

NITRIFICATION-DENITRIFICATION IN WSP: A MECHANISM FOR PERMANENT NITROGEN REMOVAL IN MATURATION PONDS

**Miller Alonso Camargo-Valero, Fiona Read, Duncan Mara,
Rob Newton, Tom Curtis and Russell Davenport**

Universidad Nacional de Colombia – University of Leeds – University of Newcastle



**8th IWA Specialist Group Conference on Waste Stabilization Ponds
April 26 to 30 2009, Belo Horizonte, Brazil**

- Ammonia volatilisation
- Biological ammonium uptake
- Sedimentation of dead biomass and accumulation on sludge layer after partial hydrolysis
- Conventional nitrification-denitrification
- Nitrification and simultaneous biological uptake
- Denitrification by nitrifiers
- Nitrification–denitrification supported by methanotrophs
- Anaerobic ammonia oxidation

Introduction – nitrification-denitrification

In view of the **low nitrate and nitrite concentrations in WSP**, it has been suggested that **nitrification is not likely to occur** in maturation ponds despite prevalent in-pond aerobic conditions and long retention times. However, simultaneous processes such as biological nitrate uptake and/or denitrification would help to explain the apparent absence of nitrification in WSP.

Indeed, **simultaneous nitrification-denitrification** has been reported as the main mechanism for permanent nitrogen removal in WSP (e.g., Lai and Lam, 1997; Zimmo *et al.*, 2004; Picot *et al.*, 2005; Strang and Wareham, 2005), although hardly any evidence regarding to nitrogen transformation pathways dominating nitrification and denitrification has been reported.

Black box approach



In this work, tracer experiments using ^{15}N stable isotopes, along with molecular microbiological analyses, were carried out in a pilot-scale maturation pond in the UK to facilitate the study of the dynamics of inorganic forms of nitrogen under conditions of low algal activity, in order to determine the relative importance of nitrogen transformations and removal mechanisms associated with nitrification and denitrification processes.

Experimental pilot-scale WSP system at Esholt



Methods and materials



FACULTATIVE POND

$$\lambda_{\text{BOD}} = 80 \text{ kg/ha d}$$

$$\lambda_{\text{N}} = 8 \text{ kg N/ha d}$$

$$\theta = 60 \text{ days}$$



MATURATION PONDS IN SERIES

$$Q = 0.6 \text{ m}^3/\text{d}$$

$$\theta = 17 \text{ days}$$

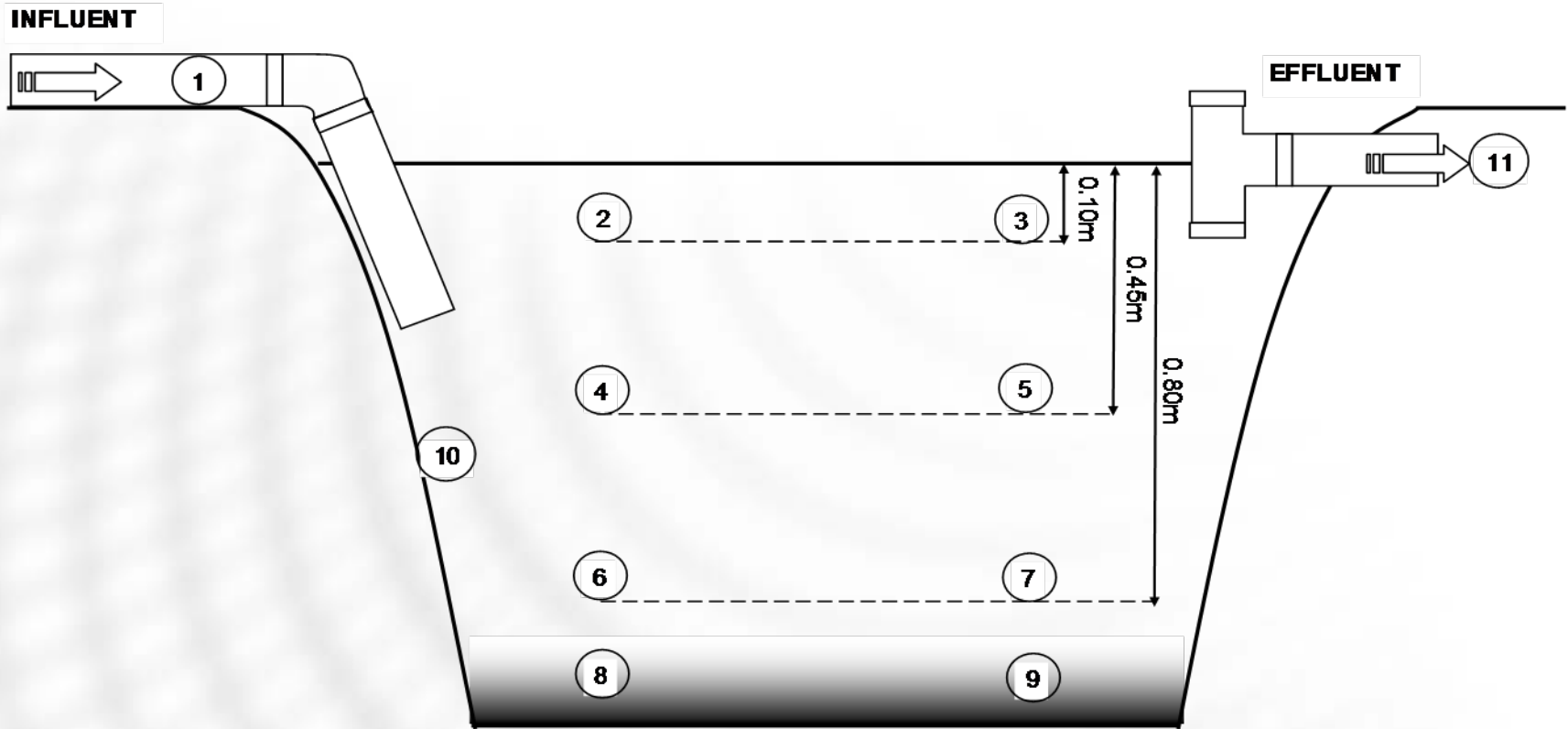
¹⁵N spiking in winter and early spring (2006–2007)

- ¹⁵NH₄Cl
- Na¹⁵NO₂

Sampling for $3 \times \theta$

- 24-h composite samples from grab samples collected every hour in M1 effluent
- Measuring in real time DO, temperature and pH in M1 effluent
- Composite sediment samples for sedimentation rates of organic N
- Collecting ex-pond gases for ammonia volatilisation rates
- Collecting water and sludge samples for molecular microbiology analysis
- Weekly sampling for performance indicators (Chlorophyll *a*, BOD₅ and filter BOD₅, SS, TKN and filtered TKN, NO₂⁻ and NO₃⁻)

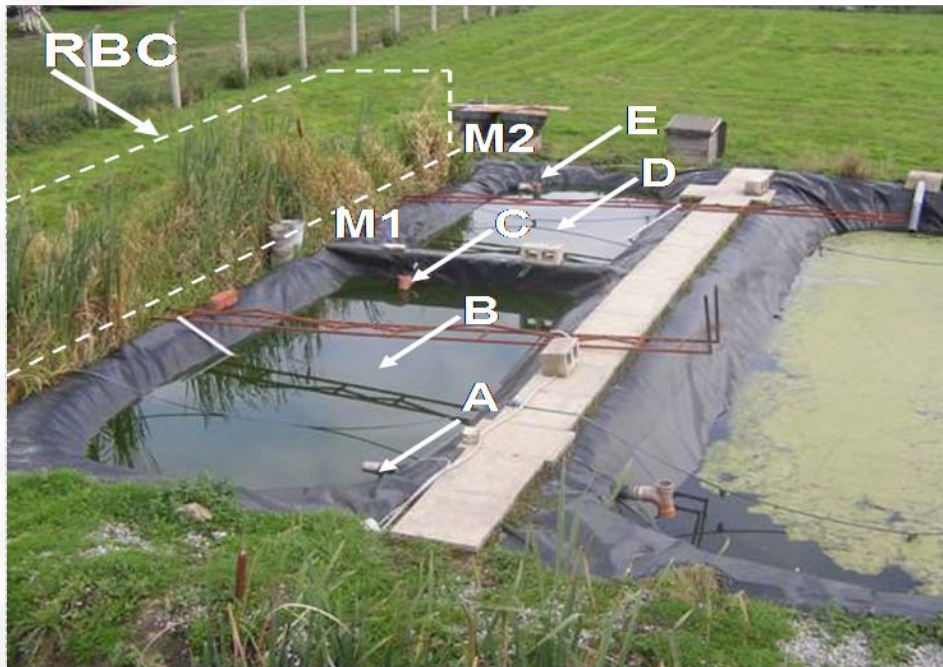
Sampling for molecular microbiology analysis



Samples for molecular microbiology analyses: Influent (1); water column at 0.10m (2 and 3), 0.45m (4 and 5) and 0,80m (6 and 7); sludge layer (8 and 9); pond's wall (10) and effluent (11).

Weekly sampling for performance indicators

Chlorophyll *a*, BOD5 and filter BOD5, SS, TKN and filtered TKN, NO_2^- , NO_3^- , temperature, DO and pH



Sampling points

M1 influent (A)

M1 water column (B)

M1 effluent (C)

Processing composite water samples from pond effluent

- Sequential extraction of four nitrogen fractions (ammonium, suspended organic nitrogen, soluble organic nitrogen and oxidised nitrogen)
- Analysis of $\delta^{15}\text{N}$ values from nitrogen fractions

Processing sediment samples

- Sediment samples were sieve (ASTM sieve No. 10)
- Settled in 1-l Imhoff cones for 3 hours
- Thickened samples were dried and processed for solids, moisture content, total nitrogen content and $\delta^{15}\text{N}$ values

Targeted functional group/organism

- β -proteobacterial ammonia oxidizing bacteria (AOB)
- Ammonia-oxidizing archaea (AOA)
- Anammox bacteria
- Most methanotrophs and some ammonia-oxidizing bacteria
- Nitrospira nitrite-oxidizing bacteria
- Nitrobacter nitrite-oxidizing bacteria
- Denitrifiers

Sample processing

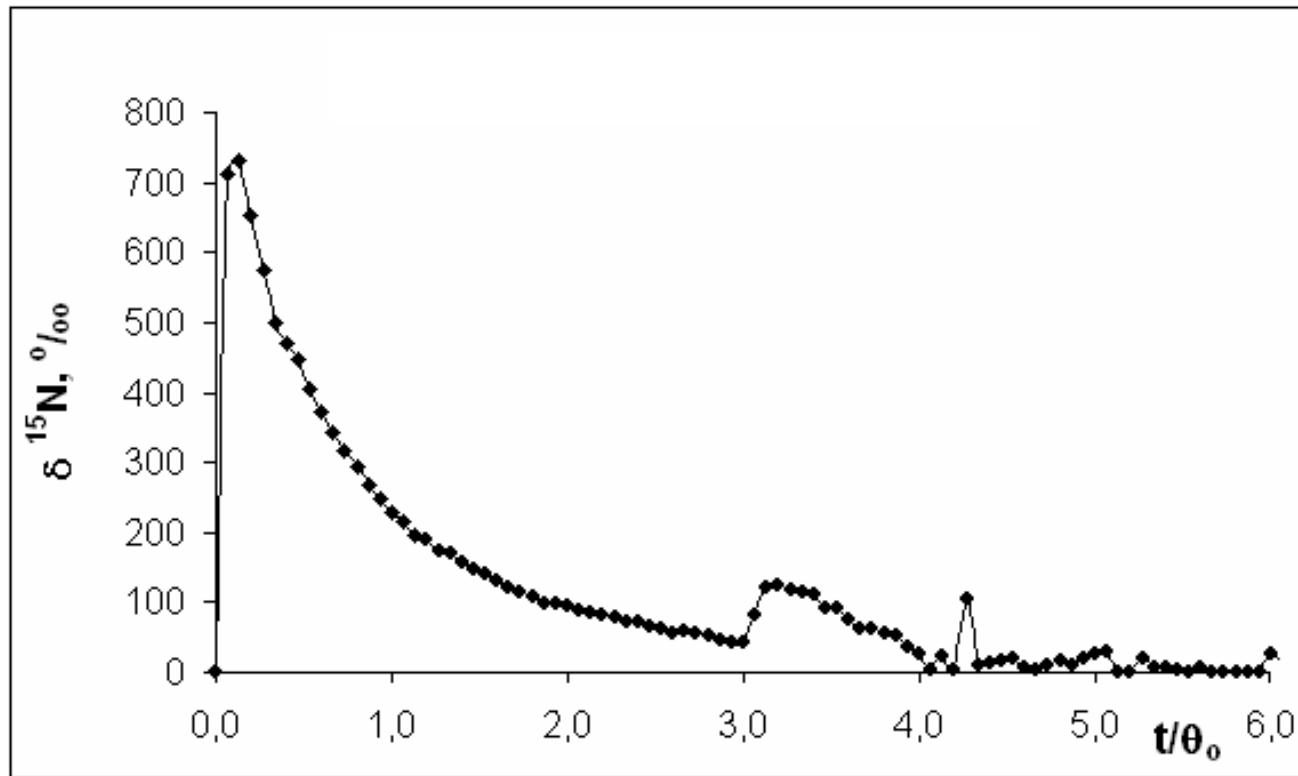
- Extraction of DNA from each sample
- Targeting 16S rRNA gene (or functional gene fragments) PCR using previously published primers and conditions
- Confirming presence or absence using PCR results
- Selected positive PCR results were used to confirm the identity of microorganisms
- DGGE analysis (selected predominant bands) and sequence checked against BLAST.

$\delta^{15}\text{N}$ values

$$\delta^{15}\text{N}, \text{‰} = \left[\frac{\left(\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right)_{\text{sample}} - \left(\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right)_{\text{std}}}{\left(\frac{{}^{15}\text{N}}{{}^{14}\text{N}} \right)_{\text{std}}} \right] \times 1000$$

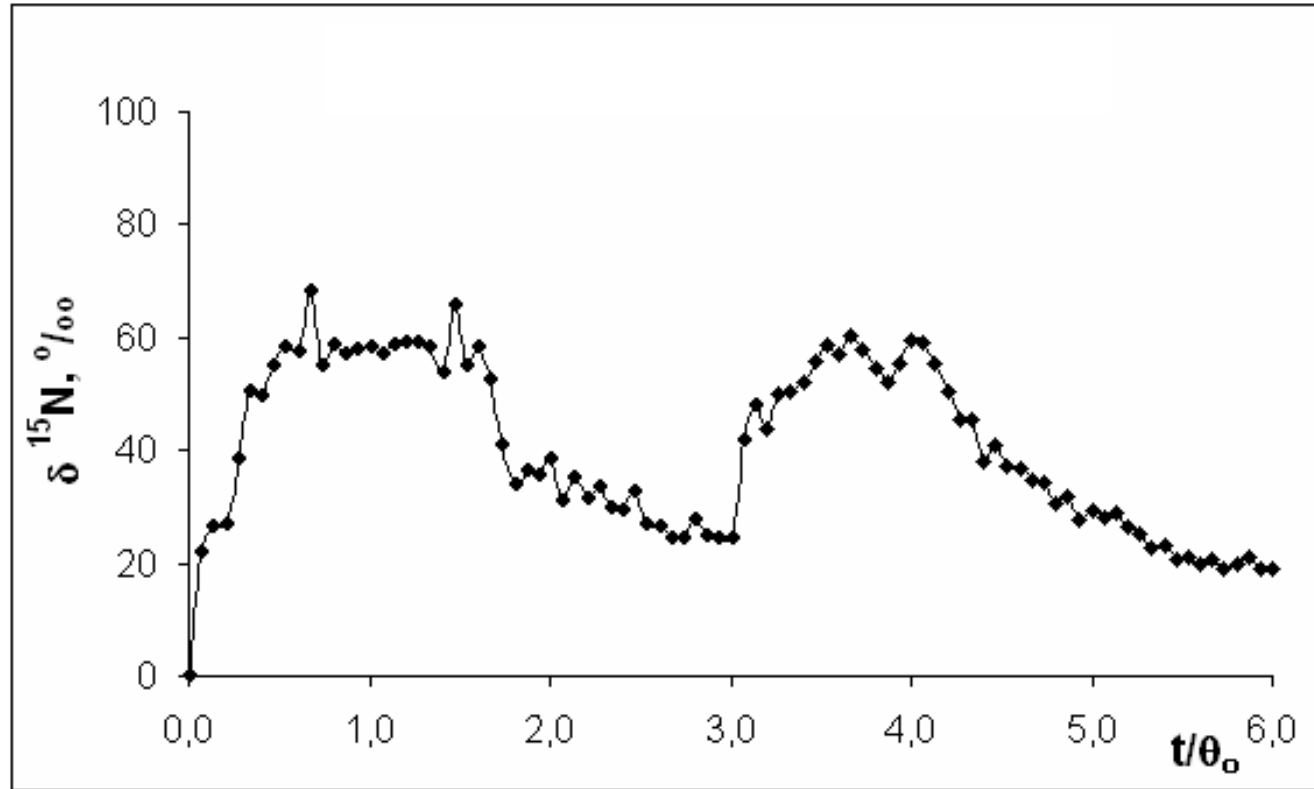
They are not concentrations of ^{15}N isotope but differences between $^{15}\text{N}:^{14}\text{N}$ ratios in the sample and atmospheric gas which has a known ^{15}N content and acts as a standard

$\delta^{15}\text{N}$ values from M1 effluent



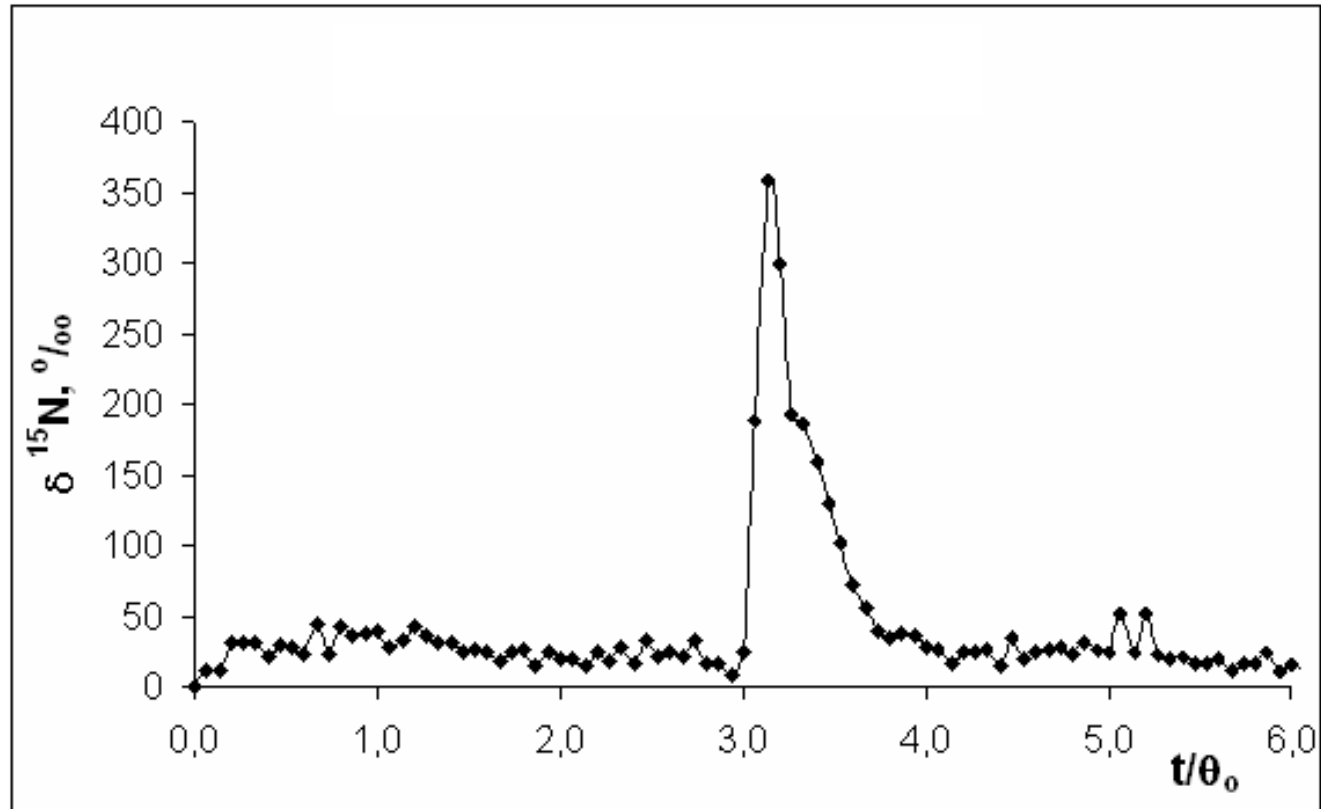
Ammonium nitrogen

$\delta^{15}\text{N}$ values from M1 effluent



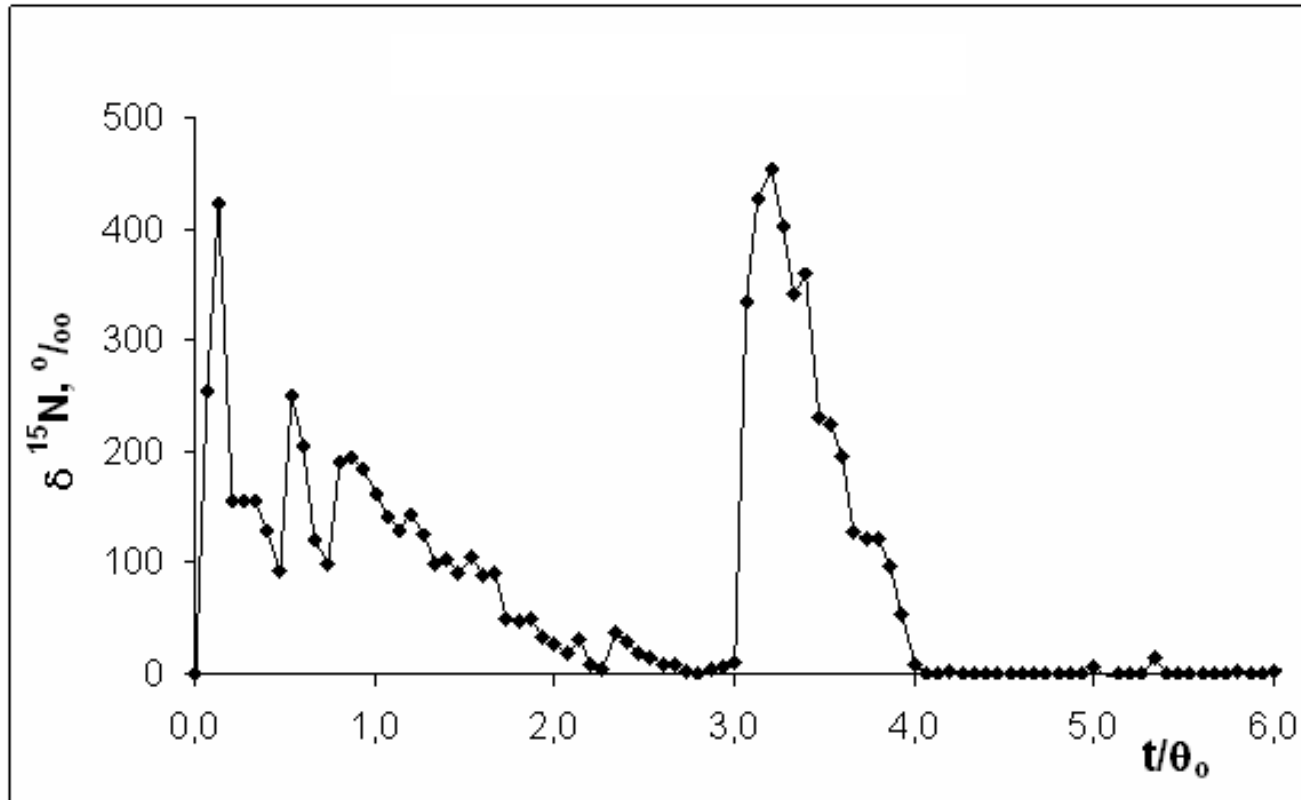
Suspended organic nitrogen

$\delta^{15}\text{N}$ values from M1 effluent



Soluble organic nitrogen

$\delta^{15}\text{N}$ values from M1 effluent



Oxidised nitrogen (nitrite + nitrate)

Results and discussion – tracer recovery

Nitrogen fractions	Tracer recovery, %	
	* $^{15}\text{NH}_4^+$	** $^{15}\text{NO}_2^-$
Recovered in M1 effluent		
Suspended organic nitrogen	2.0	3.3
Soluble organic nitrogen	0.8	4.8
Ammonium	55.7	1.9
Nitrite + Nitrate	3.0	1.5
Remaining in water column	~4.0	~1.5
Stored in sludge layer	~24.5	~30.0
Ammonia volatilisation	0.0	0.0
Net recovery	~90	~43.0

* The mass balance was calculated over the $0 < t/\theta_0 < 3$ period

** The mass balance was calculated over the $3 < t/\theta_0 < 6$ period

Enrichment of ex-pond gas with ^{15}N

- **Tracer experiment with ^{15}N -labelled ammonium**

From 16.7 to 35.7‰

- **Tracer experiment with ^{15}N -labelled nitrite**

From 10.05 to 52.79‰

Baseline: -42.40 to -31.10‰

Operational conditions and overall performance

	Experiment 1	Experiment 2
Temperature, °C	3.1 – 6.4	5 – 12
Daylight, h/day	7.8 – 10.8	10.8 – 13.9
Sunlight, h/day	2.4	5.3
Chlorophyll <i>a</i> , µg/L	46	250
pH	6.1 – 7.6	6.8 – 8.2
Ammonium removal, %	~ 0	75
Total N removal, g/ha d (%)	813 (23%)	324 (18%)
Experiment 1: 15N-labelled ammonium Experiment 2: 15N-labelled nitrite		

Results for the presence/absence of microbial groups by PCR

Sample	Ammonia-oxidizers		Nitrite-oxidizing bacteria		Anammox	Methanotrophs	Denitrifiers	
	AOB	AOA	<i>Nitrobacter</i>	<i>Nitrospira</i>			<i>nirS</i>	<i>nirK</i>
Water column	+	±	+	+	-	+	+	+
Sludge	+	-	+	+	-	+	+	+

Key: + = strong band detected, ± = weak band detected, - = no band detected

AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea.

PCR results, DGGE band sequence and comparison with BLAST

AOA: uncultured crenarchaeote clone (98% similarity)

AOB: more predominant and also capable to perform denitrification

Methanotrophs: confirmed with methanotroph-like sequences

NOB: *Nitrospira* (80%), but no *Nitrobacter*

Denitrifiers: confirmed by *nirS* and *nirK* genes (99% and 89% similarities with uncultured clones)

Conclusions

Tracer experiments with ^{15}N stable isotopes showed a clear competition for inorganic nitrogen species between the two main mechanisms dominating nitrogen removal in maturation ponds: algal uptake and nitrification-denitrification.

Denitrification supported either by AOB or methanotrophs, in addition to NOB, in WSP may be counted as a feasible mechanism for permanent nitrogen removal, but its relative supremacy over other nitrogen removal mechanisms (e.g., biological uptake) would depend upon phytoplanktonic activity.

Acknowledgements

EPSRC

Engineering and Physical Sciences
Research Council



COLFUTURO



UNIVERSIDAD
NACIONAL
DE COLOMBIA



YorkshireWater



Nitrification-denitrification in WSP: a mechanism for permanent nitrogen removal in maturation ponds
8th IWA Specialist Group Conference on Waste Stabilization Ponds
April 26 to 30 2009, Belo Horizonte, Brazil

NITRIFICATION-DENITRIFICATION IN WSP: A MECHANISM FOR PERMANENT NITROGEN REMOVAL IN MATURATION PONDS

Miller

M.A.Camargo-Valero@leeds.ac.uk

Duncan

D.D.Mara@leeds.ac.uk

Russell

R.J.Davenport@ncl.ac.uk