MAJOR PATHWAYS FOR NITROGEN REMOVAL IN WASTE WATER STABILIZATION PONDS

PATRICK C. C. LAI[1] and PAUL K. S. LAM[2]*
[1] *Department of Environmental Management, Victoria University of Technology, P.O. Box 14428, MMC, VIC 3000, Australia;* [2] *Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon*

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Abstract. A study on the factors influencing nitrogen removal in waste water stabilization ponds was undertaken in an eight-pond series in Werribee, Australia. Nitrogen species including Kjeldahl nitrogen, total ammonia nitrogen, nitrite and nitrate were monitored monthly from March 1993 to January 1994. At the same time, pH, temperature, chlorophyll *a* content and dissolved oxygen were also recorded. Highest nitrogen removal occurred during the period with highest levels of chlorophyll *a* content and dissolved oxygen, but the rate of nitrogen removal was not related to temperature and pH. Enhanced photosynthetic activities resulting from an increased phytoplankton abundance due to prolonged detention time caused an increase in dissolved oxygen, and created an optimum condition for nitrification to occur. In this process, ammonia was oxidized to nitrite and nitrate which were subsequently reduced to elemental nitrogen. Apart from nitrification-denitrification which was the major nitrogen removal pathway in the study system, algal uptake of ammonium, nitrate and nitrite as nutrient sources also contributed to the nitrogen removal. The role of phytoplankton and zooplankton in the treatment process in waste stabilization ponds was discussed.

Key words: nitrogen removal, waste stabilization pond, phytoplankton, nitrification and denitrification

1. Introduction

Previously, biochemical oxygen demand, suspended solids and faecal coliform bacterial count have commonly been used as criteria for assessing the efficiency of sewage treatment facilities, while nutrient parameters have largely been overlooked (Toms *et al.*, 1975; Hussainy, 1979). Recently, more attention has been paid to the eutrophication of receiving water bodies which is mainly caused by nutrient enrichment from the effluent of waste treatment facilities. To date, nutrient parameters have become an integral part of effluent discharge guidelines set by environmental protection agencies. In particular, more attention should be paid to the control of nitrogen in water bodies receiving point source discharges where nitrogen is often limiting for algal growth (Gakstatter *et al.*, 1978).

Although waste stabilization ponds have been widely used over the world and proved to be an economical way of sewage treatment (Gloyna, 1971), waste stabilization ponds often do not have special design configurations for nutrient removal. Furthermore, the biological and chemical mechanisms involved in waste stabilization ponds have not been well studied as compared with other conventional sewage

* Author for all correspondence.

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treatment processes such as activated sludge systems. For instance, there is no consensus with regard to the detailed mechanisms of nitrogen removal among studies on the same waste stabilization ponds in United States (see DiGiano, 1982; Ferrara and Avci, 1982; Pano and Middlebrooks, 1982). Possible nitrogen removal pathways reported in previous studies included nitrification-denitrification, ammonia volatilization at enhanced pH, algal uptake and sedimentation to the benthic layer. Although, it is generally believed that the efficiency of nitrogen removal is related to temperature, pH and detention time, the principal removal mechanisms involved can seldom be clearly defined (Reed, 1985).

In this study, we are not trying to resolve those conflicts regarding the mechanisms of nitrogen removal reported in previous studies of which conclusions were mostly based on mathematical modelling of field data. Instead, we use a different approach to study the nitrogen dynamics of a series of waste stabilization ponds in Australia. We will describe the dynamics of different nitrogen species along a stabilization pond series in detail and correlate these variations with the factors that may influence the removal of nitrogen, and explore the principal mechanisms of nitrogen removal in the system.

2. Study Area

The study was carried out in a series of waste stabilization ponds at the Werribee Treatment Complex which is located 35 km south-west of the city of Melbourne, Australia. The Complex practises stabilization pond treatment, land-filtration and grass filtration and handles the waste water from a population of 1.5 million. The stabilization pond system in the Complex consists of a number of pond series which consist of 8 to 12 ponds. The pond system occupies a total area of 16.54 km^2 and it is the largest stabilization pond system in Australia. More detailed account of the Complex can be obtained from Parker *et al.* (1959), Hussainy (1979) and Lai and Lam (1994).

The study system (145W) consists of eight roughly square ponds connected in a series (Figure 1), and their sizes and depths are summarized in Table I. The system receives unsedimented raw sewage after primary screening. Waste water passes through the pond series by gravitation; sewage fills up the first pond and then enters the second pond through four outflows. The outflow of the first pond is equipped with scum boards which are used to screen out the scum and large particles. The monthly mean detention time of the system based on the total capacity of the system divided by the daily flow rate of sewage delivered to the system is shown in Figure 2. Marked seasonal variation of the detention times was observed with the longest detention periods between May and June 93. The ponds are fishless, however, high abundances of phytoplankton and zooplankton appear in the later section of the series.

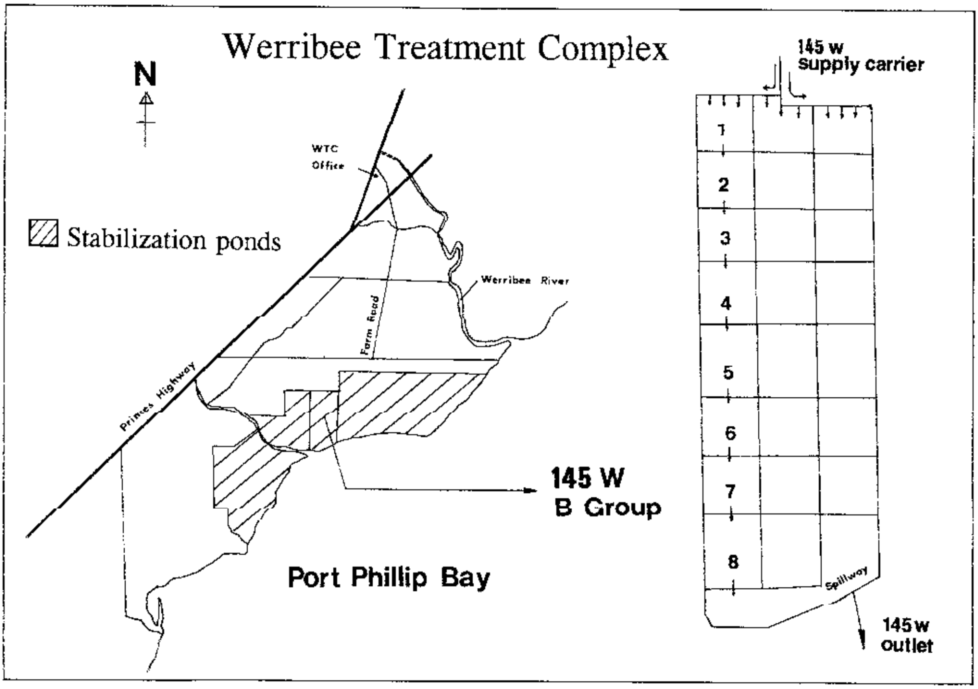

Figure 1. Location of the study stabilization pond series (145W) and its physical layout.

Table I
Physical characteristics of the study stabilization pond series

Pond no.	Area (m^2)	Depth (m)
1	43800	1.5–2
2	46400	1
3	53200	1
4	58400	1
5	62100	1
6	54500	1
7	55000	1
8	55000	1

The local climate type is marine with mild winters and warm summers, all seasons are moist. The mean maximum and minimum January temperatures are 26 and 13 °C respectively, while those recorded in July are 14 and 6 °C. Two or three times each summer there are hot and dry spells when temperatures reach over 35 °C for consecutive days. Annual rainfall totals are approximately 660 mm and usually evenly distributed throughout the year (Linacre and Hobbs, 1977). The

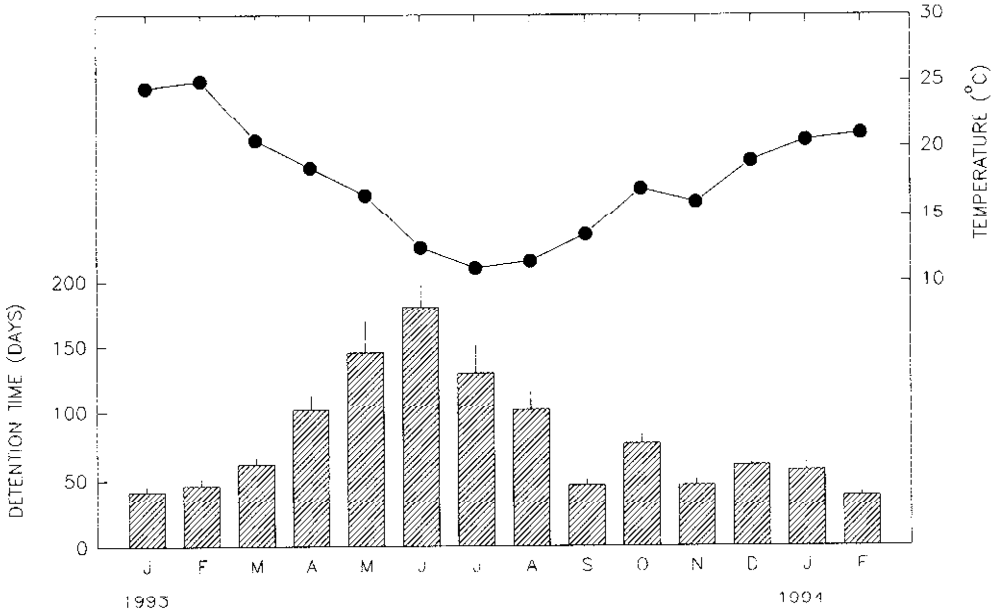

Figure 2. Monthly average detention time (bars) and water temperature (line) of the study system over the period January 1993 to February 1994. Verticals line are 1 S.E.

monthly mean water temperature of the stabilization pond system during the study period is shown in Figure 2.

3. Materials and Methods

3.1. Samples Collection

Samples were taken monthly between 1000 and 1400 over the period from March 1993 to January 1994 in the stabilization pond series under investigation. Water samples were collected in the middle of each of the four banks of pond 1 through to pond 8. Samples were chilled with an ice bath while being transported to the laboratory, and kept at 4 °C for subsequent analyses within 48 hours. During each field visit, dissolved oxygen and temperature of the surface water were measured at each sampling point using a Dissolved Oxygen Meter (YSI Model 58). Dissolved oxygen at surface and bottom layers were also determined in the middle of pond 7 and pond 8 while on board a rubber dingy. Two surface water samples, each of 250 mL, were collected at each sampling point for laboratory analyses of pH, Kjeldahl nitrogen, total ammonia nitrogen (ammonia and ammonium ion), nitrite, nitrate and chlorophyll *a* content. The chlorophyll *a* content is used as an estimate of phytoplankton abundance.

3.2. Laboratory investigations

All chemical analytical procedures followed the standard methods of the American Public Health Association *et al.* (1989). Concentrations of different nitrogen species of unfiltered water samples were determined using an automatic flow injection analyzer (Aquatec 5400 Analyzer) following the Aquatec Instruction Manual (Tecator, 1990). pH was measured in the laboratory with a bench-top pH meter (Orion model SA520).

3.3. Statistical analyses

Since total ammonia nitrogen is toxic to aquatic organisms and is the major component of nitrogen in raw sewage, it was chosen as the parameter for the assessment of the efficiency of nitrogen removal. Determination of the percentage removal of total ammonia nitrogen is achieved by comparing its concentration in the waste water before and after the treatment. This requires the use of time-lag analyses which would be complicated in the present study as the detention time varied widely during the study period. Instead, since the concentration of total ammonia nitrogen of the influent (pond 1) was more or less constant in the Werribee system (Lai, 1994), the monthly percentage removal of total ammonia nitrogen of the system was determined by comparing the concentration of the effluent (pond 8) in each month with the mean value of the influent (pond 1) during the study period. Simple correlation analyses between the monthly percentage removal of total ammonia nitrogen and the average levels of chlorophyll *a* content, dissolved oxygen, pH and temperature among the eight ponds in each month were conducted.

4. Results

4.1. Dynamics of chlorophyll *a* content, dissolved oxygen and pH level

Variations of chlorophyll *a* content of the water from pond 1 to pond 8 during the study period are shown in Figure 3. Chlorophyll *a* contents generally exhibited two peaks across the eight ponds. Phytoplankton was practically absent in pond 1, while the first peak in chlorophyll *a* content occurred in pond 2 or pond 3. The second peak appeared in the last few ponds (pond 6 to pond 8) of the series. The high chlorophyll *a* contents in pond 6 and pond 7 from May to July coincided with the period during which the longest detention time was observed in the system. Indeed, the average chlorophyll *a* content among the eight ponds in each month is significantly correlated with the detention time of the system ($r = 0.60$, $n = 12$, $P<0.05$).

Pond 1 was strictly anaerobic in all seasons while there was a general increase in dissolved oxygen contents from pond 1 to pond 8. No significant vertical stratifi-

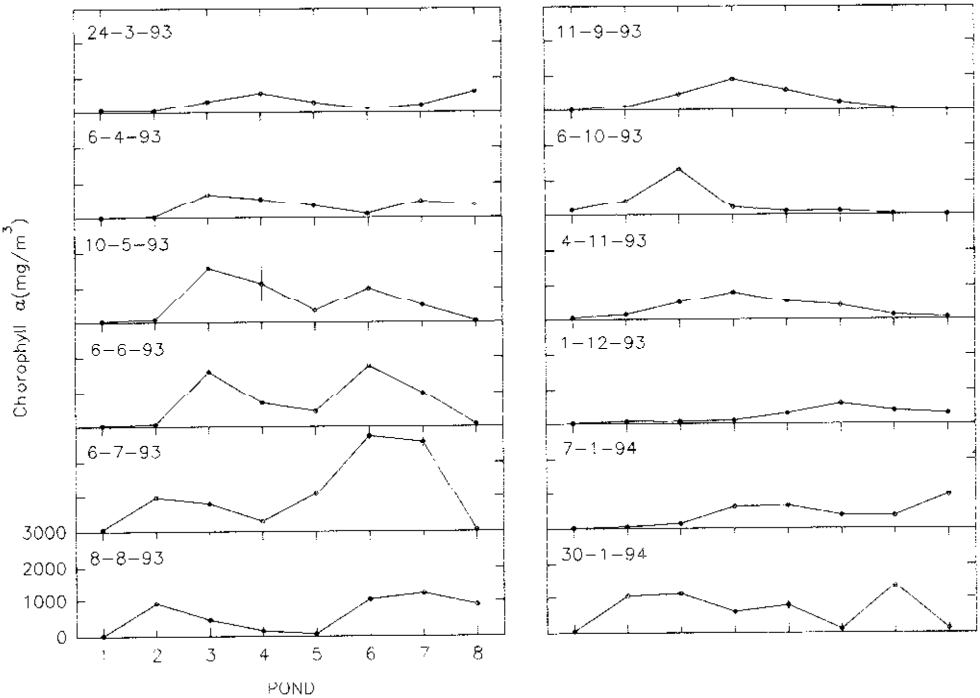

Figure 3. Variations of the chlorophyll *a* content from pond 1 to pond 8 over the period March 1993 to January 1994. Vertical lines are 1 S.E.

cation in dissolved oxygen level was observed in pond 7 and pond 8 and indeed the dissolved oxygen level at the bottom layer was not significantly different from that at the surface layer in both ponds ($t<1.3$, d.f. = 11, $P>0.05$). Raw sewage in pond 1 was more or less neutral in pH, but the treated sewage became more alkaline as it passed through the ponds. Seasonal mean values of dissolved oxygen and pH of the eight ponds are shown in Table II. The seasonal variations of dissolved oxygen level and pH along the pond series shared a similar pattern with that of the chlorophyll *a* content. This is evident by the fact that chlorophyll *a* content is significantly correlated with dissolved oxygen ($r = 0.60$, $n = 96$, $P<0.001$) and pH ($r = 0.61$, $n = 96$, $P<0.001$).

4.2. Dynamics of Nitrogen

Variations of the concentration of Kjeldahl nitrogen, total ammonia nitrogen, nitrite and nitrate of the water in different ponds during the study period are shown in Figure 4. Raw sewage in the system contained about 50 mg/L of total nitrogen of which 70% is total ammonia nitrogen and 30% is organic nitrogen, while only trace amounts of nitrate and nitrite were present (below 0.3 mg/L).

Table II
Seasonal mean values (S.E.) of dissolved oxygen and pH of surface water in the eight ponds

POND	D.O. (mg/L)	pH
1	0.30 (0.07)	7.30 (0.09)
2	1.52 (0.34)	7.67 (0.10)
3	3.15 (0.51)	7.92 (0.01)
4	4.65 (0.75)	7.93 (0.10)
5	5.42 (0.83)	8.05 (0.12)
6	7.19 (1.27)	8.28 (0.15)
7	7.62 (1.09)	8.32 (0.17)
8	6.78 (0.83)	8.20 (0.14)

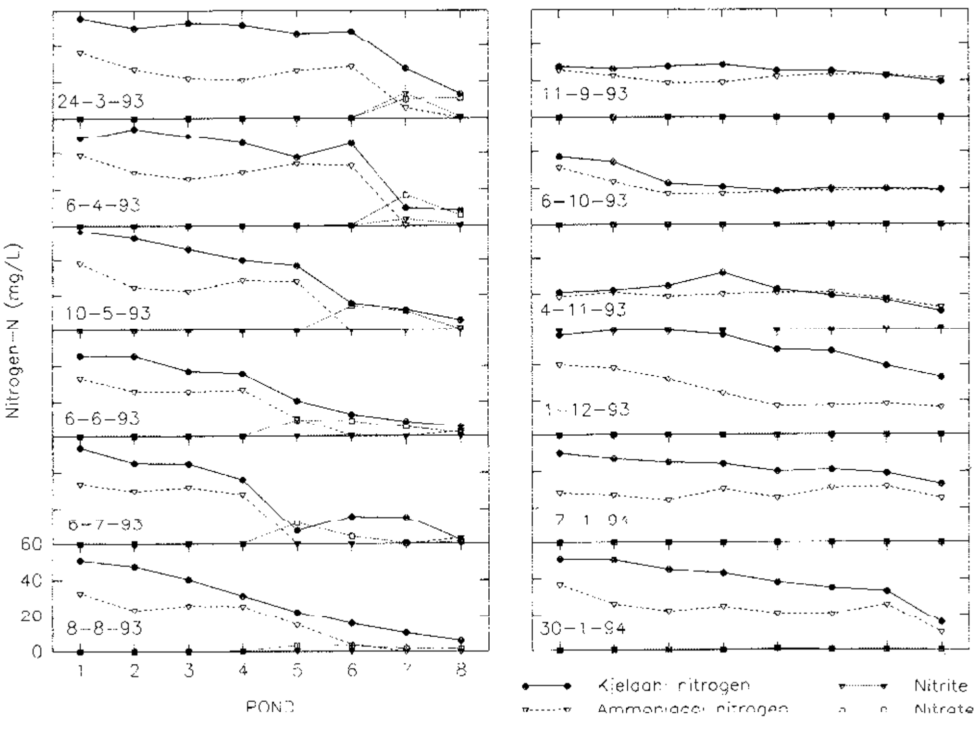

Figure 4. Variations of the concentration of Kjeldahl nitrogen, total ammonia nitrogen, nitrite and nitrate from pond 1 to pond 8 over the period March 1993 to January 1994. Vertical lines are 1 S.E.

Variations of Kjeldahl nitrogen shared a similar pattern with total ammonia nitrogen although there was a general reduction in the difference between the two parameters (i.e. organic nitrogen) from pond 1 to pond 8. In most cases, the variations in the organic nitrogen concentration were closely related to those of the chlorophyll *a* content. Particularly, the low concentrations of organic nitrogen

Table III

Summary of the correlations between the percentage removal of total ammonia nitrogen and the possible factors influencing the removal efficiency

Factors	Correlations with the pencentage removal of total ammonia nitrogen		
Dissolved oxygen level	r = 0.73	n = 12	$p<0.001$
Chlorophyll *a* content	r = 0.57	n = 12	$p<0.05$
Temperature	r = 0.27	n = 12	$p>0.1$
pH	r = 0.04	n = 12	$p>0.1$

recorded in pond 8 between September and November coincided with the period of low phytoplankton abundance.

Dynamics of different nitrogen species exhibited marked seasonal variations in the pond system. From April 93 to August 93 when the detention time was the longest and chlorophyll *a* content highest, the concentrations of total ammonia nitrogen were reduced to very low levels, and these were accompanied by an increase in the concentrations of nitrite and nitrate. By contrast, no obvious reduction in total ammonia nitrogen was observed between September 93 and December 93 and the concentrations of nitrite and nitrate remained low throughout the pond series. This was particularly evident during September 93 and October 93 when phytoplankton abundance was extremely low.

4.3. Factors influencing the removal of nitrogen

The correlations between the percentage removal of total ammonia nitrogen and the average levels of chlorophyll *a* content, dissolved oxygen, pH and temperature among the eight ponds in each month are summarized in Table III. The strongest correlation was observed between the total ammonia nitrogen removal and dissolved oxygen level, followed by chlorophyll *a* content, while pH and temperature were not significantly correlated with the percentage removal of nitrogen.

5. Discussion

Temperature has been regarded as the most important physical factor influencing the efficiency of waste stabilization ponds as it affects the metabolic rate of the micro-organisms in the system, and thus the rate of degradation of organic matter and subsequent stabilization of inorganic nutrients (Gray, 1992). Since stabilization ponds usually operate in a relatively uncontrolled environment as compared to advanced treatment facility such as activated sludge plant, water temperature and hence the efficiency of the system changes with the weather. Previous studies in Europe and America have shown that the nutrient removal efficiency by stabilization ponds was higher in summer than in winter (Toms *et al.*, 1975; Pano and Middlebrooks, 1982; Santos and Oliveira, 1987). Similarly, Hussainy (1979) reported that the removal efficiency of total ammonia nitrogen, and the rate of nitrification in particular, was higher during summer at Werribee.

Contrary to the above findings, removal of nitrogen in the present study was more efficient during autumn and early winter as compared with summer during which nitrogen removal was incomplete. The complete removal of total ammonia nitrogen in the study system during the colder months was mainly attributed to the longer detention time which allowed a significant increase in phytoplankton abundance. The discharge guidelines at the outlet for organic nitrogen and ammonia nitrogen are 15 and 40 g/m^3 respectively. During the period of long detention time, growth of phytoplankton in the ponds exceeded the loss due to flushing through the outflow, resulting in a significant increase in phytoplankton abundance (Toms *et al*., 1975). Since ammonium is the most preferentially utilized form of nitrogen for phytoplankton (Boney, 1989), uptake of total ammonia nitrogen by the abundant phytoplankton contributed to the removal of ammonium from the waste water (Ferrara and Avci, 1982). The increase of organic nitrogen (the difference between Kjeldahl and total ammonia nitrogen) at the last few ponds coincided with the increase of chlorophyll *a* content during May and July, suggesting that some inorganic nitrogen were converted to organic nitrogen in the algal cells through phytoplankton uptake and growth. However, estimates of the dry weight of phytoplankton in the study ponds was only about 100 mg/L (see Lai, 1994) which could represent 4 to 7.5 mg/L of nitrogen depending on the phytoplankton species (Hemens and Mason, 1968). Hence, algal uptake could not explain the complete removal of total ammonia nitrogen which could be as high as 30 mg/L.

The observation that a reduction in the concentration of total ammonia nitrogen was accompanied by a significant increase in nitrite and nitrate levels in the present study suggest that nitrification was the principal removal mechanism of nitrogen in the study system. In this process, total ammonia nitrogen is oxidized to nitrite, and then nitrate, by nitrifying bacteria (Sharma and Ahlert, 1977). The observed strong correlation between the nitrogen removal efficiency and dissolved oxygen level also lends support to our hypothesis as oxygen is essential to nitrification. During the period with high phytoplankton abundance, enhanced photosynthetic activities of the phytoplankton not only increased the dissolved oxygen level, but also elevated the pH by consuming the acidic carbon dioxide in the ponds. These conditions are optimal for the nitrifying bacteria and could have speeded up the rate of nitrification (Wild *et al*., 1971). It should be noted that nitrification does not occur commonly in facultative ponds due to the low density of nitrifying bacteria in the aerobic zone of the waste stabilization ponds (Ferrara and Avci, 1982). The bacteria tend to adsorb onto the surface of particles which settle to the bottom anoxic sludge layer where the process of the nitrification is arrested. However, during the period with extremely high phytoplankton abundance in the facultative pond at Werribee, the high intensity of photosynthetic activity could have created an aerobic condition throughout the depth of the pond allowing nitrification to take place down to the water-sludge interface. Indeed, our results showed that aerobic condition did prevail at the bottom of the facultative pond at Werribee and this could be attributed to the shallowness of the pond and the often windy condition which enhanced vertical mixing.

In view of the low nitrate and nitrite concentrations recorded in the pond system studied elsewhere, it was suggested that nitrification-denitrification was unlikely to be a principal mechanism of nitrogen removal in waste water stabilization pond (Toms *et al.*, 1975; Ferrara and Avci, 1982; Pano and Middlebrooks, 1982; Reed, 1985). However, the results of the present study suggest that nitrification can be an important mechanism in nitrogen removal if the detention time is long enough to allow significant growth of phytoplankton. Permanent removal of nitrogen from the system can then be achieved through denitrification during which nitrate is reduced to nitrite and then to elemental nitrogen, and the nitrogen gas is ultimately exported to the atmosphere. Although denitrification is an anoxic process and may not prevail in aerobic ponds, Hussainy (1979) showed that there was significant diurnal variations in dissolved oxygen which was particularly apparent during phytoplankton blooms in the Werribee ponds, and that dissolved oxygen could drop to a very low level during night time which might permit denitrification to take place. Such an alternation of aerobic and anaerobic conditions during day and night time respectively resembles the controlled environment in the activated sludge reactor for nitrogen removal (Horan, 1990). Notwithstanding, the decrease in nitrite and nitrate concentrations might also be due to direct uptake by phytoplankton when the more preferred ammonium was at a low concentration level (Fitzgerald and Rohlich, 1964).

Pano and Middlebrooks (1982) suggested that another mechanism namely ammonia volatilization may also be important in the removal of total ammonia nitrogen in stabilization ponds. It is proposed that carbon dioxide consumed by actively photosynthesising algae exceed those supplied by organic degradation, resulting in an increase in pH. Total ammonia nitrogen in water existed in an equilibrium with dissolved ammonia (NH_3) and ammonium ion (NH_4^+), and alkaline pH shifts the equilibrium towards ammonia. The volatilization of ammonia to the atmosphere depends on the mass transfer coefficient which is enhanced by the mixing effect of wind action and high temperature. Although this mechanism is unlikely to be the major nitrogen removal pathway in the Werribee system, it helps to explain the minor reduction of total ammonia nitrogen concentration when there are no noticeable increases in phytoplankton biomass nor nitrate and nitrite.

In addition to the function of algae as an oxygen-producing source for aerobic bacterial decomposition (Bartsch, 1961), the results of this study concur with previous findings that algae can play a direct role in nitrogen removal in waste stabilization ponds (Tom *et al.*, 1975; Ferrara and Avci, 1982; Santos and Oliveira, 1987). The principal biochemical and physical pathways of different species of nitrogen in a waste stabilization pond system at Werribee are summarized in Figure 5. With regard to water-quality improvement, an increase in phytoplankton

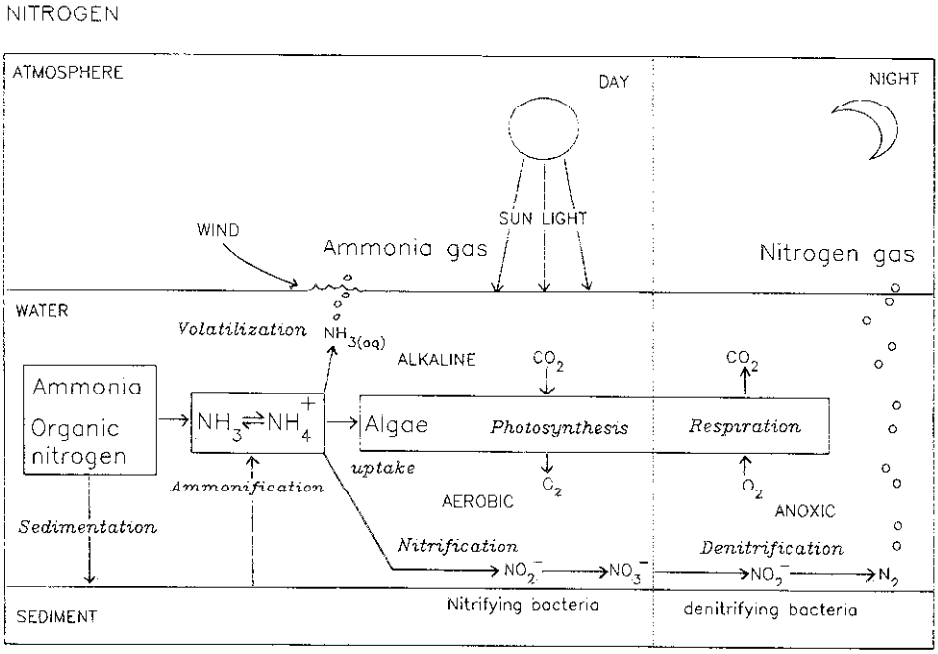

Figure 5. Principal biochemical and physical pathways for nitrogen removal in waste stabilization ponds at Werribee.

biomass will nevertheless result in an increase in total suspended solids in the effluent. Indeed, the potential impact of an effluent containing high phytoplankton biomass would depend on the size of the receiving water body, and whether the algae can survive in the receiving system (Toms *et al.*, 1975; Mitchell, 1980). In Werribee where the effluent is discharged directly into Port Phillip Bay, the freshwater algae in the effluent are unlikely to survive in the receiving marine environment. The dead algal cells will increase the biological oxygen demand of the bay in the vicinity of the outlet.

In the Werribee ponds, zooplankton appeared in the aerobic sections of the pond system in high densities and played a significant role in the reduction of the total suspended solids (see Lai, 1994). These animals grazed on the phytoplankton and produced compact faecal pellets which settled to the pond bottom (Loedolff, 1965). Consequently, phytoplankton abundance were low in the effluent, particularly in September and October when zooplankton abundance was highest. However, the inorganic nitrogen regenerated by zooplankton through their grazing activities on phytoplankton could be significant (Moegenburg and Vanni, 1991), and in this study, high amounts of unstabilized inorganic total ammonia nitrogen were observed in the last few ponds in the series. Indeed, Mitchell and Williams (1982) reported that the zooplankton could only account for less than 6% of nitrogen in the pond system. Through grazing, the stabilized nitrogen (organic) in algal cells are released back into the water column in inorganic forms which may cause eutrophication in the receiving water body. Intense grazing on the phytoplankton which is essential in efficient nitrogen removal may also be detrimental to the treatment process.

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