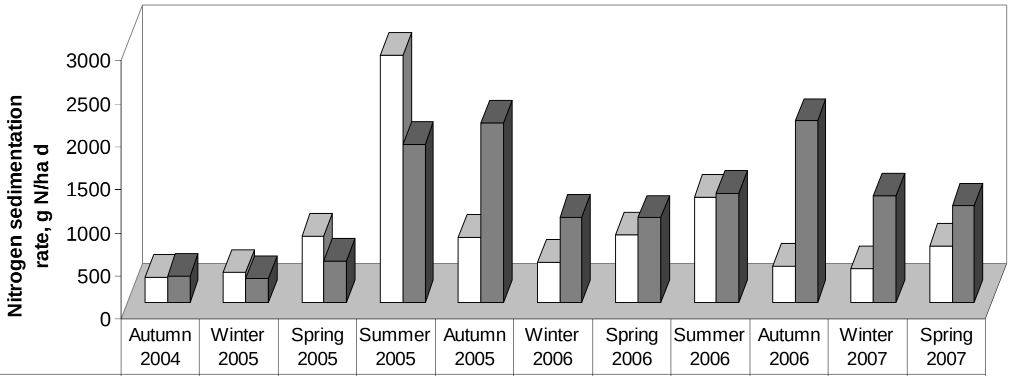
7. RESULTS AND DISCUSSION: NITROGEN REMOVAL BY SEDIMENTATION OF ORGANIC NITROGEN

Nitrogen removal in WSP has been attributed to sedimentation of organic nitrogen via biological uptake, and its subsequent sedimentation and retention after partial hydrolysis in the sludge layer, and high rates of ammonia volatilisation (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). Considering that ammonia volatilisation is not the main mechanism for ammonium removal in our pilot-scale WSP system (see chapter 6), a set of experiments was undertaken to determine the importance of organic nitrogen sedimentation on ammonium and total nitrogen removals in WSP; therefore, this chapter shows the results obtained and reports the seasonal variation of net nitrogen sedimentation rates over the experimental timeframe. Findings are also compared with similar works reported in the literature.

7.1 Sedimentation of Organic Nitrogen in Maturation Ponds

Composite samples for settled organic nitrogen from maturation ponds M1 and M2 were collected seasonally and processed in the laboratory as described in section 3.5. For this experiment, 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). 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 period of time.

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 7.1). 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 7.1 Mean seasonal nitrogen sedimentation rates in ponds M1 and M2

Considering that nitrogen organic sedimentation is achieved in WSP after biological (mainly algal) uptake of inorganic nitrogen, better conditions for phytoplankton activity during the warm months in summer would have stimulated algal growth in M1 at a faster rate than in M2, as in-pond chlorophyll-*a* values decrease from pond to pond in WSP systems – as well as nutrient availability – when they are arranged in series. It would have led not only to increases in in-pond organic nitrogen as suspended algal biomass in M1, but also nitrogen sedimentation rates. 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 Corinne WSP system, Utah (US EPA, 1977c); this pond had a surface area of 1.49 ha and an average depth of 1.2 m. Nitrogen fractions (total nitrogen, TKN, ammonium, nitrite and nitrate) from the pond influent and effluent were used to calibrate a model developed by Ferrara and Harleman (1978, 1981), which includes the following nitrogen removal and transformations mechanisms: (a) net loss to sediments; (b) ammonia volatilisation; (c) denitrification; (d) cell uptake of ammonia nitrogen; (e) cell uptake of nitrate nitrogen; and mineralization of organic compounds.

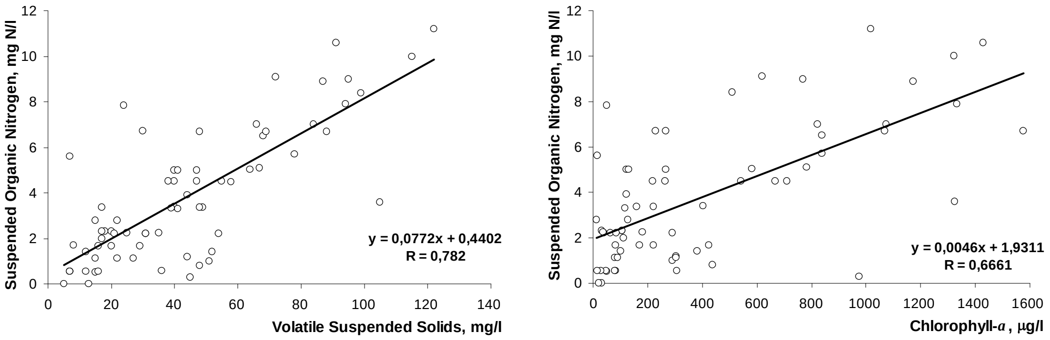
Results showed that over a 13-month period, total nitrogen was removed in Corinne Pond 1 at an average rate of 3.34 kg N/ha d (47%; N loading rate = 7.08 kg N/ha d), mainly through sedimentation of organic nitrogen (3.21 kg N/ha d). Average total nitrogen removal rate (from October 2004 to May 2007) in M1 was 0.911 kg N/ha d (16%) and sedimentation of organic nitrogen was also the most important mechanism for nitrogen removal – 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 7.1); 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.

7.2 Biological Nitrogen Uptake in Maturation Ponds

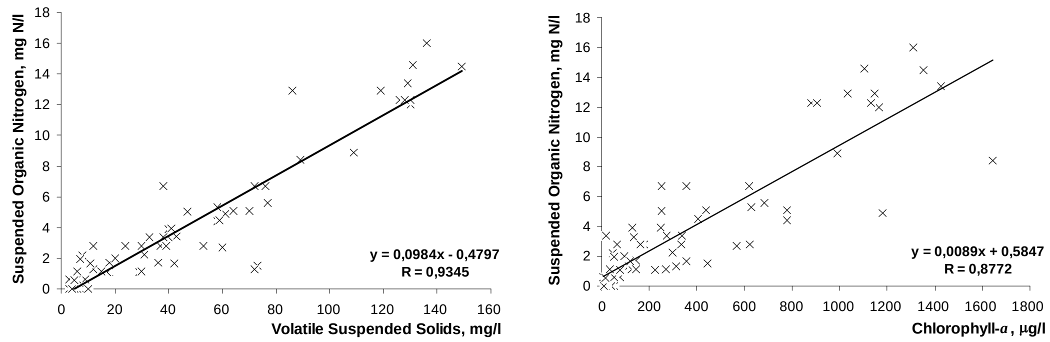
Samples collected weekly from M1 influent (A), M1 effluent (C) and M2 effluent (E) were processed for TKN, filtered TKN, ammonium, nitrite, nitrate, chlorophyll-*a*, suspended solids (SS) and volatile suspended solids (VSS), among other parameters, as described in section 3.3. Results were analysed in order to determine the importance of biological nitrogen uptakes in the maturation WSP under study. First of all, 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 VSS, from A, C and E sampling points.

From 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.

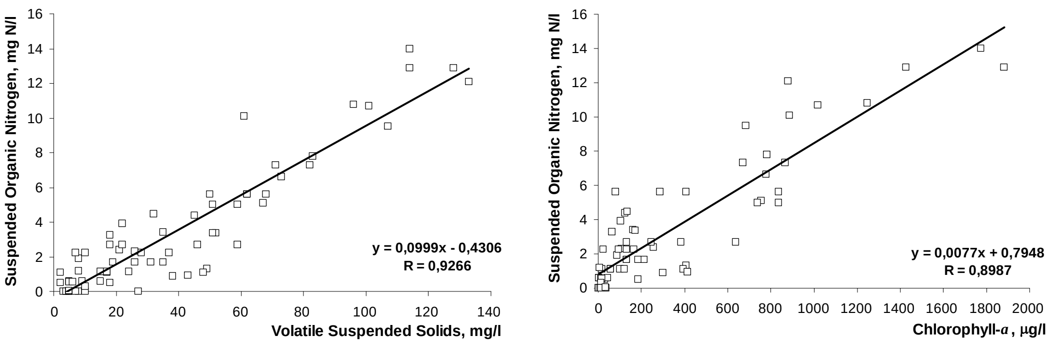
Linear regressions for suspended organic nitrogen vs. VSS and Chl-*a* were also calculated and the results suggest that the suspended organic nitrogen fraction (SuspON) entering M1 (Figure 7.2 (a)) was not entirely from algal biomass as the intercept (≈2 mg N/l) from the Chl-*a* vs. SuspON graph is far 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 7.2 (b)) and M2 effluent (Figure 7.2 (c)) indicates that the nature of suspended organic nitrogen in maturation ponds effluents is mainly algal biomass.



(a) Sampling point A: M1 influent



(b) Sampling point C: M1 effluent



(c) Sampling point E: M2 effluent

Figure 7.2 Linear regressions for suspended organic nitrogen vs. VSS and Chl-*a*

The relationship between VSS and suspended organic nitrogen shows that nitrogen content in VSS from M1 influent, M1 effluent and M2 effluent was 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 alga 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 7.1 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 7.1 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 as 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 job’, performed by biological 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 (see chapter 6). 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.

7.3 Permanent Accumulation of Organic Nitrogen in Pond Sludge Layer

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 as described in section 3.3. 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, U.C. 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 at continuous flow (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 and an important portion (51% in M1 and 39% in M2) of the nitrogen taken up by in-pond algae was finally accumulated as sludge after anaerobic digestion. This can be observed when the nitrogen contents in samples from VSS in pond effluent, sediments and sludge are compared (Figure 7.3); it is even more evident in M2 where sludge feedback did not affect laboratory analysis results. Microscopic examination of sediments from M1 and M2 also confirmed the presence of algae in decomposition (Figure 7.4).

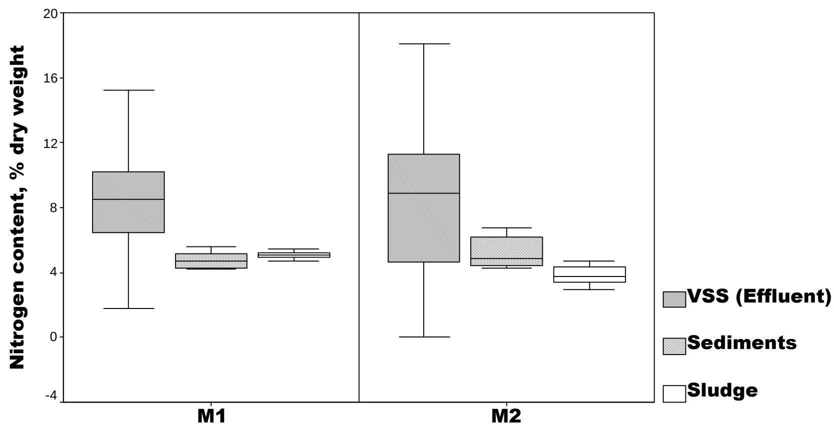
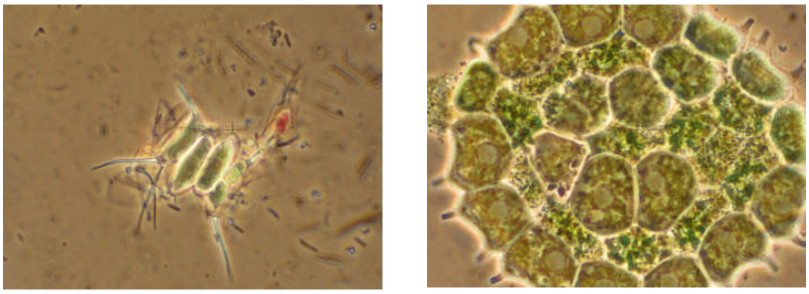


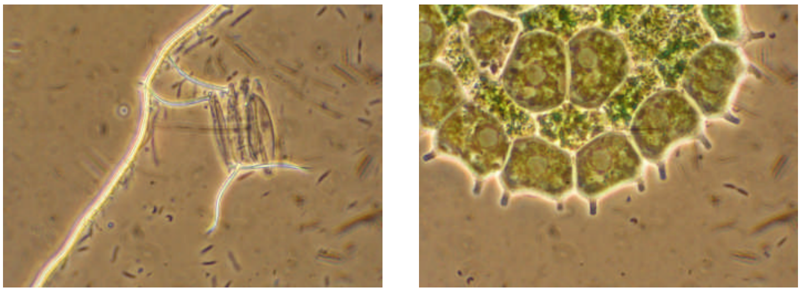
Figure 7.3 Box-plots for nitrogen content in VSS, sediments and sludge

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 which 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.



Scenedesmus protuberans *Pediastrum boryanum*

(a) M1 sediment samples



Scenedesmus protuberans *Pediastrum boryanum*

(b) M2 sediment samples

Note: a bright green colour corresponds to alive algal cells

Figure 7.4 Algal cells in decomposition from sediment samples

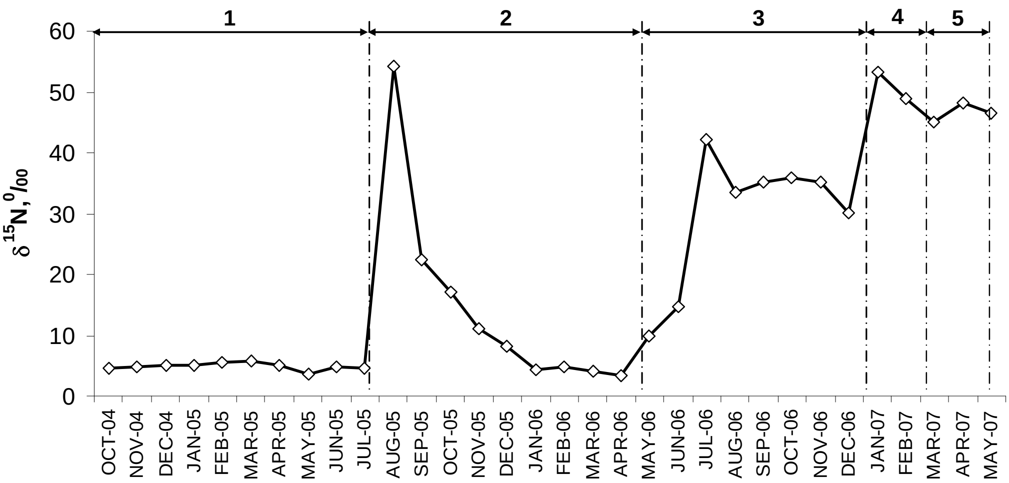


Figure 7.5 $\delta^{15}N$ values in sludge samples from M1 maturation pond

In fact, $\delta^{15}N$ values (Figure 7.5) in sludge samples collected monthly from the maturation pond M1 shows how fast nitrogen was recycled from the sludge layer after anaerobic digestion. These results are from a set of four tracer experiments with ^{15}N stable isotopes carried out as part of this research project (see chapter 8). Figure 7.5 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; (4) tracer spike with^{15}N-labelled ammonium; and (5) tracer spike with ^{15}N-labelled nitrite. Sections 2 and 3 give the overall idea considering that inorganic and organic sources of nitrogen (^{15}N-labelled ammonium and ^{15}N-labelled algae) were spiked within M1 influent under favourable conditions for algal growth. Therefore, $^{15}NH_4^+$ was firstly taken up by algae (section 8.2.1.) and then dead algal cells settled down on the bottom of M1 pond increasing δ^{15}N values in the sludge layer up to 53.2‰. After that, δ^{15}N values decreased following a decreasing exponential pattern until they reached again similar baseline values after about six months.

On the other hand, dead cells of ^{15}N-labelled algae settled down uneven on the bottom of the pond and after anaerobic digestion, ^{15}N-labelled ammonium was released to the water column where nitrogen algal uptake and sedimentation of dead algal cell were performed. It indicates that nitrogen compounds are continuously recycling from the water column to sludge layer and viceversa. For that reason δ^{15}N values from sludge samples slowly increased after M1 was spiked (section 2, Figure 7.5) until reaching a maximum peak (≈42‰).

In summary, biological uptake of inorganic nitrogen species and further sedimentation of dead biomass is one of the major mechanisms controlling ammonium and nitrogen removal in WSP, particularly when environmental and operational conditions are favourable for algal growth.

7.4 Related Publications

This research work was partially published as part of two conference proceedings as follows:

Camargo Valero M. A. and Mara D. D. (2005). Nitrogen removal by sedimentation of organic nitrogen via biological uptake in maturation ponds. *In* Proceedings of the 6th UK Meeting, IWA Young Researchers Conference, April 6−7, Leeds, UK.

Camargo Valero M. A. and Mara D. D. (2005). Sedimentation of organic nitrogen via biological uptake in maturation ponds. *In* Proceedings of *Agua 2005*, October 31−November 4, Cali, Colombia.