as a reliable technical option for nutrient removal from domestic wastewater; however, recent studies on WSP in the UK have shown that nitrogen is removed to low levels (<5 mg ammonium N per litre) in both winter and summer, but it is not yet known how to optimize WSP design criteria for nitrogen and ammonium removal (Abis and Mara, 2003). In a WSP system, maturation ponds are most commonly designed to reduce the number of pathogenic organisms from secondary effluents (facultative pond effluents), but they also make important improvements in physicochemical wastewater quality by reducing the concentrations of organic matter (BOD) and suspended solids and, additionally, they can make a significant contribution to cumulative nitrogen and phosphorus removal in WSP systems (Mara and Pearson, 1986, 1998; Pearson *et al*., 1987a; Mara *et al*., 1992).

Feasible mechanisms and pathways by which nitrogen in its various forms can be transformed in and removed from WSP have been proposed and supported by research carried out in many parts of the world, including: (a) ammonia volatilization (Pano and Middlebrooks, 1982; Soares *et al*., 1996; Rockne and Brezonik, 2006); (b) biological uptake (Digiano *et al*., 1982; Ferrara and Avci, 1982); (c) simultaneous nitrification-denitrification (Zimmo *et al*., 2003; Picot *et al*., 2005); and/or (d) sedimentation of dead biomass and accumulation of organic nitrogen in sludge layer after partial ammonification (Ferrara and Avci, 1982; Reed, 1985; Pearson *et al*., 1988). The majority of these studies are based on the measurements of nitrogen fractions (organic, ammonium, nitrite and nitrate) in water samples collected from both the pond influent and effluent; occasionally, average in-pond characteristics such as ammonium, pH and temperature have been used as inputs into mathematical models to estimate nitrogen removal rates (especially ammonia volatilization rates), regardless of their well known diurnal and seasonal variations. However, such approaches may in fact make understanding of the fate (or fates) of nitrogen in WSP particularly difficult in situations in which water quality changes are so small that they do give any evidence about simultaneous processes such as, for instance, nitrification-denitrification and nitrification-biological nitrate uptake. Therefore, the debate is still open regarding the relative importance of the various pathways through which, and the mechanisms by which, nitrogen is transformed and removed in WSP.

A much better approach to further our understanding of the fate(s) of nitrogen compounds in WSP is based on using stable nitrogen isotopes (^{15}N), which have been largely used to illustrate the behaviour of nitrogen in aquatic ecosystems. In wastewater treatment, nitrogen transformations can be tracked and removal rates estimated by using two types of stable isotope studies: (a) isotope fractionation (Kanazawa and Urushigawa, 2007) and/or (b) tracer methods (Reddy, 1983). In this work, tracer experiments using ^{15}N-labelled ammonia and ^{15}N-labelled algae were carried out in a pilot-scale maturation pond in summer to facilitate the simultaneous study of the dynamics of inorganic and organic forms of nitrogen, in order to determine the relative importance of nitrogen transformations and removal associated with ammonia volatilization, nitrification, and algal uptake and its subsequent sedimentation and retention/hydrolysis in the sludge layer.

Methods

This research was undertaken on an experimental pilot-scale WSP system at Esholt Wastewater Treatment Works in Bradford, West Yorkshire, UK. The pilot-scale WSP system comprises one primary facultative pond (PFP) which is fed with screened wastewater (50% domestic, 50% industrial), two maturation ponds in series (M1 and M2) and a reedbed channel. The PFP was loaded at 80 kg BOD/ha d (8 g BOD/m^2 d) and 8 kg N/ha d (0.8 g N/m^2 d), with an average nominal retention time (θ) of 60 days within the experimental timeframe reported herein. Pond M1 (6.3 × 3.5 × 1.00 m) received effluent from the PFP which was pumped at an average rate of 0.6 m^3/d (θ = 17.5 d); the effluent from M1 discharged by gravity into M2.

Pond M1 was spiked to increase the δ^{15}N of dissolved ammonium effluent by 1000‰ with a single pulse of 0.6812 g of $^{15}NH_4Cl$ (98% ^{15}N; Cambridge Isotope Laboratories, Cambridge, USA) in summer 2005, and with 1.2516 g (dry solids) of ^{15}N-labelled algae in summer 2006. The ^{15}N-labelled algae were made up by culturing *Chlorella vulgaris* (CCAP 211/11B; SAMS Research Services Ltd, Oban, Scotland) in

10 litres of Bold's Basal Medium (Andersen *et al*., 2005) substituting the nitrogen source with $^{15}NH_4Cl$ (98% ^{15}N). M1 effluent was sampled hourly for 1×θ before spiking and for 3×θ afterwards by using an auto-sampler (Aquacell P2-Multiform; Aquamatic, Manchester, England). Samples were preserved in situ by the addition of 5 ml of preservative solution (6N HCl containing 2 g $CuCl_2$/l) per litre of sample. Simultaneously, a multi-parameter sonde probe (YSI 6820; YSI Inc., Yellow Springs, USA) was used to measure in real time dissolved oxygen (DO), temperature and pH in the M1 effluent.

The samples were taken to the Public Health Laboratory, University of Leeds, where 24-hour composite samples were made. The composite samples were processed for ammonium (method 4500-NH3 B; *Standard Methods*, 1998), suspended solids (SS) (2540 D), TKN and filtered TKN (4500-Norg C), and nitrite and nitrate by ion chromatography (IC-ED; DX500, Dionex Cop., Sunnyvale, USA) following the analytical procedure described by Raessler and Hilke (2006). Samples were also sequentially partitioned to extract four nitrogen species separately: (a) suspended organic nitrogen, by filtering on pre-ashed (550ºC) fibre-glass filters (GF/C; Whatman International Ltd, Maidstone, England); (b) soluble organic nitrogen, by solid phase extraction (Isolute C18 cartridge; Biotage, Uppsala, Sweden), followed by elution with absolute ethanol and further concentration on pre-ashed fibre-glass (Whatman GF/D) by volatilization at 40ºC; (c) ammonium nitrogen, by ammonia diffusion (Holmes *et al*., 1998); and (d) oxidised nitrogen, by nitrate and nitrite reduction into ammonium with Devarda`s alloy (Brooks *et al*., 1989) and simultaneous ammonia extraction by diffusion (Holmes *et al*., 1998). Each fraction was analyzed to determine ^{15}N:^{14}N ratios using an elemental analyzer coupled with a stable isotope ratio mass spectrophotometer (EA-IRMS; EuroEA3000-Micromass Isoprime, Eurovector, Milan).

Ammonia losses by volatilization were estimated on site following a procedure described by Camargo Valero and Mara (2007); samples were processed for ammonium and ^{15}N:^{14}N ratios, as described above. Settled organic nitrogen samples were collected in 10-litre metal buckets which were strategically placed on the bottom of M1 and taken out at the end of each experiment. Collected sediment samples were sieved (ASTM sieve No. 10) to remove coarse solids and settled in 1-litre Imhoff cones. Thickened samples were dried at 105ºC and processed simultaneously for nitrogen content and ^{15}N:^{14}N ratios. Sediment sub-samples were also processed for solids and moisture content (2540 B, 2540 D, 2540 F).

Results and Discussion

The results for ^{15}N:^{14}N ratios from samples collected in M1 effluent are shown in Figures 1 and 2 as delta values in parts per thousand ($\delta^{15}N$, ‰), which are defined as (subscript 'spl' = sample, 'std' = standard):

$$\delta^{15}N = \left[\frac{\left(^{15}N/^{14}N\right)_{spl} - \left(^{15}N/^{14}N\right)_{std}}{\left(^{15}N/^{14}N\right)_{std}} \right] \times 1000$$

Thus they are not concentrations of the ^{15}N isotope but differences between ^{15}N:^{14}N ratios in the sample and atmospheric N_2 gas (which has a known $\delta^{15}N$ value). Instrument calibration was done with two certified standards of labelled ammonium sulphate: IAEA-USGS26 ($\delta^{15}N$ = +53.7) and IAEA-USGS25 ($\delta^{15}N$ = −30.4), provided by the U.S. Geological Service (Denver, CO) and certified by the Section of Isotope Hydrology, International Atomic Energy Agency (Vienna). The standard error in $\delta^{15}N$ readings of the certified standards was ±0.12‰ at most. The $\delta^{15}N$ values have been corrected for background content based on results from samples collected before tracer injection; therefore negative values are graphed as zero as they include only tracer ^{15}N.

After the tracer was injected into M1 in summer 2005 (Figure 1), the ammonium nitrogen fraction was highly enriched with ^{15}N, as expected, but it decayed rapidly within the experimental timeframe. This may

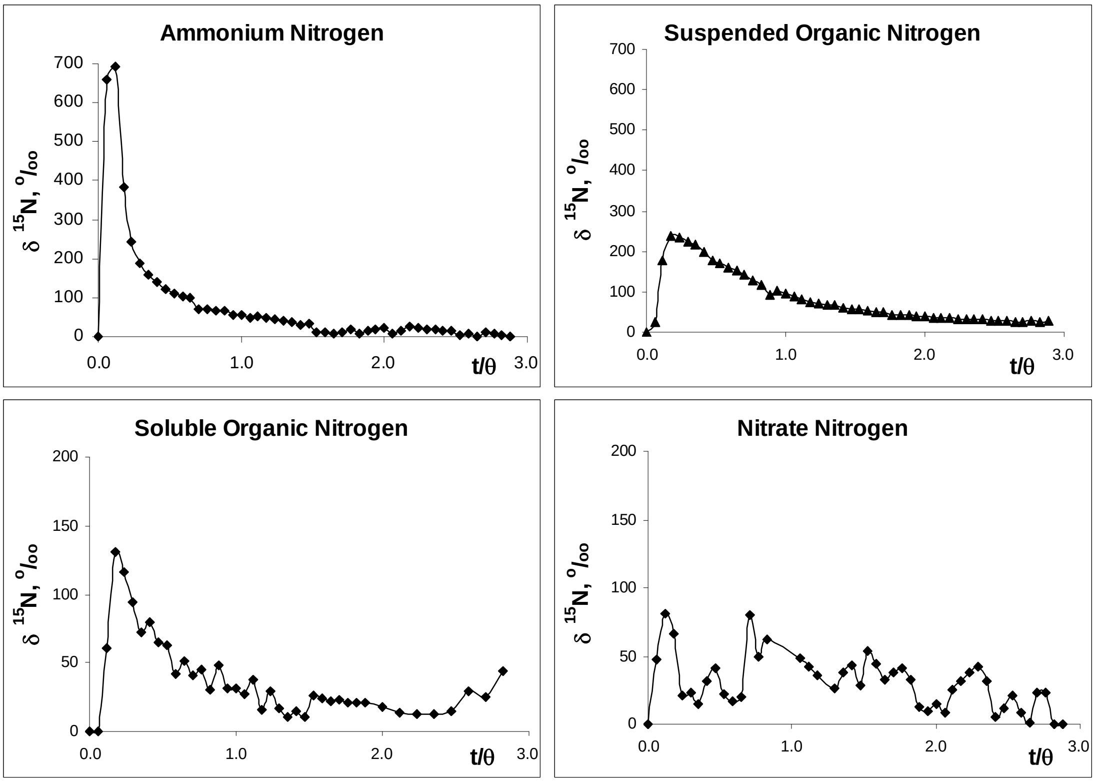

Figure 1. $\delta^{15}N$ values in nitrogen fractions from M1 effluent in summer 2005

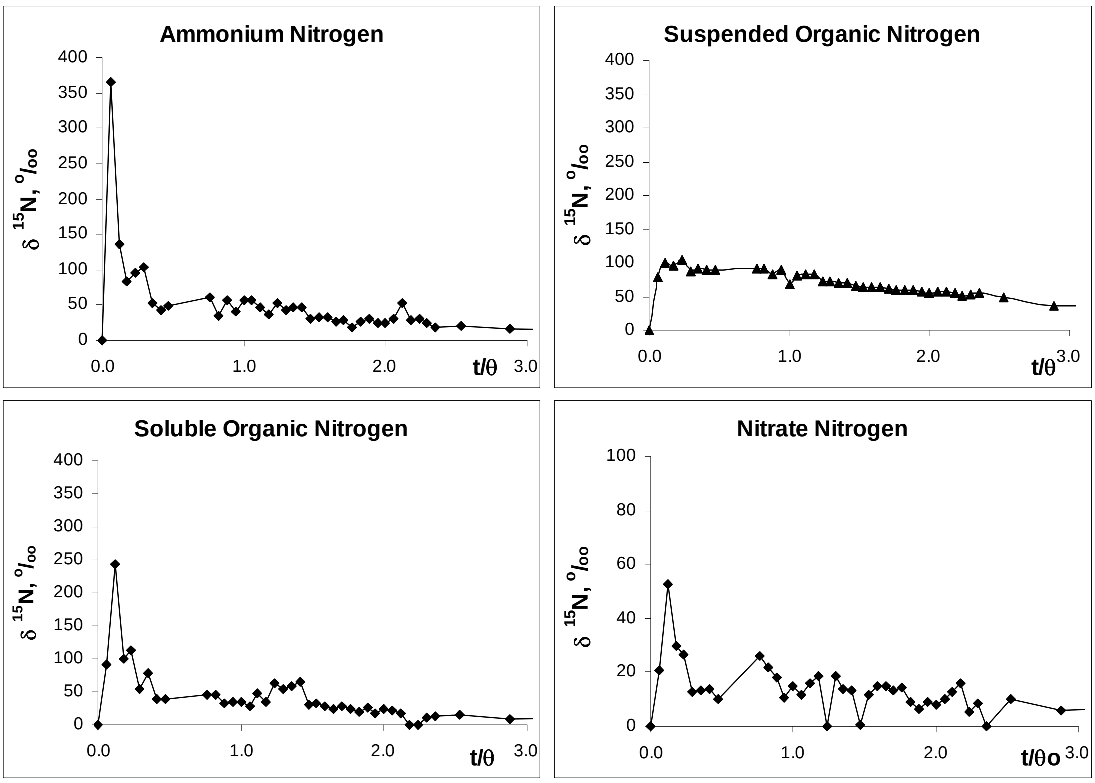

Figure 2. $\delta^{15}N$ values in nitrogen fractions from M1 effluent in summer 2006

mean that the hydraulic regime in M1 pond was very close to complete mixing and the ammonium nitrogen was involved in a process with a very high reaction rate. Ammonia volatilization could have been expected to be the dominant mechanism for ammonium removal as both temperature (15.2–18.2°C) and pH (8.9–10.1) were very favourable for this process; however, the ammonia volatilization rates were found to be very low (0–27 g N/ha d) and the corresponding $\delta^{15}N$ values (−42.40 to −31.10‰) were not significantly different from the background samples (p <0.05). In contrast, the suspended organic fraction was ~250‰ enriched with ^{15}N, suggesting that biological (algal) uptake was an important mechanism for ammonium nitrogen removal. Samples collected from the sediments in M1 were enriched with ^{15}N from +5.0 to +54.1 ($\delta^{15}N$, ‰); decay of dead algal cells would have released ammonia and soluble organic nitrogen which therefore may be considered as intermediate steps in the nitrogen removal process. The oxidised nitrogen fraction (mainly nitrate) was also enriched with ^{15}N, but the corresponding $\delta^{15}N$ values were lower than for the other fractions, suggesting that the ^{15}N in nitrate did not decrease as fast as the soluble organic fraction and the decreasing sinusoidal graph (Figure 1) may indicate that nitrate formation is also an intermediate step but restrained by the ammonium nitrogen availability. A ^{15}N mass balance for $3\times\theta$ showed that the tracer was recovered in the M1 effluent as follows: suspended organic fraction, 48.9%; soluble organic fraction, 4.9%; ammonium nitrogen fraction, 8.8; and nitrate fraction, 0.2%; the remaining ^{15}N accumulated in the water column (~29.8%) and sludge layer (~7.4%).

The results from summer 2006 (Figure 2) confirmed a similar nitrogen behaviour despite the ^{15}N source being organic nitrogen rather than inorganic nitrogen as in summer 2005. In this experiment dead cells of *Chlorella vulgaris* containing ^{15}N were rapidly hydrolyzed in the sludge layer, so releasing labelled organic compounds as an intermediate step to provide labelled ammonium nitrogen which was simultaneously transformed into nitrate and taken up by the biomass in water column. Again, ammonia removal by volatilization was negligible and nitrogen removal via sedimentation was confirmed from sludge samples enriched with ^{15}N from +14.7 to +42.1 ($\delta^{15}N$, ‰). A ^{15}N mass balance for $3\times\theta$ showed that the tracer was recovered in M1 effluent as follows: suspended organic fraction, 17.9%; soluble organic fraction, 6.2%; ammonium nitrogen fraction, 3.2; and nitrate fraction, 0.2%; the remaining ^{15}N accumulated in the water column (~58.0%) and the sludge layer (~14.4%).

Conclusions

Under summer conditions, the nitrogen cycle in a primary maturation pond is dominated by biological uptake since ammonium nitrogen is rapidly transformed into algal biomass as suspended organic nitrogen which then either leaves in the pond effluent or is sedimented as dead cells. Ammonia removal by volatilization makes little or no contribution to nitrogen removal and biological nitrate uptake prevents classical nitrification. Thus algal uptake of ammonium and subsequent sedimentation and retention in the sludge layer, after partial ammonification of the algal organic nitrogen, appears to be the dominant mechanism for permanent nitrogen removal in maturation ponds during warm summer months in England.

Acknowledgements

We are extremely grateful to all our funders, The Engineering Physics and Science Research Council - EPSRC (GR/S98382/01), The University of Leeds, Universidad Nacional de Colombia, COLFUTURO, and especially to Yorkshire Water who also generously provided the site and give us almost day-to-day operational support in one way or another at Esholt. Also to Dr. Rob Newton for supervising stable isotope determinations.

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