Nitrogen removal in maturation WSP ponds via biological uptake and sedimentation of dead biomass

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Abstract
In this work a set of experiments was undertaken in a pilot-scale WSP system to determine the importance of organic nitrogen sedimentation on ammonium and total nitrogen removals in maturation ponds and its seasonal variation under British weather conditions. Two maturation ponds in series (M1 and M2) were monitored seasonally from September 2004 to May 2007. The nitrogen content in collected sediment samples varied from 4.17 to 6.78 percent (dry weight) and calculated nitrogen sedimentation rates ranged from 291 to 2,868 g N/ha d in M1 and from 273 to 2,077 g N/ha d in M2. The increment of chlorophyll-*a* in M1 and M2 maturation pond effluents had a very good correlation with the corresponding increment of VSS (algal biomass) and suspended organic nitrogen; therefore, the occurrence of biological (algal) nitrogen uptake was confirmed. After sedimentation of the dead algal biomass, it was clear that algal-cell nitrogen was recycled from the sludge layer into the pond water column. Recycled nitrogen can be either taken up by algae or washed out in the pond effluent. Biological (mainly algal) uptake of inorganic nitrogen species and further sedimentation of dead biomass (together with its subsequent mineralization) is one of the major mechanisms controlling in-pond nitrogen recycling in maturation WSP, particularly when environmental and operational conditions are favourable for algal growth.

Keywords
Algal nitrogen uptake; maturation pond; sedimentation; nitrogen removal

INTRODUCTION

Nitrogen removal in waste stabilisation ponds (WSP) has been attributed to high rates of ammonia volatilisation and the sedimentation of organic nitrogen via biological uptake, and its subsequent retention in the sludge layer after partial hydrolysis (Pano and Middlebrooks, 1982; Ferrara and Avci; 1982; Reed, 1985). However, researchers have found it difficult to determine whether sedimentation or volatilisation is the dominant mechanism for nitrogen removal because of the very complex interactions in the biochemical pathways involved, although it was thought that volatilisation may dominate during the warm summer months and deposition during the winter (Maynard *et al.*, 1999). 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 ^{15}N tracer experiments in an undisturbed natural stream showed that biological nitrogen uptake and retention of nitrogen in fine benthic organic material was highly efficient as it represented approximately 32 percent of the spiked ^{15}N-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 large amount of industrial wastewater and domestic sewage was discharged into the lake. They reported a positive correlation between ammonium fluxes and algal biomass and chlorophyll-*a* concentrations and they therefore concluded that higher fluxes of ammonium may support a higher biomass of the phytoplankton and consequently a higher algal nitrogen uptake rate.

Ferrara and Avci (1982) reported algal nitrogen uptake, and the subsequent sedimentation of the biologically incorporated organic nitrogen, as the principal mechanism for total nitrogen removal in maturation ponds. 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 intervenes in nitrogen transformation and removal as it does in all algae-induced processes (e.g., faecal bacteria removal, oxygen supply, etc.). This would be particularly expected in maturation ponds, considering that it has been shown that ammonia volatilisation does not to make any significant contribution to total nitrogen removal (Camargo Valero and Mara, 2007a). In this work a set of experiments was undertaken in a pilot-scale WSP system to determine the importance of organic nitrogen sedimentation on ammonium and total nitrogen removals in maturation ponds and its seasonal variation under British weather conditions.

METHODS AND MATERIALS

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) fed with screened wastewater (50% domestic, 50% industrial), two maturation ponds in series (M1 and M2), and a reedbed channel (RBC). 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_0) 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_0 = 17.5 d); the effluent from M1 discharged by gravity into M2 and thence also by gravity into the RBC.

Composite samples for settled organic nitrogen from maturation ponds M1 and M2 were collected seasonally from September 2004 to May 2007, in order to estimate seasonal sedimentation rates of organic nitrogen. Each period under study was defined as follows: autumn (September, October and November), winter (December, January and February), spring (March, April and May) and summer (June, July and August). Settled organic nitrogen samples were collected in 10-litre metal buckets, which were strategically placed on the bottom of each maturation pond and taken out at the end of each season. Collected sediment samples were sieved (ASTM sieve No. 10) to remove coarse solids and settled in 1-litre Imhoff cones for 3 hours. Thickened samples were dried at 105ºC and processed for solids and moisture content (methods 2540 B, 2540 D and 2540 F; APHA, 1998). Additional sludge samples were collected on a monthly basis from the bottom of M1 and M2 and processed as described previously. Thickened sub-samples (seasonally and monthly sampling) were dried at 105ºC and processed simultaneously for nitrogen content and ^{15}N:^{14}N ratios ($\delta^{15}N$, ‰) by using an elemental analyzer coupled with a stable isotope ratio mass spectrophotometer (EA-IRMS; EuroEA3000-Micromass Isoprime, Eurovector, Milan).

Additionally, a weekly sampling for performance indicators was carried out by collecting grab samples from M1 influent (sampling point A), M1 column (B), M1 effluent (C), M2 column (D) and M2 effluent (E). These samples were analyzed for chlorophyll *a* (Pearson *et al*., 1987), BOD_5 and filtered BOD_5 (5210 B), 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). Temperature, dissolved oxygen (DO) and pH from sampling points were determined on site with a multiparameter sonde (model YSI 6130, YSI Inc., Yellow Springs, USA).

RESULTS AND DISCUSSION

The nitrogen content in collected sediment samples varied from 4.17 to 6.78 percent (dry weight) (mean value = 5.04%); these figures were used to calculate nitrogen sedimentation rates over the corresponding season. Nitrogen sedimentation rates ranged from 291 to 2,868 g N/ha d in M1 and

from 273 to 2,077 g N/ha d in M2. Although there was no a significant difference when corresponding mean values were compared with the *t*-test (t (20) = −1.133, p = 0.270), it was clear that sedimentation rates in M1 and M2 varied independently through the year (Figure 1). Maturation pond M1 had the highest nitrogen sedimentation rates during summer periods, while M2 had the highest in autumn.
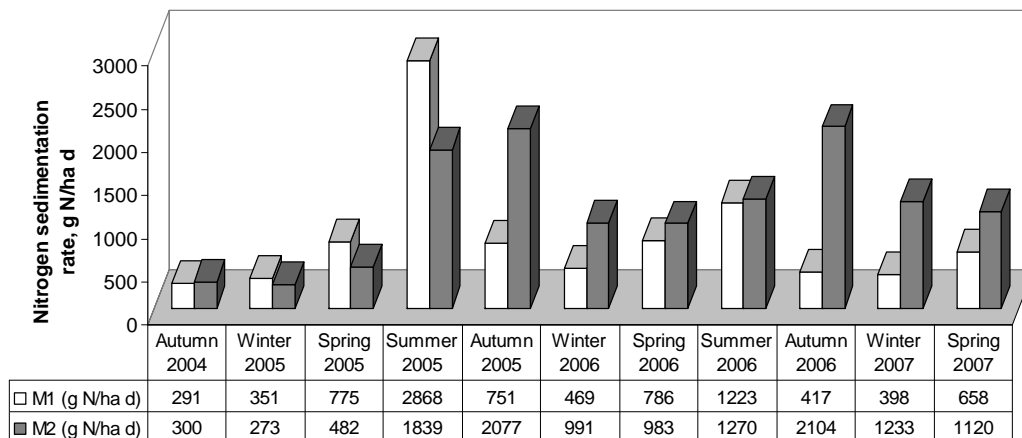

	Autumn 2004	Winter 2005	Spring 2005	Summer 2005	Autumn 2005	Winter 2006	Spring 2006	Summer 2006	Autumn 2006	Winter 2007	Spring 2007
☐ M1 (g N/ha d)	291	351	775	2868	751	469	786	1223	417	398	658
▦ M2 (g N/ha d)	300	273	482	1839	2077	991	983	1270	2104	1233	1120

Figure 1. Mean seasonal nitrogen sedimentation rates in ponds M1 and M2

Considering that sedimentation of organic nitrogen is achieved in maturation ponds after biological (mainly algal) uptake of inorganic nitrogen, more favourable conditions for phytoplankton activity during the warm summer months would have stimulated algal growth in M1 at a faster rate than in M2, as in-pond chlorophyll-*a* values – as well as nutrient availability – decrease from pond to pond in WSP systems when they are arranged in series. It would have led to increases in in-pond organic nitrogen as suspended algal biomass in M1 and hence a higher rate of nitrogen sedimentation. In fact, mean in-pond chlorophyll-*a* values in summer 2005 were 912 μg/l for M1 and 758 μg/l for M2; corresponding values in summer 2006 were 367 and 254 μg/l. On the other hand, when environmental conditions were less favourable for algal growth (e.g., autumn, winter), the accumulated in-pond algal biomass in M1 could have been washed out in the pond effluent, so increasing organic nitrogen sedimentation rates in the M2 pond.

Ferrara and Avci (1982) modelled and analysed data collected from Pond 1 of the Coring WSP system, Utah (US EPA, 1977); this pond had a surface area of 1.49 ha and an average depth of 1.2 m. Results showed that over a 13-month period, total nitrogen was removed in Coring Pond 1 at an average rate of 3.34 kg N/ha d (47%), mainly through sedimentation of organic nitrogen (3.21 kg N/ha d). Average total nitrogen removal rate (from October 2004 to May 2007) in M1 was 0.911 kg N/ha d (16%) and sedimentation of organic nitrogen was also the most important mechanism for nitrogen removal – the average net sedimentation rate in M1 was 0.817 kg N/ha d. However, a closer analysis shows that organic nitrogen sedimentation rates in M1 and M2 are season-dependent (Figure 1); hence other feasible mechanisms may contribute to total nitrogen removal when environmental conditions are not favourable for biological uptake and further sedimentation of organic nitrogen (e.g., denitrification).

Results from weekly sampling were analysed in order to determine the importance of biological nitrogen uptakes in the maturation WSP under study. Firstly, the Pearson correlation was used to make an evaluation of the linear relationship between algal biomass (chlorophyll-*a*) and suspended organic nitrogen (TKN – filtered TKN), as well as volatile suspended solids (VSS), from sampling

points A, C and E. For M1 influent (A), Pearson's correlation coefficient (r) indicates a statistically significant linear relationship between chlorophyll-*a* and suspended organic nitrogen ($r = 0.844$), but a less strong relationship with VSS ($r = 0.662$). In the maturation pond effluents (C and E), VSS and suspended organic nitrogen correlated very significantly with chlorophyll-*a*; in M1 effluent, the results for Pearson's correlation coefficient were: (a) Chl-*a* vs. suspended organic nitrogen: $r = 0.877$ and (b) Chl-*a* vs. VSS: $r = 0.902$. Corresponding figures for r in M2 effluent were: (a) 0.895 and (b) 0.883. In all cases, the size of the sample (N) was 70 and r (70) = −0.356. Similar results for the correlation between Chl-*a* and VSS ($r = 0.980$) were reported by Bich *et al.* (1999) in the final effluent of a high-rate algal pond. Therefore, the increment of chlorophyll-*a* in maturation pond effluents undoubtedly indicates an increment of VSS (algal biomass) and consequently the occurrence of biological (algal) nitrogen uptake.
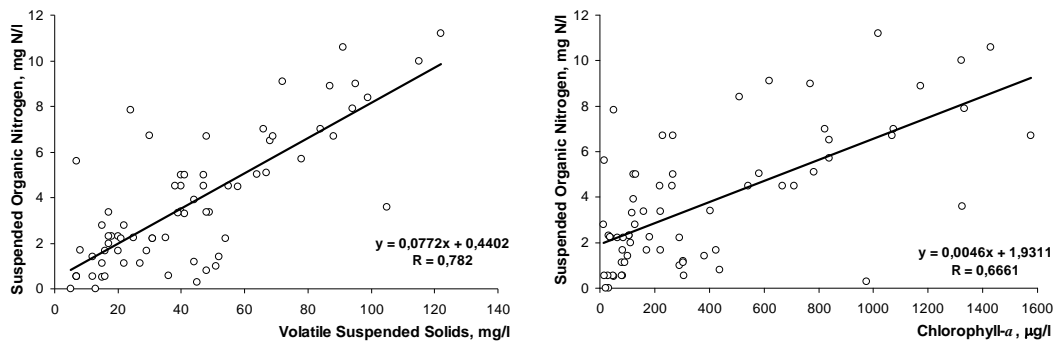
(a) Sampling point A: M1 influent
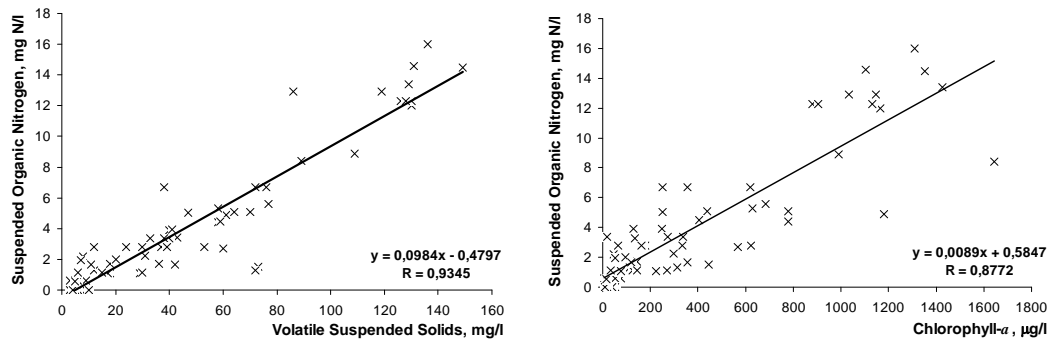
(b) Sampling point C: M1 effluent
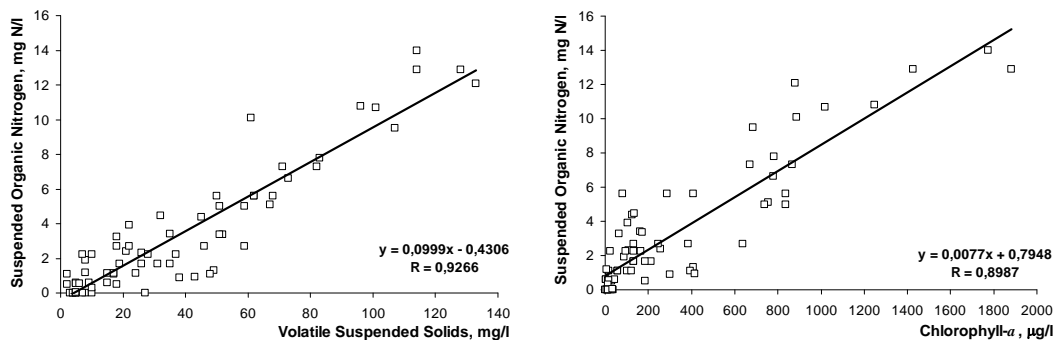
(c) Sampling point E: M2 effluent

Figure 2. Linear regressions for suspended organic nitrogen vs. VSS and Chl-*a*.

Linear regressions for suspended organic nitrogen vs. VSS and Chl-*a* were also calculated and the results suggest that the suspended organic nitrogen fraction (SuspON) entering M1 (Figure 2a) is not entirely from algal biomass as the intercept ($\approx$2 mg N/l) from the Chl-*a* vs. SuspON graph is far from the origin. It seams that bacterial biomass from the primary facultative pond effluent may also make an important contribution to the suspended organic nitrogen input in the maturation pond M1. However, a similar analysis to linear regressions from M1 effluent (Figure 2b) and M2 effluent (Figure 2c) indicates that the nature of suspended organic nitrogen in maturation ponds effluents is mainly algal biomass.

The relationship between VSS and suspended organic nitrogen shows that nitrogen contents in the VSS in M1 influent, M1 effluent and M2 effluent were 7.7, 9.8 and 10.0 percent (dry weight), respectively. The theoretical nitrogen content in algal biomass is 9.2 percent, assuming that the molecular formula for algal cells is $C_{106}H_{181}O_{45}N_{16}P$ (Oswald, 1988). The cellular nitrogen content in algal species grown in domestic wastewater can vary over a relatively wide range (1.22–11.00% dry weight); in general, it appears that nitrogen accounts for about 7.5 percent of the dry weight in the cyanophytes and approximately 4.2 percent in the chlorophytes (Hemens and Mason, 1968). However, the cellular nitrogen content in micro-algae depends on the composition of the medium, among many other factors. Richardson *et al*. (1969) reported that the content of nitrogen in *Chlorella sorokiniana*, in a continuous culture with nitrate as nitrogen source, increased from 5.6 to 10.1 percent when the nitrate concentration was changed from 70 to 280 mg NO_3^--N/l.

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 indeed the 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, and Table 1 shows how 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.

Table 1. Nitrogen load removal in maturation ponds M1 and M2

Season	Total nitrogen load removal, %					
	Unfiltered effluent			Filtered effluent		
	M1	M2	M1 + M2	M1	M2	M1 + M2
Autumn 2004	16	21	36	29	35	45
Winter 2005	19	18	34	25	25	39
Spring 2005	31	30	52	54	68	78
Summer 2005	13	20	30	82	79	82
Autumn 2005	9	8	18	70	72	74
Winter 2006	11	14	23	38	45	51
Spring 2006	19	24	39	63	38	49
Summer 2006	12	−14[*]	−6[*]	51	41	48
Autumn 2006	−18[*]	−27[*]	−45[*]	19	0	19
Winter 2007	24	−5[*]	25	32	2	25
Spring 2007	21	13	31	70	59	68

[*] Negative values (−) correspond with periods reporting sludge feedback.

Mara (2006) has drawn attention to the inclusion of solids removal units (e.g., rock filters) as an integral part of WSP systems, which would play the same role than secondary sedimentation tanks

in activated sludge systems. In other words, they both would serve the same purpose: the removal of biomass produced in the preceding biological treatment stage (bacteria in the case of activated sludge and algae in the case of WSP). In the case of enhanced nitrogen removal in WSP, solids removal units would complement the highly efficient algal nitrogen uptake. Moreover, under suitable environmental and operational conditions for primary productivity in WSP (e.g., summer), algal uptake of inorganic nitrogen species was also responsible for the bulk of ammonium removed in our pilot-scale maturation ponds (Camargo Valero and Mara, 2007b). Indeed, ammonium and VSS in M1 and M2 effluents had a statistically significant inverse correlation, which was identified by using the Pearson correlation. Corresponding results were: r (70) = −0.356 for M1 effluent and r (70) = −0.366 for M2 effluent; both correlations were significant at the 0.01 level (p = 0.002). 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.

Grab samples from the sludge layer in the bottom of the maturation ponds M1 and M2 were collected monthly and analysed for nitrogen content and $^{15}N:^{14}N$ ratios ($\delta^{15}N$) as described previously. Samples from M1 reported a mean nitrogen content of 5.1 percent, while for M2 sludge samples the average nitrogen content was 3.9 percent. Mean figures of nitrogen content from M1 and M2 samples were compared with the t-test: a statistically significant difference between them was found (t (21) = 6.004; p = 0.039). Similar differences were reported in sludge samples collected from an Advanced Integrated Water Pond System (AIWP) at Richmond Field Station, UC Berkeley (Hsieh, 2000). The AIWP system comprises an advanced facultative pond (AFP), two high rate ponds (HRP) and three settling basins (SB). Mean nitrogen content in sludge samples was also decreasing along the treatment line from 4.0 percent in AFP to 3.9 and 3.0 percent in HRP and SB units, respectively.

Hemes and Mason (1968) also reported nitrogen content in algal sediments from a shallow plastic-lined trench (3.14 m in length) fed continuously (98 m^3/d; 24-hour retention time) with settled biofilter effluent from a sewage treatment works in Pretoria, South Africa. The corresponding values for nitrogen in algal sediments showed the same tendency, with a maximum of 5.2 percent in the upper sections and a minimum of 1.4 percent (dry weight) in the middle and lower sections. Apparently, lower nitrogen contents in algal sludge should be expected in final algae-based treatment units operating in series. Considering that mean nitrogen content in VSS from M1 and M2 effluents was 9.8 and 10.0 percent respectively, which leads to the assumption that the nitrogen content in 'fresh' dead algal biomass is about the same figure, it is clear that algal-cell nitrogen was recycled from the sludge layer into the pond water column via anaerobic digestion.

In fact, the $\delta^{15}N$ results (Figure 3) from sludge samples collected monthly from the maturation pond M1 shows how fast nitrogen is recycled from the sludge layer after anaerobic digestion. The natural abundance of ^{15}N in sludge samples was affected by a set of four tracer experiments with ^{15}N stable isotopes carried out by Camargo Valero and Mara (2008) in the same pilot-scale WSP system. Figure 3 shows five sections where the behaviour of $\delta^{15}N$ values in sludge samples is a consequence of spiking ^{15}N compounds in M1 pond as follows: (1) $\delta^{15}N$ baseline; (2) tracer spike with ^{15}N-labelled ammonium; (3) tracer spike with ^{15}N-labelled algae (*Chlorella vulgaris*); (4) tracer spike with ^{15}N-labelled ammonium; and (5) tracer spike with ^{15}N-labelled nitrite. Section 2 gives the overall idea considering that an inorganic source of nitrogen (^{15}N-labelled ammonium) was spiked within M1 influent under favourable conditions for algal growth. Camargo Valero and Mara (2008) found that $^{15}NH_4^+$ was firstly taken up by algae and then dead algal cells settled down to the bottom of M1 pond, so increasing $\delta^{15}N$ values in the sludge layer up to 53.2‰. The $\delta^{15}N$ values reported herein show how ^{15}N content in collected sludge samples decreased following a

decreasing exponential pattern until they reached again similar baseline values after about six months.
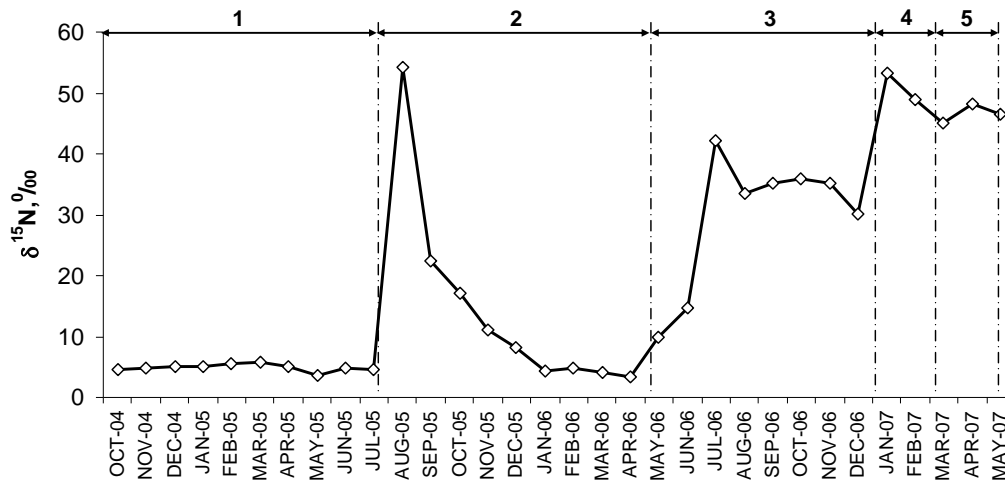

Figure 3. $\delta^{15}N$ values in sludge samples from M1 maturation pond

Anaerobic digestion of algal sludge has been identified as a major contribution of organic carbon and nutrients in WSP systems, particularly in temperate climates where it may cause overloading problems (Reed *et al*., 1988; Walmsley and Shilton, 2005). During cold periods, sediments are mainly stored in the sludge layer and then when temperature rises, algal sludge is digested faster; that may cause a large extra input of nutrients and oxygen demand on the pond. In fact, Somiya and Fujii (1984) stated that the regeneration rate of nutrients from sediments in a maturation pond is so active that the removal of nutrients by algal uptake is not effective and consequently the overall nitrogen removal efficiency decreases. In summary, biological uptake of inorganic nitrogen species and further sedimentation of dead biomass (together with its subsequent mineralization) is one of the major mechanisms controlling in-pond nitrogen recycling in WSP, particularly when environmental and operational conditions are favourable for algal growth.

CONCLUSIONS

Algal nitrogen uptake was clearly identified as the major mechanism for ammonium removal under favourable environmental and operational conditions for phytoplanktonic activity in maturation WSP. In summer, algal nitrogen uptake was found responsible for the majority of the ammonium removed and along with sedimentation of dead algal biomass, it constitutes the dominant nitrogen removal mechanism in maturation ponds. However, it is important to highlight that once dead algal biomass reaches the bottom of the pond, anaerobic digestion of pond sediments partially recycles ammonium nitrogen to the water column. Therefore, it is to be expected that total nitrogen removal rates would be low, as most of the ammonium nitrogen removed by algal uptake would be washed out as suspended solids in the pond effluent. In the case of enhanced nitrogen removal in WSP, solids removal units would complement the highly efficient algal nitrogen uptake.

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