7th IWA Specialist Group Conference on Waste Stabilisation Ponds

Nitrogen Cycling in Waste Stabilisation Ponds

A. Yamamoto[1], N. J. Cromar[1], J. B. Nixon[2], D.G . Sweeney[2] and H. J. Fallowfield[1]
[1]Department of Environmental Health, Flinders University, Adelaide, South Australia
[2]United Water International, Adelaide, South Australia

Correspondence: Prof. Howard Fallowfield
Department of Environmental Health, Flinders University, Adelaide, South Australia
GPO Box 2100, Adelaide, South Australia, 5001
Phone: +618 8204 5730
Fax: +618 8204 5226
E-mail: howard.fallowfield@flinders.edu.au

ABSTRACT:
This study on nitrogen transformations in waste stabilisation ponds (WSPs) has specifically focused on the microbial processes relating to nitrification. *In situ* incubations in mesocosms, clear polyethylene tubes isolating the sediment and water column from the bulk water of the WSP, were shown to be an appropriate methodology for studying biological inorganic nitrogen transformations in WSPs. The conditions within the mescosms were shown to mirror those of the bulk water of the WSP. The wastewater within respective mesocosms was amended to give final concentrations of 10 mg NO_2-N, or 100 mg/L of either NO_3-N or NH_4-N. The changes in inorganic nitrogen speciation was measured over 24 days. The *in situ* mesocosm incubations suggested that nitrification was occurring within the wastewater. It was implied from the results that nitrate removal from the wastewater is more probably due to phytoplankton uptake rather than high rates of denitrification occurring in the sediment. In another experiment, samples of wastewater and wastewater overlying sediment were collected from Bolivar WSP and the Mount Barker septic tank effluent disposal scheme (STEDS) lagoon. The wastewater and wastewater plus sediment were amended to give final concentrations, in the respective microcosms, of either 10 mg NO_2-N or NH_4-N/L. The microcosms were incubated in the dark, aerobically (7 mg DO/L), at 25ºC. The changes in inorganic nitrogen speciation was followed over 21 days. Nitrification was evident, however, for Bolivar incubations, the rate of nitrite oxidation to nitrate was greater in the absence of sediment, whereas NH_4-N oxidation was only measured in the microcosm containing wastewater and sediment. A similar result was also obtained for Mt. Barker STEDS laboratory microcosms. This suggest that there may be spatial partitioning of nitrite and ammonia-oxidising bacteria within the wastewater and sediment, respectively, in these incubations.

KEYWORDS: Nitrification, nitrogen cycling, waste stabilisation ponds, septic tank effluent drainage schemes, mesocosms.

INTRODUCTION:

Wastewater treatment is necessary for protection of the environment. One of the primary functions of a wastewater treatment plant (WWTP) is to remove nutrients such as nitrogen and phosphorous from wastewater, with the goal of minimising their ultimate discharge into the aqueous environment. Waste Stabilisation Ponds (WSPs) are an ideal wastewater treatment technology, particularly in rural regions where land costs are less expensive. WSPs have proven to be an efficient and economical wastewater treatment methodology. The Bolivar WWTP, north of Adelaide, South Australia, utilises the largest WSP system in the Southern Hemisphere, covering a total area of 350 ha. The performance of the Bolivar WWTP has increased significantly since trickling filtration was discontinued and replaced with an activated sludge (AS) plant in 2001. The

AS plant has decreased suspended solids (SS) loading in the WSP effluent, and subsequently reduced the total operational and management costs associated with the dissolved air flotation/filtration (DAF/F) plant at Bolivar (Sweeney *et al*., 2003). The quality of WSP influent at Bolivar has also dramatically improved, especially with respect to biological oxygen demand (BOD), total Kjeldahl Nitrogen (TKN), SS, and NH_4–N concentration. On the other hand, nitrite and nitrogen concentrations in the WSP effluent have increased (Cromar *et al*., 2003).

It is important to understand the nitrogen cycle within WSPs, since the removal of nitrogen from the water column may potentially lead to a change in the microbial ecology of the WSPs towards nitrogen-fixing cyanobacteria (Cromar *et al*., 2003). Biological nitrification and denitrification may play an important part in WSP nitrogen cycling. Nitrification is a two-stage aerobic process by which ammonia is oxidised first to nitrite and then finally to nitrate by nitrifying bacteria. Denitrification is an anaerobic process whereby nitrate is reduced to gaseous oxides of nitrogen and/or di-nitrogen by bacterial respiration of nitrate (Stolp, 1988). Maynard *et al.* (1998) concluded that ammonia volatilisation and sedimentation of biologically-assimilated organic nitrogen are primary processes for nitrogen removal in tertiary lagoons. However, surprisingly little is known regarding inorganic nitrogen cycling in WSPs.

It is necessary to determine the performance of WSPs with respect to nitrogen removal and nitrogen cycling in order to establish a methodology for the design of advanced WSP systems. This study on nitrogen transformations in WSPs has specifically focused on the microbial process of nitrification. While it is most desirable to undertake full-scale WSP studies, due to difficulties associated with field-scale sampling and monitoring protocols, it was deemed impractical for the purposes of this research. Also, since this study was concerned specifically with investigation and quantisation of biological processes, it was preferable to operate smaller-scale closed systems. This paper presents results from a study incorporating both *in situ* 'meso-scale' field WSP and laboratory 'micro-scale' incubations.

MATERIALS AND METHODS

Study sites

Site 1 utilised WSPs located at the Bolivar WWTP. The Bolivar WWTP train comprises secondary treatment by an AS plant, followed by six tertiary WSPs, treating approximately 150 ML/d of wastewater of residential and industrial origin. The WSPs cover an area of 350 ha, with an average depth of 1.2 m and an hydraulic residence time of around 30 days. Within this six-pond Bolivar WSP system, Lagoon 1 was selected as the location for the *in situ* mesocosm incubations. Site 2 involved WSPs located at Mt. Barker, East of Adelaide, South Australia. The Mt. Barker septic tank effluent disposal scheme (STEDS) receives settled septic tank effluent from a population of 25,000. The septic tank effluent is treated within a local council managed WSP system before being discharged to a wetland.

Analytical methods

Grab samples were collected and stored on ice during transport to the laboratory for same-day analyses. Samples for dissolved nutrient analyses were pre-filtered using GF/C filters (Whatman). Ammonia-N (NH_4-N), nitrite-N (NO_2-N), and nitrate-nitrogen (NO_3-N) were measured according to methods 4500-NH_3 C, 4500-NO_2^- B, and 4500-NO_3^- H, respectively (APHA, 1995). Alkalinity was determined titrimetrically using the methyl orange method. Samples for chlorophyll *a* analyses were filtered (Whatman GF/C), and then analysed according to the trichromatic method 10200 H (APHA, 1995). Dissolved oxygen (DO) concentration and temperature were measured using a WTW Oxi 330 hand-held meter. pH was measured using a WTW pH 320 meter.

Bolivar WWTP *in situ* incubations

Four tubular mesocosms (10 cm × 130 cm) made from clear polyethylene were driven into the sediment of Lagoon 1 (34° 45'856"S 138° 34'004"E), and secured against metal stakes to prevent physical dislodgment. This served to isolate the wastewater within the column from the bulk water phase of the WSP, and allowed for *in situ* determination of nitrification and denitrification. The mesocosms were located within Lagoon 1 as shown in Figure 1. The tops of mesocosms were covered with plastic PVC elbows to prevent any influx of rainwater. Ambient inorganic nitrogen concentrations were determined on Day 0. Initial concentrations of inorganic nitrogen in three of the four mesocosms were then augmented as follows: Mesocosm 1 (M1), 10 mg-NO_2/L; Mesocosm 2 (M2), 100 mg-NO_3/L; and Mesocosm 3 (M3), 100 mg-NH_4/L. The nitrogen concentration was not amended in the control Mesocosm 4 (M4) . Water samples were collected after the amendment (Day 0) and at Days 2, 9, and 24. Temperature, DO concentration, and pH were measured before sampling the mesocosms on each sampling interval. Irradiance, both inside and outside of the mesocosms, was determined at four different depths (0, 20, 40, and 80 cm) on Days 0 and 24.

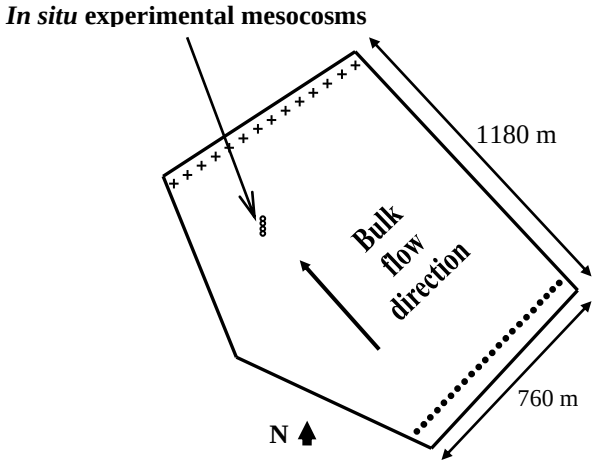


Figure 1 Outline of Bolivar Lagoon 1, showing: location of the mesocosms (○); upward jetting inlets (●); and downward draining outlets (+) (Sweeney, 2005).

Laboratory microcosm studies

Wastewater from Lagoon 1 at Bolivar WWTP or Mt. Barker STEDS lagoons were collected in 20 L polyethylene carboys, transported to the laboratory, pre-filtered (GF/C) and stored at 4°C in the dark until required. Samples of sediment and overlying wastewater (5 L) were also collected using a plastic corer (3 cm × 130 cm), transported and stored in polyethylene carboys. In the laboratory, the samples (wastewater or wastewater plus underlying sediment) were incubated in a water bath at 25°C, a temperature similar to that at the sample sites in summer. The inorganic nitrogen concentrations were augmented as shown in Table 1.

The microcosm vessels were gently sparged with air, without disturbing underlying sediments, at a rate that was determined not to result in a change in the initial DO concentration (ca. 7 mg/L). Experiments were conducted in the dark to inhibit the activity of algae within vessels. Temperature, DO, and pH were measured before the determination of nitrite, nitrate, and ammonia concentrations and alkalinity every third day for a total of 21 days.

Table 1 Inorganic nitrogen amendments for the laboratory microcosm studies (Day 0).

Vessel Number	Augmentation	Wastewater/Sediment	Origin
B1	10 mg-NO_2-N/L	Wastewater + Sediment	Bolivar
B2	100 mg-NH_4-N /L	Wastewater + Sediment	Bolivar
B3	10 mg-NO_2-N /L	Wastewater	Bolivar
B4	100 mg- NH_4-N /L	Wastewater	Bolivar
MB1	10 mg- NO_2-N /L	Wastewater + Sediment	Mt. Barker
MB2	100 mg- NH_4-N /L	Wastewater + Sediment	Mt. Barker
MB3	10 mg- NO_2-N /L	Wastewater	Mt. Barker
MB4	100 mg- NH_4-N /L	Wastewater	Mt. Barker

RESULTS AND DISCUSSION

Mesocosm *in situ* study

The profiles of nitrogen species in the four mesocosms and for the bulk water are shown in Figure 2. The nitrogen composition at the sampling site in the lagoon, before amendment, was 7.1 mg NH_4-N/L, 0.48 mg NO_2-N/L, and 6.35 mg NO_3-N/L. The nitrite concentration in M1 increased sharply following augmentation with nitrite. The subsequent reduction observed in the concentration of nitrite in M1 implies that nitrification was occurring. The concentration of nitrate in M1 decreased over time as a result of either uptake by suspended phytoplankton or through denitrification of nitrate in the anaerobic sediment—a subject of further study. Greater than 85% of the added nitrate was removed in M2; the majority of the removal occurred after 9 days incubation and when the NH_4-N was depleted. The reduction in NO_3-N following ammonia depletion and the lack of NO_3-N removal from Day 0 to 9 suggests low rates of denitrification. Furthermore, comparison with the ammonia removal pattern within M2 suggests that the ammonia was removed preferentially, and nitrate removal increased following ammonia depletion. This pattern of preferential removal of ammonia is typical of uptake by phytoplankton. These results suggest that much of the removal of inorganic nitrogen is via uptake by phytoplankton rather than by denitrification. Ammonia decreased in M3 over 9 days of incubation, with a 55% decrease in ammonium concentration after 24 days incubation. This, together with the increase in nitrite observed on Day 24 is circumstantial evidence for nitrification in M3. The decrease in alkalinity in M3 after Day 2 (60 mg-$CaCO_3$/L $\rightarrow$ 35 mg-$CaCO_3$/L) may also be associated, in part, with biological nitrification. The 'native' nitrate present decreased from approximately 5 mg/L to zero over the same incubation period. This may be due to phytoplankton uptake or, since ammonia was available for preferential uptake, denitrification within the anaerobic sediment.

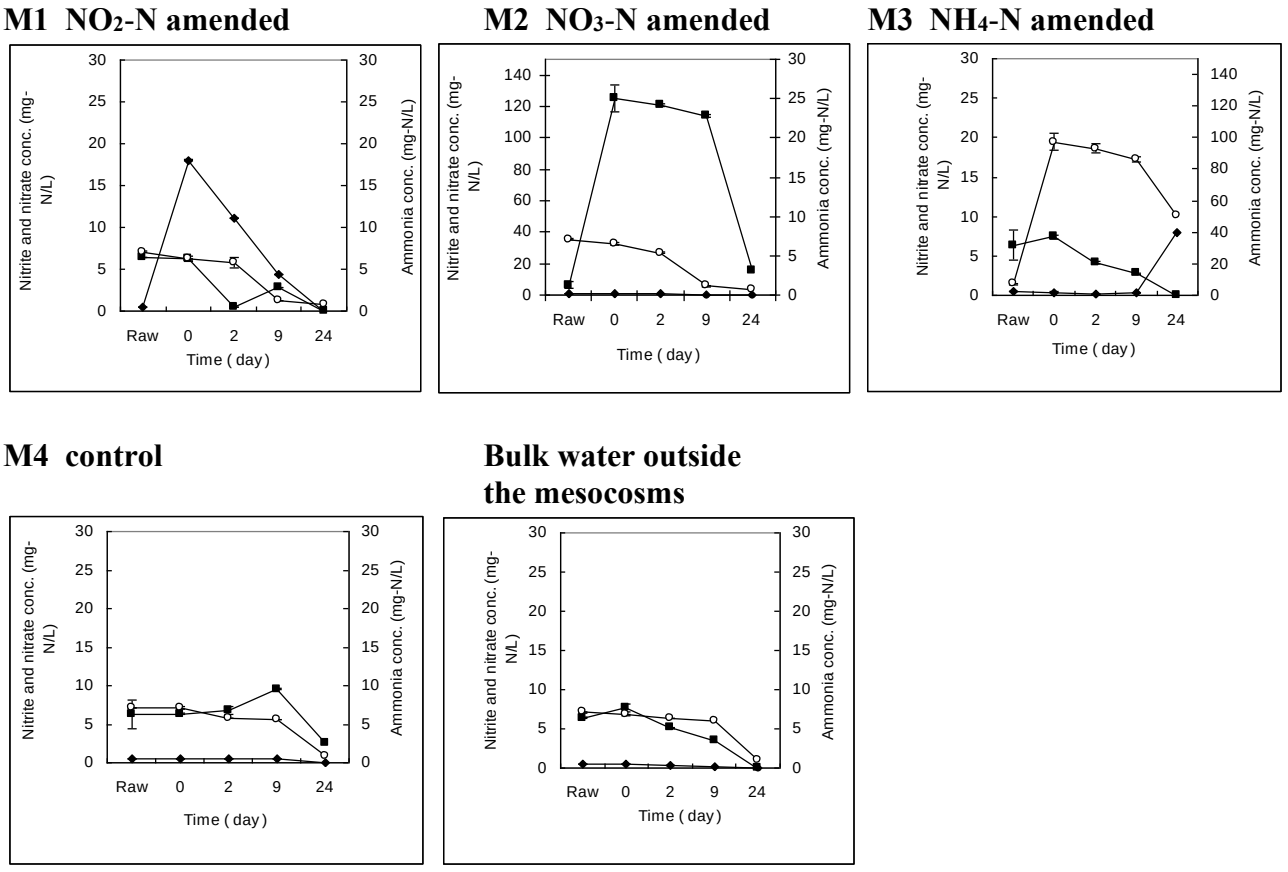
The DO during the day in the mesocosms was over 20 mg/L (data not shown). Chlorophyll *a* concentration at the sampling site was 13.4 μg/L. High chlorophyll *a* concentrations were found in all the mesocosms at Day 24. This resulted in high pH (9–11) in the mesocosms, which may have also contributed to high ammonia volatilisation rates. Comparison of the control mesocosm (M4) with the bulk water outside the mesocosms (Figure 2) showed remarkable similarity regarding the concentrations of NH_4-N and NO_2-N over the incubation period, however, minor differences in the behaviour of NO_3-N were apparent between the mesocosm and the bulk water. These results, coupled with the similarities in chlorophyll *a* concentration in the control mesocosm and bulk waters, demonstrates that the control mesocosm was generally mirroring the behaviour of the bulk water. Furthermore, the results demonstrate that mescosms offer an appropriate method for studying processes within WSPs.

Laboratory microcosms

Bolivar effluent nitrogen transformations

Transformations of nitrogen species in wastewater alone and wastewater with underlying sediment from Bolivar Lagoon 1 incubated aerobically at 25°C are shown in Figure 3. The nitrogen composition of the WSP wastewater before the amendments was 1.88 mg-NO_3/L and 1.31 mg-NH_4/L with no nitrite detectable. Microcosms B1 and B3 were amended to a final concentration of 10 mg NO_2-N /L. Nitrification is evident in both microcosms with NO_2-N concentrations decreasing and NO_3-N concentration simultaneously increasing, however, the rate of nitrification was much higher in B3 in the absence of sediment. Microcosms B2 and B4, containing wastewater plus sediment and wastewater respectively, were amended to a final concentration of ca. 100 mg NH_4-N/L. In contrast to the nitrite amendments, only B2 showed evidence for nitrification with ammonium concentrations decreasing, followed by an initial rise in nitrite content and a subsequent increase in nitrate. Microcosm B4, containing wastewater only, showed no similar evidence of nitrification. These results suggest that the ammonia oxidising bacteria may reside differentially within the sediment in these microcosms, whereas the nitrite oxidising bacteria were more likely suspended in the wastewater.

Figure 2 Concentrations of various nitrogen species: ammonia (○); nitrite (♦); and nitrate (■); in the mesocosms, and in the bulk water phase in Bolivar Lagoon 1.



Mount Barker STEDs effluent nitrogen transformations

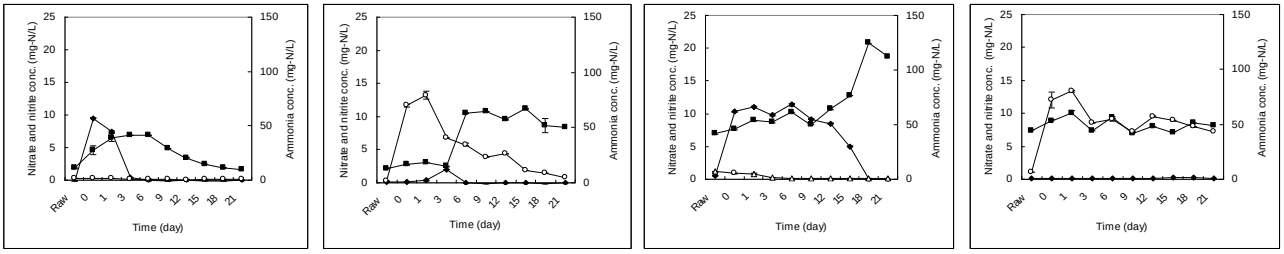
Data from the incubation of Mt. Barker STEDS effluent and effluent with underlying sediment at 25°C is shown in Figure 3. Only ammonium (72.1 mg-NH_4/L) inorganic nitrogen was detected in the wastewater from Mt. Barker STEDS lagoon. Microcosm MB1 showed evidence for nitrification after a 6 day lag period, with ammonium concentration decreasing followed by a sequential increase

in nitrite and nitrate concentrations. A similar pattern emerged for the microcosm B3, containing wastewater and sediment, which showed high rates of ammonia oxidation to nitrite followed by oxidation to nitrate. There was some evidence for a differential response in the presence or absence of sediment, with lower nitrite oxidation occurring in MB1 in the presence of sediment. Microcosms amended with ammonium showed similar response to those of the Bolivar microcosms. Nitrification was evident in the wastewater plus sediment microcosm MB2 after 3 days incubation but occurred at much lower levels in the microcosm MB4, which contained wastewater only. MB4 also showed high rates of ammonium assimilation.

Figure 3 Nitrogen concentration (mg/L) profiles in the microcosm of wastewater and sediment from (a) Bolivar and (b) Mt. Barker WWTP, incubated at 25°C, following amendment with nitrite or ammonia: NH_4-N (○); NO_2-N (♦); and NO_3-N (■).

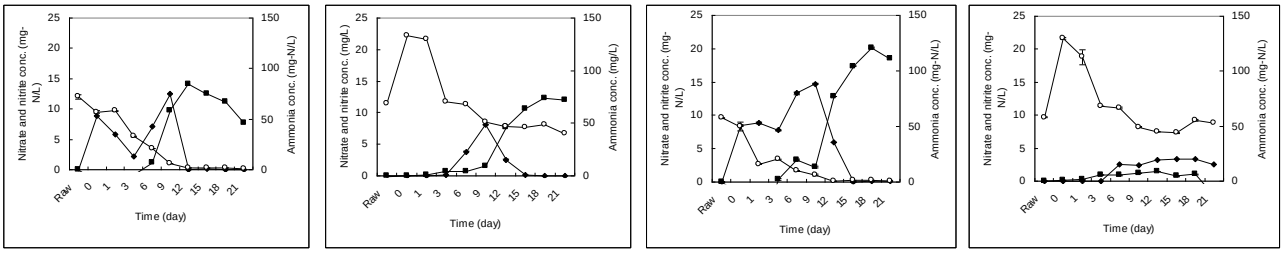
(a) Bolivar Laboratory Incubations

B1 WW + Sediment NO_2-N amended **B2 WW + Sediment NH_4-N amended** **B3 WW NO_2-N amended** **B4 WW NH_4-N amended**



(b) Mt. Barker Laboratory Incubations

MB1 WW + Sediment NO_2-N amended **MB2 WW + Sediment NH_4-N amended** **MB3 WW NO_2-N amended** **MB4 WW NH_4-N amended**



CONCLUSIONS

- The results demonstrate that *in situ* mesocosms are an appropriate methodology for studying biological nutrient transformations in WSPs.
- The *in situ* mesocosm incubations suggest that nitrification was occurring within the wastewater. The results indicate that much of the nitrate removal from the wastewater was more probably due to phytoplankton uptake rather than denitrification occurring in the sediment. More research is required to confirm this inference.

- The laboratory microcosm incubations of wastewater and sediment from Bolivar WSP and Mt. Barker STEDS lagoon also provided evidence for nitrification. More significantly, for Bolivar samples, the rate of nitrite oxidation to nitrate was greater in the absence of sediment, whereas NH_4-N oxidation was only measured in the microcosm containing wastewater and sediment. A similar result was also obtained for Mt. Barker STEDS laboratory microcosms. This suggests that there may be spatial partitioning of nitrite and ammonia oxidising bacteria within the wastewater and sediment respectively in these incubations.

ACKNOWLEDGMENTS

This work is supported by United Water International and a Rotary Foundation Ambassadorial Scholarship (AY). The authors would also like to thank Richard Evans for assistance with the fieldwork and Michael Short for reviewing the manuscript.

REFERENCES

APHA. (1995). *Standard methods for the examination of water and wastewater*. 18th edn. American Public Health Association/American Waterworks Association/Water Environment Federation, Washington, DC, USA.

Cromar, N.J., Sweeney, D.G., O'Brien, M.J. and Fallowfield, H.J. (2003), Changes in waste stabilization pond performance resulting from the retrofit of activated sludge treatment upstream—Part II: Water quality issues. *Water Science and Technology,* **51**(12), 11–16.

Hao, X. and Martinez, J. (1998). Removing nitrite and ammonium from drainage water by simulation of natural biological process. *Water Research*, **32**(3), 936–943.

Maynard, H.E., Ouki, S.K. and Williams, S.C. (1998). Tertiary lagoons: A review of removal mechanisms and performance. *Water Research*, **33**(1), 1–13.

Stolp, H. (1988). Microbial Ecology Organisms, Habitats, Activities. Cambridge University Press, pp. 232–241.

Sweeney, D.G., O'Brien, M.J., Cromar, N.J. and Fallowfield, H.J. (2003). Changes in waste stabilization pond performance resulting from the retrofit of activated sludge treatment upstream—Part I: Management and operating issues. *Water Science and Technology,* **51**(12), 17–22.

Sweeney, D.G. (2005). *Integrating biological and hydraulic aspects of waste stabilisation pond design*. PhD thesis, Department Environmental Health, Flinders University of South Australia, Adelaide, South Australia.