

Nitrification potential in waste stabilisation ponds: comparison of a secondary and tertiary pond system

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Abstract This study compared the nitrification potential of two separate Waste Stabilisation Ponds (WSPs) operating under differing physical and chemical conditions. In order to probe the nitrification potential of each system, the oxidation of ammonium and also the intermediate product nitrite was assessed using both *in situ* and laboratory micro-scale incubations. The role of sediment in determining the nitrification potential of the two WSPs was also investigated. Results from laboratory microcosm incubations revealed a competent and strikingly similar nitrification potential for both WSPs in spite of their differing nitrogen and organic loadings, and also suggested a significant role for sediment in WSP nitrogen cycling. Results from *in situ* field experiments identified biomass uptake to be the dominant nitrogen removal mechanism in natural pond environments. Other aspects of WSP nitrogen cycling are also discussed.

Keywords Ammonium; nitrate; nitrification; nitrogen cycling; waste stabilisation pond; sediment.

Introduction

Nitrogen (N) is a major nutrient in wastewater and must be reduced to acceptable levels during treatment to reduce its eutrophication potential in receiving water bodies (Harremoës, 1988). In WSP influent, N is mainly present as either inorganic ammonium (NH₄-N) or as organic N in the form of protein or urea (Reed, 1985). There are three accepted pathways for NH₄-N removal in WSP environments: volatilisation; biomass sequestration (microbial and algal); and nitrification–denitrification (Middlebrooks *et al.*, 1982); however, there remains ongoing debate regarding the relative contributions of each pathway to overall N removal. Previously, the majority of inorganic N removal was considered to be achieved via microbial biomass assimilation and/or NH₄-N volatilisation (Ferrara and Avci, 1982; Reed, 1985; Shin and Polprasert, 1988; Silva *et al.*, 1995). However, Hussainy, (1979) identified nitrification–denitrification as a dominant mechanism for N removal in certain WSPs. More recent research has also shown algal biomass uptake and nitrification–denitrification to be more important factors for N removal in WSPs than volatilisation (Lai and Lam, 1997; Baskaran and Farago, 2007; Camargo Valero and Mara, 2007a, 2007b).

The performance of the Bolivar wastewater treatment plant (WWTP), north of Adelaide, South Australia has increased significantly since trickling filtration was replaced with activated sludge (AS) treatment in 2001. The WSP influent quality has dramatically improved with respect to biochemical oxygen demand (BOD), total Kjeldahl Nitrogen, suspended solids, total phosphorous and NH₄-N concentration (Cromar *et al.*, 2005; Sweeney *et al.*, 2005b). Concomitantly, concentrations of oxidised N (nitrite and nitrate) in the WSP influent have increased 1000-fold since the AS plant commissioning (Cromar *et al.*, 2003). It is important to understand the N cycle within WSPs, since the removal of inorganic N from the water column may—in some instances—have the potential to change the ecology of WSPs towards N-fixing cyanobacteria (e.g., Cromar *et al.*, 2005).

This study on N transformations in WSPs has specifically focused on the microbial process of nitrification. During this research, the nitrification potential of two different WSP systems—with

differing physical designs and operating conditions—was of investigated. In order to more closely probe nitrification potential within the two systems, the oxidation of both $\text{NH}_4\text{-N}$ and also the intermediate product nitrite was assessed. The role of sediment in the nitrification potential of the two WSP systems was also investigated. Since the study was concerned specifically with investigating microbiological processes, small-scale systems were operated in order to maximise experimental control. Results from both *in situ* and laboratory micro-scale incubations are presented in this paper.

Materials and Methods

WSPs from two sites (Bolivar and Mt. Barker) were selected for this research. The Bolivar WSPs are located north of Adelaide, South Australia ($34^{\circ}45'23''\text{S}$ $138^{\circ}34'15''\text{E}$) and comprise six ponds operating in two parallel trains covering a total area of around 350 ha; treating effluent from a population equivalent of 800,000 people. The first and largest pond (Pond 1) was selected for this research (see Sweeney *et al.*, 2003 for detailed description). With a nominal operating depth of 1.3 m and an hydraulic residence time of 12–15 days, the tertiary-level facultative WSP receives effluent from an AS plant. The second study site, the Mt. Barker WSP system, is located east of Adelaide, South Australia ($35^{\circ}04'08''\text{S}$ $138^{\circ}52'35''\text{E}$) and is a secondary-level WSP system designed to treat settled septic tank effluent from a local population of around 25,000. The Mt. Barker system (1.5 ha) consists of two WSPs operating in parallel; with the Eastern pond being utilised for this research. The Mt. Barker WSP receives influent wastewater at much higher N concentrations than the Bolivar WSP ($50\text{--}80\text{ mg N L}^{-1}$) and also operates at a deeper hydraulic depth (2.4 m). A comparative summary of the two WSP systems is provided in Table 1.

Table 1. Characterisation of the Bolivar and Mt. Barker WSP systems showing typical average water quality across a number of parameters (Herdianto, 2003; Sweeney, 2004; Cromar *et al.*, 2005).

WSP system	Treatment level	Depth (m)	Typical influent water quality			<i>in situ</i> DO (mg L^{-1})	
			$\text{NH}_4\text{-N}$ (mg L^{-1})	NO_x (mg L^{-1})	BOD_5 (mg L^{-1})	Sediment–water interface	Sub-surface
Bolivar	Tertiary	1.3	2.7	8.8	44	0.3–7	4.2
Mt. Barker	Secondary	2.4	55–75	0.02–0.2	45–100	0.5–2.5	2.5

Laboratory microcosm studies

Cylindrical polyethylene vessels ($25\text{ cm} \times 25\text{ cm}$) served as microcosm reactors for the laboratory incubation experiments. Wastewater from Bolivar Pond 1 or the Mt. Barker WSP was collected and transported to the laboratory where it was pre-filtered (Whatman[®] Qualitative Grade 1; $11\ \mu\text{m}$) and stored at 4°C in the dark until required. Samples of sediment and overlying wastewater (5 L) were also collected using a tubular sediment corer ($3\text{ cm} \times 130\text{ cm}$) and transported to the laboratory. In the laboratory, samples (wastewater or wastewater plus sediment) were incubated in water bath acclimated microcosms under dark conditions at 25°C —a temperature similar to that at the sample sites during summer (Herdianto, 2003; Sweeney *et al.*, 2005a). Microcosm inorganic N concentrations were augmented either with $10\text{ mg NO}_2\text{-N L}^{-1}$ or $100\text{ mg NH}_4\text{-N L}^{-1}$. Microcosms were gently sparged with air at to maintain the initial DO concentration ($\approx 7\text{ mg L}^{-1}$) without disturbing the sediment. Water quality parameters were measured (temperature, DO, pH, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and alkalinity) every third day for a total of 21 days using standard methodology (APHA, 1995).

Bolivar *in situ* incubations

Three cylindrical microcosms ($10\text{ cm} \times 150\text{ cm}$) made from clear acrylic were driven into the sediment of Pond 1, isolating them from the bulk water phase and allowing *in situ* determination of N dynamics. Open PVC elbows were attached to the mesocosms to prevent rainwater influx. Following initial determination of ambient inorganic N concentrations at Day zero, inorganic N within two of the three microcosms was

augmented as follows: Microcosm 1 (M1), 100 mg NH₄-N L⁻¹; and Microcosm 2, 20 mg NO₂-N L⁻¹ (M2). Microcosm 3 (M3) served as the control. Water quality parameters were monitored as above, with the addition of chlorophyll *a* analysis (method 10200 H; APHA, 1995).

Results and discussion

Laboratory microcosm study: ammonium oxidation performance

In all experiments, a N budget (Figure 1.a) confirmed that much of the NH₄-N removed from the microcosms (28–80 %) was not recovered in the form of oxidised N (NO_x) by Day 21, suggesting that nitrification was not the major process responsible for NH₄-N removal. Due to this imbalance in the N budget, differences in biological NH₄-N oxidation performance between the microcosms was instead derived from NO_x generation; since this provided a true measure of nitrification activity. This approach effectively disregards all other NH₄-N removal process (e.g., volatilisation, biomass assimilation, sediment adsorption, and/or phosphate mineral co-precipitation) which may have otherwise biased the implied biological NH₄-N oxidation performance. Following a challenge spike of NH₄-N (100 mg L⁻¹) on Day 1, NO_x generation in the Bolivar and Mt. Barker microcosms was observed to plateau after Days 9 and 12 respectively for microcosms containing sediment (Figure 1.b), indicating that the substrate NH₄-N had been exhausted. During this time, NO_x generation potential for microcosms containing sediment was significantly higher than those microcosms containing wastewater only (Figure 1.c). The magnitude of NO_x generation also correlated well with the reduction in both pH and alkalinity (Table 2), providing additional evidence of biological nitrification within the microcosms (Lui, 1997). As shown in Table 2, the relative strength of these correlations closely reflected the extent of nitrification within the microcosms (Figure 1), with more highly significant correlations between these parameters for microcosms containing sediment. These results demonstrated that the presence of sediment significantly enhanced the nitrification capacity of both the Bolivar and Mt. Barker wastewater and suggests an important role for sediments in nitrogen cycling within WSPs. Zimmo *et al.* (2004) also reported higher nitrification rates in wastewater incubations containing sediment than for those containing wastewater alone; something they speculatively attributed to a higher density of nitrifying microbes existing within the sediment.

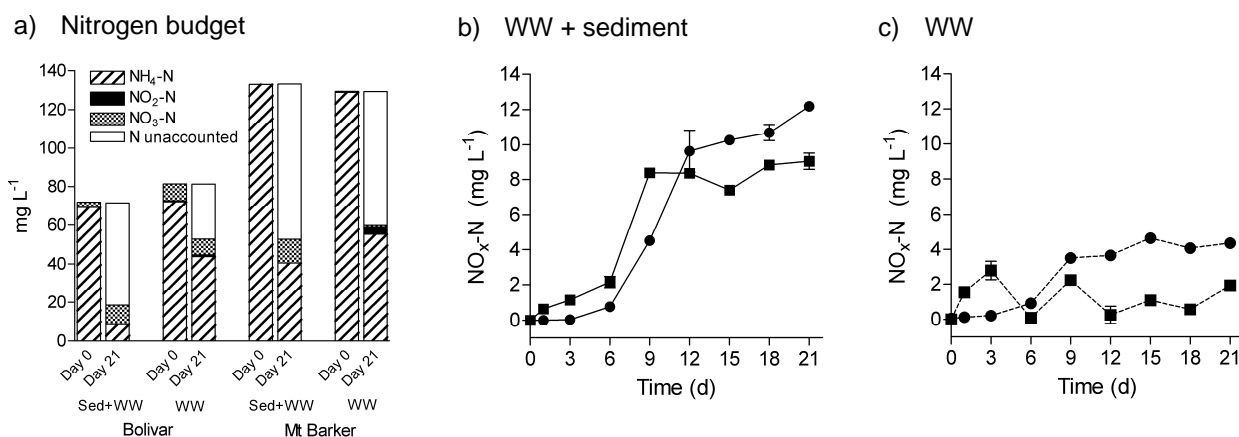


Figure 1. (a) Nitrogen budget highlighting temporal changes in inorganic nitrogen species for microcosms augmented with 100 mg L⁻¹ of NH₄-N on Day 0; and comparison of aerobic nitrification performance at 25°C between microcosms containing: (b) WSP sediment plus wastewater; and (c) wastewater only; (■) Bolivar microcosms; and (●) Mt. Barker microcosms. Data points show means ± 1 SD.

The NH₄-N oxidation potential of wastewater and the wastewater + sediment was similar for both WSP isolates and appeared independent of native N concentrations (Figures 1.b and 1.c). Although the nitrification performance of the Mt. Barker microcosms appeared slightly better than those from Bolivar, the mean performance of the two systems over the 21 day period was statistically similar for experiments

containing wastewater + sediment (unpaired *t*-test; $t_{(0.05,16)}=0.104$; $p = 0.918$) and wastewater only ($t_{(0.05,16)}=1.634$; $p = 0.122$). The Mt. Barker system contained higher native $\text{NH}_4\text{-N}$ concentrations than the Bolivar WSP, which may predispose the Mt. Barker WSP to nitrification. Conversely, the Bolivar system had greater NO_x influent loading and is also generally much more aerobic than the Mt. Barker pond (Table 1), so it could be speculated that the Bolivar pond may be more likely to be nitrifying than would the Mt. Barker system. Results from this research supported neither of these hypotheses; instead both systems were shown to possess similar nitrification potentials, suggesting a possible ‘trade-off’ between a higher indigenous $\text{NH}_4\text{-N}$ exposure and elevated DO concentration in the maintenance of nitrifying populations within the sediments of both systems.

Table 2. Spearman rank (r_s) correlation coefficients highlighting relationships between NO_x production with incubation time, pH and alkalinity for all microcosm experiments augmented with $100 \text{ mg NH}_4\text{-N L}^{-1}$, $n=10$.

	Microcosm	Incubation time (d)	pH	Alkalinity ($\text{mg CaCO}_3 \text{ L}^{-1}$)
NO_x generation (mg L^{-1})	Bolivar WW + SED	r_s 0.78*	-0.75*	-0.76*
	Bolivar WW	r_s 0.06	-0.22	0.02
	Mt. Barker WW + SED	r_s 0.99***	-0.94***	-0.97***
	Mt. Barker WW	r_s 0.78*	-0.91**	-0.89**

Correlation is significance at: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$ (2-tailed).

Laboratory microcosm study: nitrite oxidation performance

Figure 2.a shows a nitrogen budget obtained from the $\text{NO}_2\text{-N}$ augmented (10 mg L^{-1}) microcosm experiments. Results showed that all of the $\text{NO}_2\text{-N}$ added on Day zero was recovered in the form of $\text{NO}_3\text{-N}$ by Day 21. Unaccounted N in these experiments was most likely a result of $\text{NH}_4\text{-N}$ volatilisation for the Bolivar microcosms and a combination of $\text{NH}_4\text{-N}$ volatilisation, sediment adsorption and/or phosphate co-precipitation for the higher native $\text{NH}_4\text{-N}$ in Mt. Barker microcosms. Comparisons of nitrite oxidation (nitrification) performance between microcosms were based on comparing nitrite removal over time. Figures 2.b and 2.c compare the biological $\text{NO}_2\text{-N}$ oxidation potential (defined as $\text{NO}_2\text{-N}$ removal) between microcosms containing wastewater + sediment with those containing wastewater only following a challenge spike of $\text{NO}_2\text{-N}$ (10 mg L^{-1}) on Day 1. $\text{NO}_2\text{-N}$ removal in both the Bolivar and Mt. Barker microcosms containing sediment was comparatively rapid (Fig 2b), with $\text{NO}_2\text{-N}$ limited by Day 3. In contrast, $\text{NO}_2\text{-N}$ removal in microcosms containing only wastewater (Figure 2.c) was much slower, taking a further 9–12 days for the substrate $\text{NO}_2\text{-N}$ to become fully oxidised. The secondary spike in $\text{NO}_2\text{-N}$ on Day 9 within both Mt. Barker microcosms was noticeably absent in the parallel Bolivar incubations (Figures 2.b and 2.c) and was attributed to the sequential microbial oxidation of indigenous $\text{NH}_4\text{-N}$ (47 mg L^{-1}) to $\text{NO}_2\text{-N}$ and finally to $\text{NO}_3\text{-N}$.

Similar to the microcosms augmented with $\text{NH}_4\text{-N}$, results from $\text{NO}_2\text{-N}$ spiking experiments confirmed that the presence of sediment significantly enhanced the nitrification capacity of both the Bolivar and Mt. Barker wastewaters; highlighting again the importance of sediment for N cycling in WSP systems. This finding is aligned with previous reports of nitrifiers being more biologically active when attached to solid surfaces rather than existing as free-living cells (Bazin *et al.*, 1982; Underhill and Prosser, 1987) and suggests a role for WSP sediments in providing a reservoir of nitrifying microbes *in situ*. It should also be noted that the $\text{NO}_2\text{-N}$ removal potential in both the Bolivar and Mt. Barker microcosms containing wastewater only (Figure 2.c) was much greater than the $\text{NH}_4\text{-N}$ removal (NO_x generation) potential for the same experimental treatment (Figure 1.c). This observation suggests a possible spatial partitioning of nitrite- and ammonium-oxidising microbes within the WSP water column and sediment. The nitrite-oxidisers—since they are most susceptible to low DO concentrations—may preferentially inhabit the

upper depths of the water column where DO is more abundant, whereas the ammonium-oxidisers may be more likely to persist in the lower and less oxygenated region of the water column (and sediment) since they are recognised to be more resilient to hypoxic conditions (Stenstrom and Poduska, 1980). Support for this theory also lies in the reported half saturation constants for nitrite- and ammonium-oxidisers of 1.1 and 0.3 mg O₂ L⁻¹ respectively (Wiesmann, 1994); implying a greater nitrification performance in hypoxic sediments for ammonium- rather than nitrite-oxidisers.

Interestingly, and as was recorded for the NH₄-N augmented microcosms (Figure 1), the NO₂-N oxidation performance of WSP isolates drawn from a relatively N-limited *tertiary-level* system (Bolivar) and a comparatively N-rich *secondary-level* system (Mt. Barker) appeared to be qualitatively similar (Figures 2.b and 2.c) and independent of ambient N levels. This finding suggests that although the Bolivar pond has been deemed at times N-limited (Cromar *et al.*, 2005) and hence is not preconditioned to a high nitrogen load, it may indeed possess the spare biological capacity required to cope with high shock loads of N should such spike events occur.

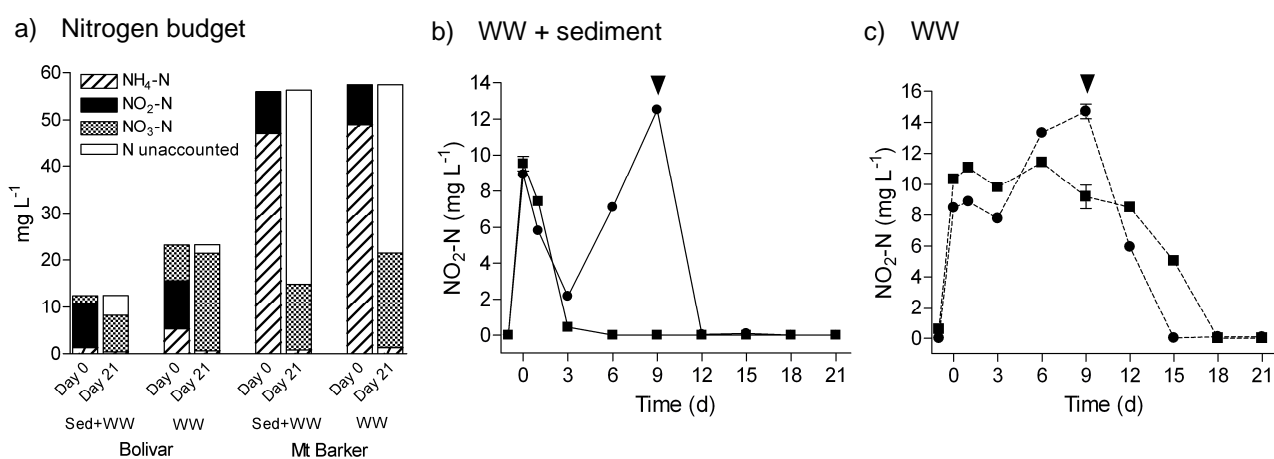


Figure 2. (a) Nitrogen budget highlighting temporal changes in inorganic nitrogen species for microcosms augmented with 10 mg L⁻¹ of NO₂-N on Day 0; and comparison of aerobic nitrite removal performance at 25°C between microcosms containing: (b) WSP sediment plus wastewater; and (c) wastewater only. Symbols denote: (■) Bolivar microcosms; and (●) Mt. Barker microcosms. Data points show parameter means ± 1 SD.

In situ microcosm study

In the laboratory-based microcosm experiments, it was considered likely that the true *in situ* nitrification potential of the two WSP systems may have been somewhat overstated. This probable overstatement of nitrification performance was likely due to both the physical removal of algal biomass removing competition for NH₄-N as well as the resuspension and redistribution of settled sediment fractions exposing the native nitrifying community to more favourable substrate and DO conditions. Field-based *in situ* experiments were therefore established to determine whether laboratory microcosm results could be extrapolated to field conditions; with results of this experiment presented in Figure 3.

For the NH₄-N augmented microcosm (100 mg NH₄-N L⁻¹; Figure 3.a), significant NH₄-N removal (48%) was recorded over the 21 day incubation; however, in contrast to the laboratory microcosm experiments (Figure 1), no significant yield in NO_x was observed, suggesting that nitrification was not responsible for NH₄-N removal *in situ*. NH₄-N removal did, however, coincide with a steady increase in chlorophyll *a* concentration within the *in situ* microcosms (Figure 3.a), indicating that NH₄-N assimilation into new algal biomass was the preferred pathway for NH₄-N removal as suggested previously by Camargo Valero and Mara (2007b). The increase in chlorophyll *a* levels strongly correlated with a decline in alkalinity compared with the control (60 to 35 mg CaCO₃ L⁻¹; *p*<0.01) and with an increase in both DO (3 to

23 mg L⁻¹; $p < 0.01$) and pH (7.4 to 9; $p < 0.01$)—all of which are characteristic indicators of elevated algal activity. This rise in pH may have also increased NH₄-N removal through volatilisation, since a significant percentage ($\approx 25\%$) of the NH₄-N would have been present as unionised free ammonia at pH 9 at 18°C (Boyd, 1990).

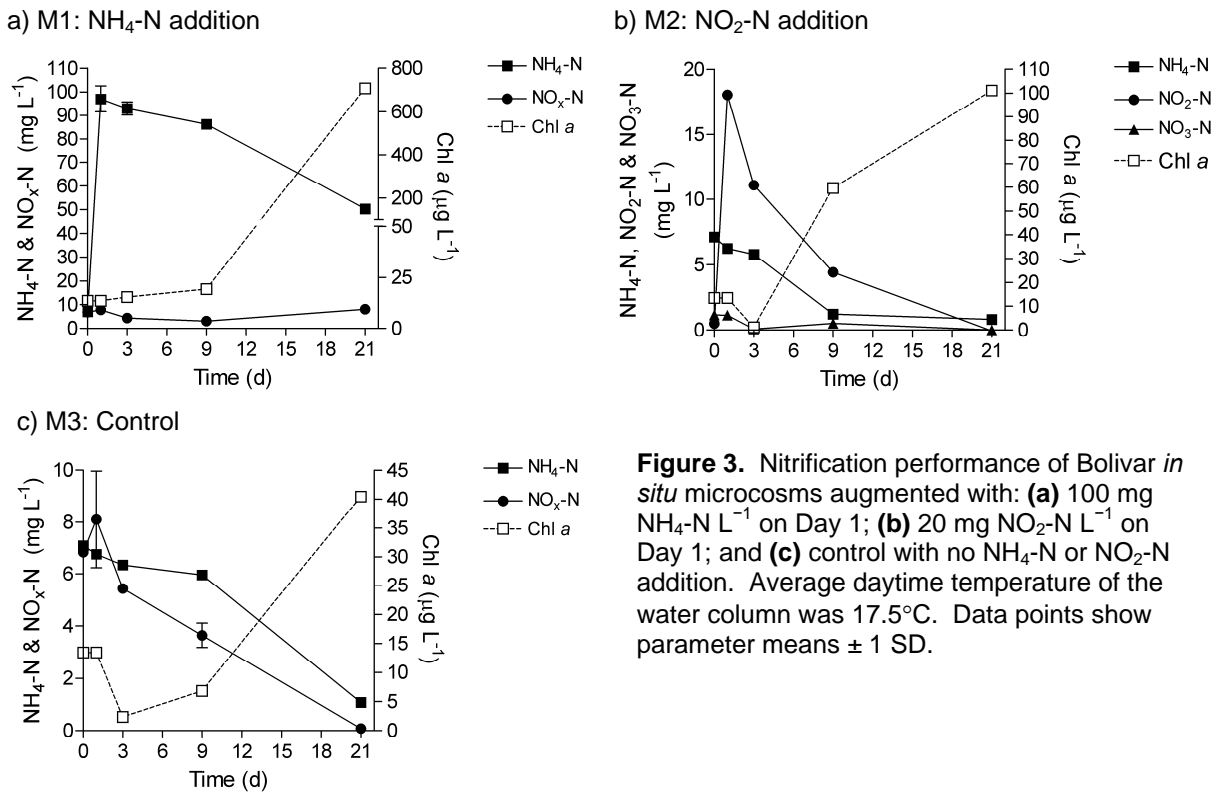


Figure 3. Nitrification performance of *Bolivar in situ* microcosms augmented with: **(a)** 100 mg NH₄-N L⁻¹ on Day 1; **(b)** 20 mg NO₂-N L⁻¹ on Day 1; and **(c)** control with no NH₄-N or NO₂-N addition. Average daytime temperature of the water column was 17.5°C. Data points show parameter means \pm 1 SD.

Stoichiometrically, a relatively small proportion ($\approx 7\%$) of the removed NH₄-N could be accounted for through algal biomass assimilation based on the Redfield elemental composition of algal cells and a 1% chlorophyll *a*:algal biomass ratio (w:w) for common WSP algal species (Reynolds, 2006). Visual inspection of the microcosm walls revealed significant attached algal biomass; however, this actively growing algal biofilm was not included in the suspended chlorophyll *a* analyses. Furthermore, it was likely that assimilated organic N contained within the algal (and microbial) biomass settled to the sediment and was similarly excluded from the N budget. Notwithstanding this, it remains possible that microbial processes may have contributed to the total NH₄-N loss from the system. Lai and Lam (1997) reported that nitrification occurs in WSPs under conditions of photosynthetically-elevated DO, and so it is possible that any NO₃-N produced through this process may have been sequentially taken up by algae before it could be measured *in situ*. It is also possible that nocturnal denitrification of NO₃-N within the sediments may have resulted in some N loss from the microcosms, since the increasingly high respiratory BOD of the expanding algal population could have promoted steep overnight declines in DO, thereby facilitating night-time denitrification of any dissolved NO₃-N (e.g., Hussainy, 1979; Lai and Lam, 1997).

Figure 3.b shows results from the NO₂-N augmented microcosm. Similar to the laboratory microcosms (Figure 2), 100% of the spiked NO₂-N was removed over the course of the 21 day experiment, with a relatively rapid rate of removal observed between Day zero and Day 3. This rapid disappearance of spiked NO₂-N correlates well with the recognised high rates of substrate-utilisation by nitrite oxidising microbes (Rittmann and McCarty, 2001). Unlike the laboratory microcosms experiments, however, NO₂-N losses did not coincide with the production of NO₃-N (compare Figures 2.a and 3.b). Since NO₂-N was

disappearing from the system, it was most likely that nitrified $\text{NO}_3\text{-N}$ was being directly and sequentially assimilated by algal populations before it could be measured *in situ*—as demonstrated by the complementary increase in chlorophyll *a* in Figure 3b. The capacity for direct $\text{NO}_2\text{-N}$ uptake by some algal species has been known for some decades (e.g. Toetz, 1976); although the rate of $\text{NO}_2\text{-N}$ uptake can be several times slower than for the preferred form of inorganic nitrogen $\text{NH}_4\text{-N}$ (Toetz, 1976; Conway, 1977). It remains unclear whether the disappearance of $\text{NO}_2\text{-N}$ in the nitrite-amended microcosms was a consequence of indirect microbial nitrification followed by algal assimilation or a result of direct $\text{NO}_2\text{-N}$ reduction (via nitrite reductase) and assimilation by resident algae. Regardless of this uncertainty, it was apparent that the spiked $\text{NO}_2\text{-N}$ was indeed removed from the system and that its disappearance was associated with an increase in measured algal biomass. Once again, only a small fraction of the added $\text{NO}_2\text{-N}$ was able to be stoichiometrically accounted for through algal biomass assimilation ($\approx 6\%$). Comparison of the control microcosm (Figure 3.c) with the bulk water outside (data not shown) showed remarkable similarity regarding the concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_x\text{-N}$ and chlorophyll *a* over the 21 day incubation period, with the control microcosm generally mirroring the behaviour of the bulk water phase. 93–100% of control microcosm NO_x existed in the form of $\text{NO}_3\text{-N}$. Similar to Figures 3.a and 3.b, the decline in $\text{NH}_4\text{-N}$ and NO_x (predominantly $\text{NO}_3\text{-N}$) coincided with a steady increase in chlorophyll *a*, suggesting again that $\text{NO}_3\text{-N}$ was the N source used for algal productivity.

The somewhat overestimated nitrification performance observed in the laboratory microcosms may be attributed to the surface area-to-volume ratio of the laboratory microcosms (4:1) being six-fold greater than the *in situ* microcosms (0.67:1); resulting in a much greater exposure of sediment surface area to the water phase during the laboratory experiments. This greater exposure of sediments to the water column would have meant that dissolved N was more available to microbial communities present within the upper sediment layer and may have also led to an elevated potential for $\text{NH}_4\text{-N}$ adsorption to the sediment layer (Mackin and Aller, 1984; Nielsen, 1996); the latter process possibly also contributing to the poor overall N recovery in that system. In the shallow Bolivar WSP, the resuspension and reconstitution of benthic sediments is commonplace due to its exposed location and large wind fetch (Sweeney *et al.*, 2003). Others have reported on the beneficial influence of wind-induced mixing for maintaining aerobic conditions and also homogenising pond biota throughout the water column (Gloyne, 1971; Banks and Herrera, 1977; Lai and Lam, 1997) and it is considered that this mixing activity could also stimulate WSP nitrification potential *in situ*.

Conclusions

- Results from this work provide evidence to support prior field-scale evidence of nitrification occurring in Bolivar Pond 1 (Sweeney *et al.*, 2007).
- Nitrification performance of both Bolivar and Mt Barker WSPs were found to be similar, despite their considerable differences in nitrogen and organic loading conditions.
- Results obtained from laboratory microcosm incubations suggested a significant role for sediment in WSP nitrification.
- *in situ* field experiments confirmed that biomass assimilation was likely to dominate nitrogen removal in natural pond environments.

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