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INSTITUTE FOR WATER EDUCATION

Effect of some environmental factors on nitrification in algae-bacterial biofilm

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Effect of some environmental factors on nitrification in algae-bacterial biofilm

Master of Science Thesis Proposal
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The findings, interpretations and conclusions expressed in this study do neither necessarily reflect the views of the UNESCO-IHE Institute for Water Education, nor of the individual members of the MSc committee, nor of their respective employers.

I would like to dedicate the work my Lord and Saviour for seeing me in and through this great period.

Abstract

Numerous wastewater treatment technologies are costly and require a high level of technical expertise. Waste stabilization ponds are low-cost systems and do not lower level of technical expertise for maintenance. They are thus appropriate technologies for use in developing countries and are also effective in a developed country context. Although WSPs are effective for BOD and pathogen removal, they have not been effective in nitrogen removal due to the unavailability of attachment sites and subsequent washing out of nitrifying bacteria.

Two continuous flow lab scale ponds were set up to investigate the effect of pH and algae on algal-bacteria biofilms ammonia and nitrogen removal in facultative ponds. Baffles were added to the ponds as biofilm attachment sites. Overall ammonia removal efficiencies were found to range for 15-28% for the algal-based ponds and 6-12% in the non-algal-based pond. Nitrification was identified to be the main mechanism for ammonia removal. This shows that adding attachment surfaces for development of algae-bacterial biofilms has great implications for improving the nitrification in WSPs.

In order to incorporation algae-bacterial biofilms into full-scale ponds, a better understanding of the functioning of these biofilms is required. Further studies into the effect of some environment conditions on algae-bacterial biofilms were carried out. Biofilm plates grown form the labscale ponds were then used to conduct a series of batch tests to study the effect of pH and the absence of algae on biofilm nitrification rates. Batch tests showed that for a pH range of 5-9, the maximum nitrification rates occurred at pH 7. This is different from maximum nitrification rates found for pure cultures of suspended cells, which ranged between pH 7.9 -8.3. This implies that the behaviour of algae-bacterial biofilms may differ from that described for suspended cells.

Algal-bacterial biofilm was found to have a 6.8 times higher nitrification rate than bacterial biofilm with rates of 4.42 and 0.65 $mgN/m^2/h$ respectively. The results suggest that algae contribute to the development of a heterogeneous biofilm community more prodcutive for nitrification than heterogeneous biofilm formed in the absence of algae. It can be concluded that attachment sites increase pond nitrification rates and that the optimum pH for activity of suspended nitrifying cultures differs from that of nitrifying cells in biofilms. Furthermore, algae may play a part in biofilm interactions that contribute to better nitrifying activity.

Keywords: algal-bacterial biofilm, nitrification, wastewater, pH, waste stabilization ponds

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List of abbreviations

ANAMMOX	- Anaerobic ammonia oxidation
AOB	- Ammonia oxidizing bacteria
BOD	- Biochemical oxygen demand
DO	- Dissolved oxygen
DW-S	- Dry weight of sample
WSP	- Waste stabilization pond
MDG	- Millennium Development Goal
MLVSS	-Mixed liquor volatile suspended solids
ρ	- Mass concentration, kgm-3
UN	- United Nations

Additional symbols and abbreviations are elaborated in the text.

1 Introduction

According to the UN, almost half the world's (49%) population was living in the urban areas by the year 2005. The rapid urban population growth and industrialization especially in developing countries causes a strain to the water resources and environmental assimilation capabilities. A major option for wastewater is the use of appropriate technologies. Appropriate technologies are not only economically suitable but environmentally and culturally as well. As described by Kayombo *et al.*, 2005, when one considers low energy and low operational requirements, waste stabilization ponds and constructed wetlands have been found to be effective options although a combination of different technologies can be used to improve wastewater treatment performance.

Nitrogen is the one of the major elements required for life and the most abundant gas in air (78%). A major source of nitrogen into the environment and watercourses is through wastewater and fertilizer application. The used of N fertilizer is increasing along with the increase in the world's population and food demand.

Discharge of nitrogen into the environment is a pollution problem for several reasons. Ammonia is highly toxic to fish at low concentrations. The presence of unionized ammonia species increases at higher pH and temperatures. Nitrogen together with phosphorus stimulates the growth of algae and other plants which can cause choking of water bodies. In situations where the oxygen becomes limitting, eutrophication can occur with the death of algae exerting a higher oxygen demand. These events can drastically alter aquatic ecosystems. High nitrate content in drinking water can cause methemoglobinemia or blue baby syndrome. Other health effects are the presence of nitrosamines which is associated to certain cancers. Large amounts of nitrogen dioxide, a greenhouse gas, can enter the environment. In wastewater treatment, nitrogen dioxide is produced under low COD/nitrate ratios, low retention times and low pH.

Waste stabilization ponds

Waste stabilization ponds have been found to be effective in the removal of BOD, TSS and faecal coliforms, but with the increasing stringency of environmental regulations, effect of nutrients on water bodies and human health, nitrogen has become a critical parameter for removal in wastewaters. WSPs have not been found to be very efficient in nitrogen removal. Various environmental and operational factors affect the nitrogen removal capacity of natural wastewater treatment systems.

Several alternatives have been investigated to increase the nitrogen removal efficiency of waste stabilization ponds. These include the use of macrophytes in case of Bejarano, 2005 and baffles for biofilm attachment. In Canada Houweling *et al.*, 2007 proposed a mechanistic model to explain the seasonal variation in nitrification in order to improve denifrication in aerated lagoon systems.

According to Gloyna (1971) as cited by Lai and Lam (1997), although waste stabilization ponds have been widely used over the world and proved to be an economical way of sewage treatment. Lai and Lam (1997) go on to say, 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 studied as compared with other conventional sewage treatment processes such as activated sludge systems (Lai and Lam, 1997).

Gross *et al.,* 1994 commented that one of the major factors limiting nitrification in lagoon systems was nitrifier washout. He also stated that the minimum HRT depended on the prevailing nitrifier growth rate which is subsequently dependent on several factors that include lagoon water temperature, pH, dissolved oxygen (DO) and ammonia concentration. This research focuses on the enhancement of nitrification by biofilm. Batch tests will be carried out to investigate the effect of some environmental factors on nitrogen removal rates of biofilms with a specific focus pH, and algae.

The application of waste stabilization ponds technologies depends on the availability of land, approximately 14 m^2 per p.e. in the UK (Mara, 2006 as cited by Johnson *et al*., 2007) and 2-5 m^2/capita (van der Steen, 2007). If WSPs are better engineered, the land requirement can be greatly reduced.

Babu *et al*., 2007, studied the enhancement of nitrification in algae waste stabilization ponds by addition of biofilm attachment surfaces in algal pond reactors. He compared the activity between bulk water and biofilm. A mass balance model equation was developed to predict the kjeldahl effluent nitrogen for a selected pilot scale system.

1.1 Problem Statement

According to Gloyna (1971) as cited by Lai and Lam (1997), although waste stabilization ponds have been widely used over the world and proved to be an economical way of sewage treatment, WSPs often do not have special design configurations for nutrient removal. Nitrogen removal in waste stabilization ponds has been attributed to sedimentation, ammonia volatilization and algal uptake. There little evidence of nitrification in WSP. This is because nitrifying bacteria reproduce slowly and are poor floc-formers Gerardi, (2006) and McLean, (2000). Nitrifying populations are unstable and susceptible to being washed out at various hydraulic regimes (Shilton, 2005).

The use of baffles for biofilm attachment and growth has been used to increase bacterial populations. Biofilms are stable and allow for growth of nitrifying bacteria without subsequent washing out. Numerous studies have been carried out to understand the optimum operating conditions for suspended cells and activated sludge systems nitrifying populations. Behaviour of biofilms differs from that of suspended cells. Few studies have been carried out to characterise the optimum conditions for nitrification in algae-bacterial biofilms. It therefore important to carry out studies to understand the effect of different environmental conditions such as pH, temperature, DO and algae on algae-bacteria biofilms for design of s full-scale baffled WSPs. Such an investigation necessitates laboratory studies under controlled conditions. The scope of this research is on the effect on pH and algae on the biofilm nitrification rates.

1.2 Objectives and hypothesis

Facultative ponds have the advantage of containing both an aerobic and anaerobic zone. This is an environment with the potential for additional nitrogen removal through nitrification and denitrification. "At high pH values, ammonia leaves the pond through volatilization. There is little evidence for nitrification (hence denitrification, unless the wastewater has a high of nitrate content). This is due to the fact that the population of nitrifying bacteria is low because of the lack of physical attachment sites in the aerobic zone (Kayombo *et al.,* 2005). The study seeks to under the behaviour of the algae-bacteria biofilm as it is a complex mixture of algae, heterotrophic and autotrophic bacteria dependent upon the dynamic competition and symbiosis all the organisms.

Overall objective

The focus of this research will therefore be to study the effects of different environmental factors affecting biofilm nitrification rates. The algae-biofilm under s is

Specific objectives

- To determine the effect of pH on biofilm nitrification rates.
- To ascertain whether conditions of light and dark have an effect on biofilm nitrification rates
- Quantify the major mechanisms of ammonia and nitrogen removal in baffled labscale ponds using mass balance analysis

Preliminary tests will be done

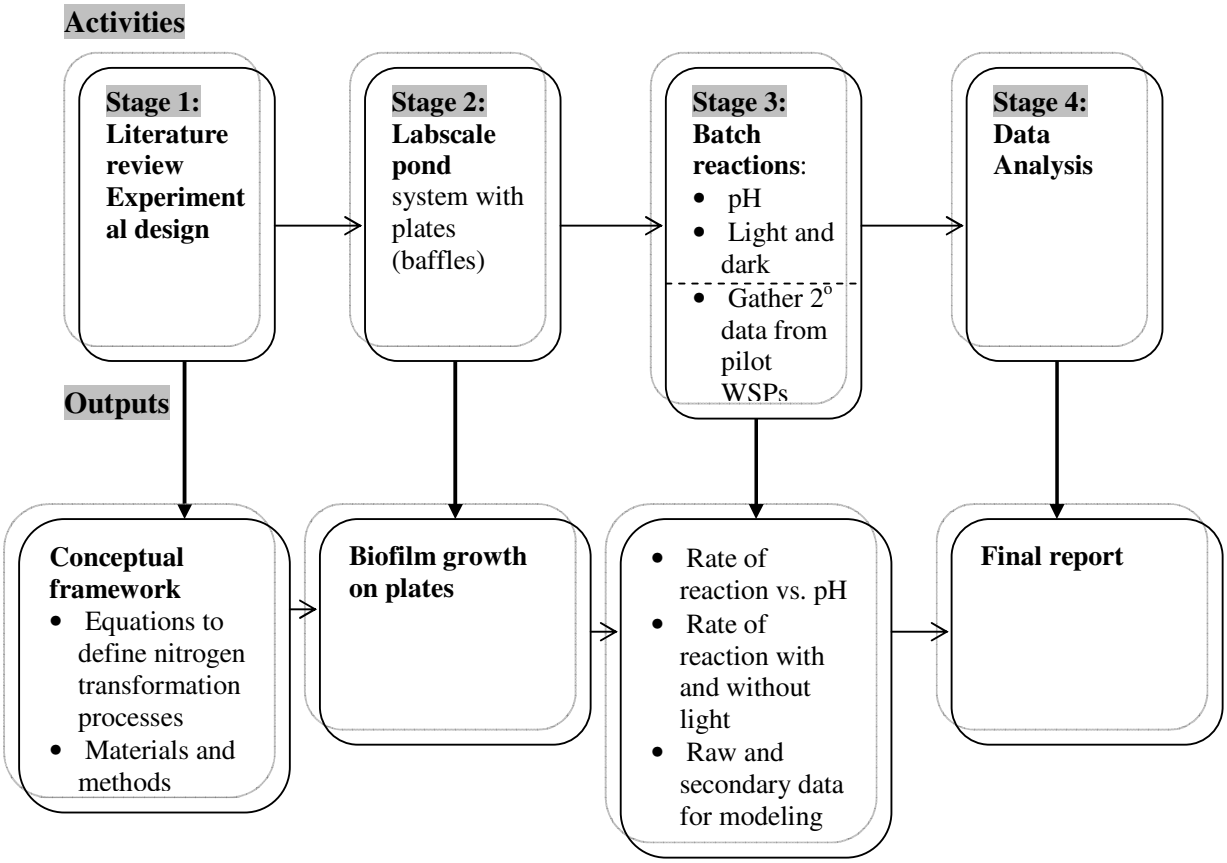
- To determine whether denitrification occurs under aerobic conditions

Hypothesis

- The effect of pH on the nitrification rates of biofilm systems is different to that of suspended cells.
- Biofilm nitrification rates are higher under light conditions than under dark conditions due to oxygen production by algae.

1.3 Research Methodology

The following diagram gives schematic overview of the research activities in four stages. The lower windows represent the outputs for the each activity. Details of the research methodology can be found in the materials and methods.



2 Literature review

2.1 Wastewater treatment

The focus of Millennium Development Goal number 7 if to ensure environmental stability. In order to see this goal reached in a significant way, organized effort and commitment that crosscuts to a sizeable portion of stakeholders is required. Target 10 of MDG 7 is aimed at water supply and sanitation and seeks to halve by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation. Numerous studies and publication have and are being conducted in order to improve the performance of wastewater systems and will provide numerous options for users.

The history of drinking water and sanitation is highlighted by Wiesmann *et. al.*, (2007). In the 19th century, rapid city growth and disease epidemics e.g. cholera, particularly in the western hemisphere necessitated an investigation into the source of drinking water contamination. It was discovered that treatment of drinking water was necessary to prevent infection. The idea that untreated wastewater could be discharged into river and rendered safe by the river's self-purification capabilities was maintained until the 1960s when it was realized that wastewater required treatment before discharging into the receiving water bodies. It is now mandatory in most countries for wastewater to meet a certain standard before being discharged into the receiving waters as stipulated by the law. The composition of domestic wastewater is given in table 2.1.

Table 2.1 Typical composition of untreated domestic wastewater

Constituent	**Weak**	**Medium**	**Strong**
	(all mg/L except for settleable solids)		
Alkalinity (as $CaCO_3$)	50	100	200
BOD_5 (as O_2)	100	200	300
Chloride	30	50	100
COD (as O_2)	250	500	1000
Suspended solids (SS)	100	200	350
Settleable solids (in $mL.L^{-1}$)	5	10	20
Total dissolved solids (TDS)	200	500	1000
Total Kjeldahl nitrogen (TKN) (as N)	20	40	80
Total organic carbon (TOC) (as C)	75	50	300
Total phosphorus	5	10	20

(*Source*: Davis and Masten, 2004)

Wastewater treatment starts of with a pretreatment process, depending on the type of wastewater and treatment system. Physical barricades such as bar rack and grit chambers removing large objects and leaving the light. Primary treatment in settling tanks allows for the settling of these organic suspended solids to form raw sludge. The secondary treatment of wastewater employs various technologies for up to 85% reduction in BOD, suspended solids. Pathogens may be removed by disinfection. Biological secondary treatment systems include activated sludge, trickling filters,

lagoons, anaerobic sludge digesters, lagoons, constructed wetlands and fixed reed bed reactors. Low removal efficiencies are achieved for nitrogen, phosphorus, soluble COD, and heavy metals (Davis and Masten, 2004). In order to reduce the levels of these pollutants requires tertiary treatment which employs advanced treatment systems.

The introduction of high concentrations of nutrients, nitrogen and phosphorus into the receiving water caused growth of cyanobacteria and algae in lakes, river and the sea which can end up undergoing eutrophication. This has a significant effect the water quality and aquatic ecology of the water body. Options for nitrogen removal maximising the process of nitrification-denitrification, ANAMMOX systems and ammonia stripping.

2.2 Waste stabilization ponds

WSPs can be described as basins excavated directly into the ground with a wastewater inlet and outlet at on opposite sides (Andersen, 2005). Ponds may be constructed fro different materials such as concrete and do not necessarily have to be lined. WSPs systems are usually composed of a combination of anaerobic ponds in parallel, a facultative pond and maturation ponds in series. Waste stabilization ponds consist of anaerobic, facultative and maturation ponds. Anaerobic ponds are deep ponds mainly serving as settling basins and facilitating the anaerobic digestion of particulate organic solids. Facultative ponds are usually the second treatment step in WSP system aimed at BOD, nutrient and pathogen removal. The final treatment step involves maturation ponds which are shallow and serve for further for effluent polishing through the stabilization of organic matter, nutrient removal and removal of pathogens. Both the facultative and maturation ponds contain algae.

WSPs ponds are effective in the removal of BOD and considerably reduce TSS, faecal coliforms and helminth eggs. The other main advantages of WSPs is that they are simple and cheap to construct, require low operational and maintenance costs, low energy inputs and simple sludge managements.

The main limitations of WSPs is that they require a large land area, temperature sensitive performance, potential odour release, low operational control and limited nitrogen removal. Various alterations have been made to improve ponds treatment efficiencies and systems such as duckweed-based pond systems, aerated lagoons and high rate ponds have been developed. Table 2.2 shows some values for total nitrogen and ammonium removal in WSPs. It can be seen that the actual removal of ammonia is higher than the total nitrogen removed. A pilot scale tracer study carried out by Mara and Valero (2007a), to explicate the important mechanisms and pathways of nitrogen removal and transformations occurring in WSP also yielded a low total nitrogen removal (8%) and a high ammonium nitrogen removal (90%). Mara and Valero (2007a) concluded that main nitrogen removal process was via algal uptake followed by sedimentation. Nitrification was not considered to be a feasible mechanism for nitrogen removal.

Table 2.2 Nitrogen removal efficiency (% of influent concentration removed) of WSP

TN	NH_3-N	Reference
21%	20%	Picot et al., 1992
31%	29%	Racault et al., 1995
40%		Garcia et al., 1991
21-73%	60-93%	Li et al., 1991
	52%	Santos and Oliveira 1987
40-80%		USEPA 1985
<79% TKN	<92%	Silva et al., 1995
	90%	Pano and Middlebrooks 1992
	95%	Middlebrooks et al., 1982
Maturation ponds		
45%		Somiya and Fuji 184
	82%	Wrigley and Toerien 1990

[*Source*: adopted from Craggs, 2005]

2.3 Nitrogen transformation processes

Facultative ponds are favourable for the enhancement of nitrification-denitrification processes due to their presence of an aerobic and anaerobic zone, see fig. 2.1. An intermediate facultative zone not included in the figure, exists between the aerobic and anaerobic zones. These zones form as a result of the attenuation of light which decreases with an increase in depth from the pond water surface. Algal densities serve to increase this attenuation via shading and absorption of as one goes deeper down Physical, chemical and biological processes are responsible for nitrogen transformation and removal. Theses process will be described in more detail in the proceeding sections.

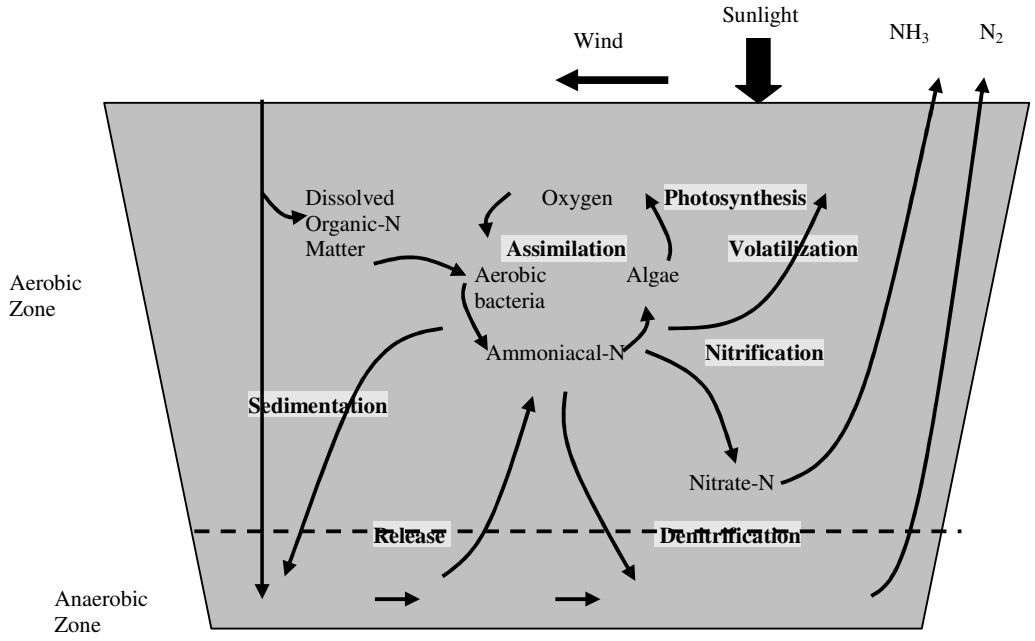


Fig. 2.1. Nitrogen transformation processes in a facultative pond

[*Source:* Adapted from Shilton, (2005)]

Various forms of nitrogen exist in wastewater. Knowledge of the various species is necessary in order to account carry out nitrogen mass balances and account for the nitrogen removal. According to Henze *et al*., (1995) total nitrogen is wastewater may be divided into the following fractions:

NO_2^-	nitrite nitrogen
NO_3^-	nitrate nitrogen
NH_4^+	ammonium nitrogen and ammonia nitrogen
DIN	dissolved inert organic nitrogen
SN	suspended easily degradable organic nitrogen
SIN	suspended inert (organic) nitrogen

DON represents a class of organic compounds that include urea, amino acids, humic and fulvic acids (Burdige and Zheng, 1998).

2.3.1 Sedimentation of wastewater solids

This is the settling of insoluble suspended particles at the bottom of the pond. Sedimentation is encouraged by reduced turbulence in ponds which allows suspended solid to settle. Mara and Valero (2007a) reported the settling of organic nitrogen to be the second major mechanism for removal of total nitrogen after assimilation by algae and bacteria. Organic nitrogen is removed by this process.

A minor removal process includes adsorption of ammonia to sludge or pond walls or at high pH ferric oxyhydroxide, aluminium hydroxide and calcium carbonate (Craggs, 2005)

2.3.2 Assimilation into algal and bacterial biomass

Algal and bacterial cells are able to assimilate nutrients and coexist in a symbiotic relationship. Bacterial growth or biosynthesis converts colloidal and soluble organic matter into particulate biomass (new cells) which can then be subsequently removed by settlement (Gray, 2005). The nutrient composition of microalgae grown on domestic wastewater varies between 0.6-16% (average 8%) for nitrogen according to Hemens and Mason, 1968 cited by Craggs, 2005.

Biosynthesis:

$$\underset{\text{(Organic matter)}}{\mathbf{COHNS + O_2 + Bacteria}} \longrightarrow \underset{\text{(New cells)}}{\mathbf{C_5H_7NO_2}} \qquad (1)$$

According to Aslan and Kapdan (2006) many studies demonstrated that microalgae have a great potential for the removal of nitrogen and phosphorus. The main mechanisms in algal nutrient removal from mechanisms in algal nutrient removal from wastewater include uptake into the cell and stripping ammonia through elevated pH. Their experimental conditions for this nitrogen removal was: an N/P ratio of 2/1, adequate supply of carbon dioxide via aeration, a pH maintained at 6.5-7.0, continuous illumination and temperatures of between 20± 2 ^oC.

Algae and bacterial symbiosis

The mechanism for BOD and ammonia removal in facultative ponds is identical to that of activated sludge system. The only difference is that the algae present in the ponds provide the additional oxygen required for oxidation of carbon and nitrogen, via photosynthesis, by heterotrophic and autotrophic bacteria respectively. Algae benefit from utilization of the carbon dioxide and ammonia as a source of cell carbon and nitrogen. Common bacterial genera in this system include *Pseudomonas, Flavobacterium and Achromobacter.* (Horan, 1990; Metcalf and Eddy, 1991). Fig.2.2 shows gives a schematic representation of the bacterial-algae relationship.

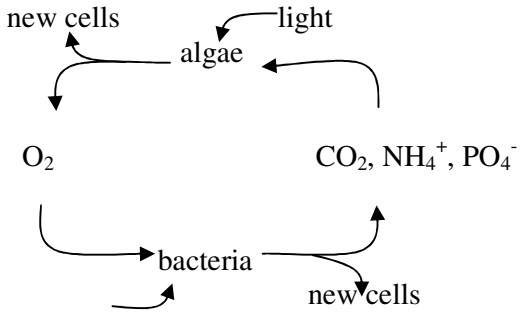


Fig. 2.2. Symbiosis of algae and bacteria in waste stabilization ponds,
[Source*:* Adopted from Horan, 1990]

2.3.3 Ammonia volatilisation

Ammonia is toxic to fish. Although algae require ammoniacal-N for assimilation rather than the oxidised forms of nitrogen, an increase in free ammonia becomes toxic to algae by the inhibition of photosynthesis. An ammonia nitrogen concentration of 36 mg/L may reduce algal growth if the pond water pH rises above 8, and may reduce algal photosynthesis by 50% at pH 9.5 (20-25^{o}C), while an ammonia nitrogen concentrations of 54 mg/L may reduce algal photosynthesis by 90% at pH 9.5 (20-25^{o}C) (Azov and Goldman, 1982; Veenstra *et al*., 1995 as cited by Shilton, 2005).

At neutral pH the chemical equilibrium of ammonia is shifted towards the left of equation 2. It shifts more towards the right at pH above 8.5 where ammonia begins to form an essential part of the total content of ammonium compounds (Henze *et al*., 1995). Ammonia volatilisation can be a dominant process of nitrogen removal in WSPs accounting for 75-98% of total N removal in domestic WSPs with pH ranges at 7 to 9, and temperature ranges from 22 to 28^{o}C (according to Pano and Middlebrooks, 1992 as cited by Shilton, 2005) .

$$\mathbf{NH_4^+ \longleftrightarrow NH_3 + H^+} \qquad (2)$$

The concentration of unionised ammonia [NH_3] in the pond water can be calculated using the equations 3 and 4 by Emerson *et al.*, 1975 as cited by Mara and Valero (2007b):

$$[NH_3] = [NH_4^+]_{Total} / (1 + 10^{(pKa - pH)}) \quad (3)$$

$$pKa = 0.09018 + 2729.92 / (273.2 + T) \quad (4)$$

where,
$[NH_4^+]_{Total}$ is the total ammonia nitrogen (mg/L)
Ka is the ammonia-ammonium equilibrium constant ($pK = -\log_{10}K$)
T is the temperature in ^oC

The mass transfer equation with the assumption that the concentration of the ammonia gas in the atmosphere is zero was used to estimate the average ammonia volatilization rates for pond systems.

$N_{NH3} = -K_l [NH_3]$ where, N_{NH3} ammonia volatilization rate of NH_3 ($mgl^{-1}d^{-1}$),
K_l is the convention mass transfer coefficient in the liquid phase (d^{-1}),
$[NH_3]$ is the ammonia concentration in the liquid phase (mgl^{-1})

Stratton, (1969) as cited by Zimmo, (2003) obtained the following expression for the mass transfer coefficient:

$$K_l = \frac{0.0566}{d} \exp[0.13(T - 20)] \quad (6)$$

where,
d is the water depth of the water column in the pond (m) and
T is the water temperature (^oC)

Studies carried out by Mara and Valero, (2007b) revealed that predictions of ammonia volatilisation using models based on mass transfer equations were higher than actual measured values in maturation ponds.

2.3.4 Nitrification

Nitrification is the conversion of ammonium to nitrate by autotrophic bacteria under aerobic conditions. The best know aerobic ammonia oxidizers are *Nitrosomonas* and Nitrospira. Common aerobic nitrite oxidizers are Nitrobacteria and Nitrospira. It is an aerobic two step reaction and can be represented as follows:

$$2\,NH_4^+ + 3O_2 \xrightarrow{Nitrosomonas+\ Nitrosospira} 2NO_2^- + 4H^+ + 2H_2O + Energy \quad (7)$$

$$2NO_2^- + O_2 \xrightarrow{Nitrobacter+\ Nitrosospira} 2NO_3^- + Energy \quad (8)$$

2.3.5 Denitrification

Denitrification is an anaerobic respiration process whereby denitrifying bacteria oxidise organic matter by reduction of nitrate to nitrogen gas as shown in equation 9.This is a dissimilatory nitrate reduction process. Common denitrifying microorganisms belong to the genera: *Pseudomonas, Bacillus, Agrobacterium, Rhizobium, Thiobacillus and Alcaligenes* (Bitton, 2005).

$$2\,NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \quad (9)$$

$$2NO_3^- + \text{organic matter} \leftrightarrow N_2 + CO_2 + H_2O \quad (10)$$

The main factors affecting denitrification in wastewater treatment plants are the nitrate concentration (Monod type-curve), anoxic conditions of DO< $1gm^{-3}$, organic matter (electron donors: acetic acid, methanol and ethanol), in wastewater pH of between 7 and 8.5 (optimum 7) and temperature > $10\,^oC$ (Bitton, 2005).

2.3.6 Heterotrophic nitrification and denitrification

Some heterotrophic bacteria are capable of oxidizing inorganic nitrogen compounds, but the rates of heterotrophic nitrification are normally four orders of magnitude lower than those of autotrophic nitrification (Atlas, 1988).

2.3.7 Anammox

Anammox stands for "anaerobic ammonium oxidation". In the absence of oxygen and available organic matter, autotrophic ammonia oxidizers can carry out denitrification by using ammonium as the electron donor and nitrite as the electron acceptor, equation 11. Some research is focused on utilising the process for nitrogen removal. The uncovered process can save up to 90% of the operation cost as compared to typical nitrogen process (Jetten *et al.,* 2001 as cited by Chamchoi and Nitisoravut, 2007). Chamchoi and Nitisoravut, (2007) cultivated Anammox sludge to monitor the nitrogen removal. They concluded that Anammox cultivation from conventional sludges was possible under a controlled environment within four months.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (11)$$

2.4 Kinetics

2.4.1 Enzyme reactions

The Michaelis-Menten equations allow the reaction rate of enzyme-catalysed reactions to be calculated:

$$r = \frac{R_{max}[N]}{Km + [N]} \quad (12)$$

Where,
r = reaction rate

R_{max} = maximum reaction rate at which the product is formed, $mgl^{-1}day^{-1}$
S = substrate concentration, mgl^{-1}

2.4.2 Bacterial growth

The growth rate of *Nitrobacter* is higher than that of *Nitrosomonas*. Thus, the rate limiting step in nitrification is the conversion of ammonia to nitrite by *Nitrosomonas* (Bitton, 2005). The Monod expression for nitrification can be written as follows:

$$\mu_N = \frac{\mu_{max} N}{N + K_N} \quad (13)$$

Where,
μ_{max} = maximum specific growth rate of *Nitrosomonas,* day^{-1}
μ_N = specific growth rate for *Nitrosomonas* in day^{-1}
N = NH_4^+-Nconcentration, mg/l
K_N = half-saturation constant, mg/l of NH_4^+-N

(Water Pollution Control Federation, 1983)

2.5 Factors affecting nitrification

2.5.1 pH

The optimum pH for *Nitrosomonas* and *Nitrobacter* lies between 7.5 and 8.5 (U.S. EPA, 1975 as cited by Bitton, 2005). Nitrification ceases at or below pH 6.0 (Painter, 1970; Painter and Loveless, 1983 as cited by Bitton, 2005). According to Winkler, 1981, nitrifiers prefer a slightly alkaline pH with an optimum pH at about 8.5.

Table 2.3 The effect on pH on nitrification

pH	Effect on Nitrification
4-4.9	Nitrifying bacteria present but inactive; limited nitrification occurs through the activity of organotrophic bacteria
5-6.7	Nitrifying bacteria but activity is sluggish
6.8-7.2	Desired pH range for nitrification in the activated sludge process
7.3-8.0	Rate of nitrification assumed to be constant
8.1-8.5	Optimum pH range for nitrification (e.g. in laboratory work nitrifying bacteria only)

(Source: Gerardi, 2006)

2.5.2 Dissolved oxygen

In wastewater treatment plants, oxygen is a limiting factor controlling the growth of nitrifiers. According to Bitton, 2005, oxygen should not go below 2mg/l in activated sludge system for nitrification to proceed. Therefore equation13 can be modified to take into account the effect of oxygen concentration (Bitton, 2005):

$$\mu_N = \frac{\mu_{max}[NH_4^+]}{K_N + [NH_4^+]} \frac{[DO]}{Ko + [DO]} \quad (14)$$

where,
[DO] = dissolved oxygen concentration, mg/l
K_0 = half saturation constant (oxygen), mg/l

K_0 has been estimated to be 0.15-2mg/l, depending on temperature (U.S.EPA, 1975 as cited by Bitton, 2005). Others have reported a range of K_0 = 0.25-1.3mg/l (Hawkes, 1983; Stenstrom and Song, 1991; Verstraete and van Vaerenbergh, 1986 as cited by Bitton, 2005).

2.5.3 Temperature

The growth rate of nitrifiers is affected by temperature in the range 8-30 ^oC. The optimum temperature has been reported to be in the range 25-30 ^oC (Bitton, 2005) and 20 ^oC (Horan, 1990). The rate of reaction with temperature varies according to Arrhenius' expression:

$$\mathbf{k_T = k_{20}\,\theta^{T-20}} \qquad (15)$$

where
T = temperature, ^oC
k_T = reaction rate and temperature T, day^{-1}
k_{20} = reaction rate constant at 20 ^oC, day^{-1}
θ =temperature coefficient

According to Gray 2005, Van't Hoff's rule states that the rate of biological activity doubles with every 10 ^oC rise in temperature within the range 5-35 ^oC. The growth rate falls to zero above 35 ^oC as the temperature approaches 45 ^oC. According to Gray (2004) *Nitrosomonas* had a 9.5% increase in growth rate per ^oC rise in temperature between a range of 8-30 ^oC.

2.5.4 Ammonium concentration

Ammonium serves as a substrate for *Nitrosomonas*. The relationship between bacterial growth and substrate concentration follows Monod's kinetics as described in section 2.4.2.

For the ammonia oxidizing bacteria (AOB), the saturation coefficients by oxygen ($\underline{K'}_{AOB}$) and substrate (K_{sAOB}) were found to be 0.99 g O_2/m^3 and 0.3 g $N\text{-}NH_3/m^3$, respectively. For the nitrite oxidizing bacteria (NOB), the saturation coefficients by oxygen ($\underline{K'}_{NOB}$) and substrate (K_{sNOB}) were found to be 1.4 g O_2/m^3 and 2.2×10^{-4} g HNO_2/m^3, respectively.

2.5.5 Other factors

Growth kinetics of nitrifiers takes into account the substrate [NH_4^+] concentration as well as environmental factors such as temperature, pH and dissolved oxygen, Barnes and Bliss as cited by Bitton, 2005):

$$\mu_N = \frac{\mu_{max}\,[NH_4^+]}{0.4e^{0.118(T-15)} + [NH_4^+]} \times \frac{[DO]e^{0.095(T-15)}}{1 + [DO]} \times (1.83)(pH_{opt} - pH) \qquad (16)$$

μ_N = specific growth rate for *Nitrosomonas* in day^{-1}
T = temperature (^oC)
pH_{opt} = optimum pH= 7.2
μ_{max} = 0.3 day^{-1}

BOD_5/TKN ratio: The fraction of nitrifying bacteria decreases as the BOD_5/TKN ratio increases.

2.5.6 Operational factors

Characteristics of nitrifying bacteria

Table 2.4: Optimal Conditions for nitrification

Characteristic	**Design**
Permissible pH range (95% nitrification)	7.2-8.4
Permissible temperatures (95% nitrification) (^oC)	15-35
Optimum temperature, ^oC (approximately)	30 ^oC
DO level at peak flow, mg/l	>1.0
MLVSS, mg/l	1200-2500
Others: Inhibitory substances	

(*Source:* Adapted from the U.S. EPA, 1977 as cited by Bitton, 2005)

2.6 Properties of biofilms

A biofilm is a surface-attached gelatinous matrix composed of microorganisms, the extracellular polymers (EPS) they excrete and foreign substances such as adsorbed molecules and even above 90% water. A more elementary definition for biofilms is given by Characklis and Marshall (1990), where a biofilm is described as consisting of cells immobilized on a substratum and embedded in an organic matrix of microbial origin. Biofilms form on available substratum and in wastewater treatment systems develop a complex mixture of prokaryotic (e.g. heterotrophic and autotrophic bacteria) and eukaryotic (e.g. algae and fungi) cells. Bacteria are usually the predominant microorganisms. According to Henze *et al.*, (1995), oxygen will normally limit the conversion of both organic matter and ammonium. As nitrifying bacteria grow slowly, they will be ousted and hence eliminated from the plant if the thickness growth of the biofilm, which is primarily conditioned by the growth of the heterotrophic bacteria, is faster than that of the nitrifying bacteria.

There has not been as much work done on algae-bacteria biofilm for wastewater treatment in comparison to bacterial biofilm. However, bacterial biofilm studies help in understanding the functioning of biofilm in general. Biofilm reactors common in wastewater treatment include trickling filters, submerged bed reactors and rotating disc reactors.

Structure of biofilms

The structure and property of biofilms is quite heterogeneous in terms of polymeric composition, cell structures and layer formation. Despite this, new analytical tools based on the use of molecular probes together with confocal laser scanning of microbial

population distributions inside biofilm matrices (Wobus *et al.*, 2000, Neu, 2000 as cited in Melo, 2003) allow for a better understanding of the biofilm structure. Microelectrodes are also used to monitor substrate and pH profiles with the biofilm layers.

Fig. 2.3 shows the basic structure of biofilm. The biofilm systems can be divided into four main compartments: the bulk liquid or overlying water, the boundary layer, the biofilm and substratum or carrier. Material exchange takes place between the biofilm and bulk liquid. Experimental observations have given strong indication of large concentration gradients for solutes just outside the biofilm (boundary layer), when these solutes are utilised or produced by the biofilm (IWA Task Group on Biofilm Modeling, 2006). This gives different solute concentrations at the biofilm surface and the completely mixed bulk liquid.

Fig. 2.3 goes onto give a break down of the different zones forming the biofilm, namely the aerobic, anoxic and anaerobic zones. These zones form due to substrate gradients. The concentration profiles for carbon, ammonium, oxygen, and nitrite/nitrate illustrate their individual depletion and formation, a consequence of biological transformation processes. These substrate gradients are caused by diffusion limitations whereby substrate availability is not uniform right through the biofilm. Unlike in suspended cells, transport (e.g. diffusive mass transport) and transfer (between bulk and biofilm) processes are critical to biofilm growth rates. Movement of solutes via fluid transportation (convection) is hindered by the biofilm cell densities, extracellular polymers and substratum. Diffusion is therefore the main transport mechanism of solutes within the biofilm aggregate. Stewart, (2003) states that, "Diffusion limitation arises in biofilm systems because fluid flow is reduced and the diffusion distance in increased in the biofilm mode of growth".

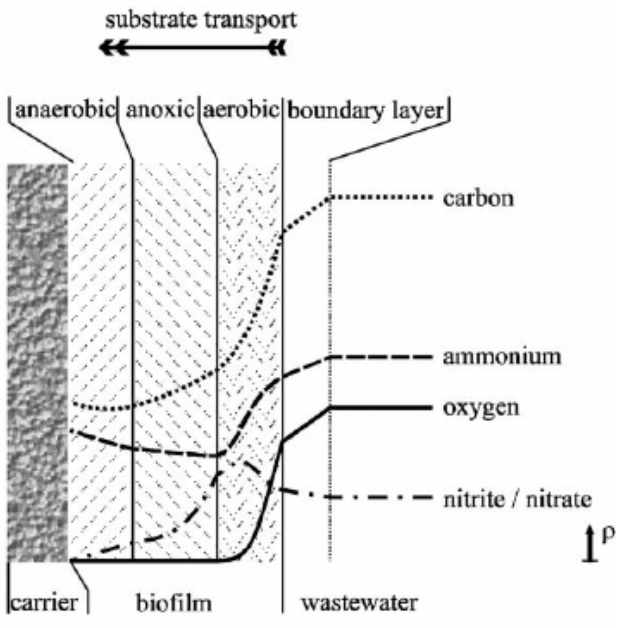


Fig. 2.3. Schematic representation of a biofilm structure at conventional aeration.

(*Source*: Walter *et. al*, 2005)

Biofilm sloughing

Biofilm development starts with the attachment of macromolecules to the substratum and then the attachment of microbial cells onto this surface. As the bacterial cells grow they produce extracellular polymeric substances (Wiesmann *et al.*, 2007). Biofilm growth involves the processes of attachment, growth, and detachment. These processes are facilitated by hydrodynamic forces and expansions due to replication. Bryers (2000) differentiates these processes from sloughing which he describes as "A more random stochastic process,..., where either large sections or the entire biofilm become displaced from the substratum and enter the liquid". Sloughing occurs because of changes in the biofilm. Sloughing has been observed to occur during high substrate loading and laminar flow or low shear or stress (Characklis and Marshall, 1990).

Advantages of a biofilm

According to Melo, 2003, microbes may form biofilms rather than live in dispersed suspension due to proximity and protection. Microenvironments with specific nutrient concentration gradients, pHs, electrical charge distributions, proton concentrations are formed. Immobilised and biofilm-bound cells remain in a continuous reactor system independent of the fluid, thus the mass loading of limiting substrate (or influent pollutant in the case of wastewater treatment reactor) can be increased well beyond the growth rate limit imposed on suspended cells (Bryers 2000).

Biofilms offer protection due to the meshwork of cells, polysaccharides and glycoproteins formed. Diffusion of toxic substances is more difficult and there is greater resistance to hydrodynamic forces. There is also the added advantage of adhesion of nutrients and organic molecules which can be utilized by the microbes. Due to the formation of microenvironments, aerobic and anaerobic zones can be formed allowing for e.g. growth of both nitrifying and denitrifying bacteria in the same biofilm.

According to Christensen and Harremoees, 1978 as cited by Bitton, 2005, denitrification may occur inside activated sludge flocs and biofilms despite relatively high levels of oxygen in the bulk liquid. They explained that the presence of denitrification may continue to occur at the microenvironment level.

3. Materials and Methods

3.1 Continuous flow experiments for biofilm generation

3.1.1 Experimental set up

Laboratory sized ponds were set up for culturing of the biofilm. The trays used were shallow lab scale ponds and mainly represent the aerobic zone of the facultative pond. Two lab scale ponds of volume14.4L* were setup as continuous plug flow systems. The pond dimensions were length 54.5cm x width 37.7cm and water depth 8cm. The *light* pond was grown in the presence of 12 hrs light-dark regimes at an illumination of 72.9µE/m^{-2}s provided by a fluorescent lamp. The treatment for the 2nd pond was the same except that the pond was covered by a black plastic cover to prevent light from reaching the pond. The connection tubing was covered with aluminium foil to prevent algal growth. (*corrected for volume displaced by plates)

Perspex plates (7x 9 x 0.05cm) were used as the biofilm attachment sites. The plates were roughened by sandpaper to prevent biofilm slurring. 4 plates were placed in a row and supported by clippers ensuring they are fully submerged in the water but not touching the bottom of the pond. In total 48 plates were placed in each pond. The plates were arranged as shown in fig. 3.1.

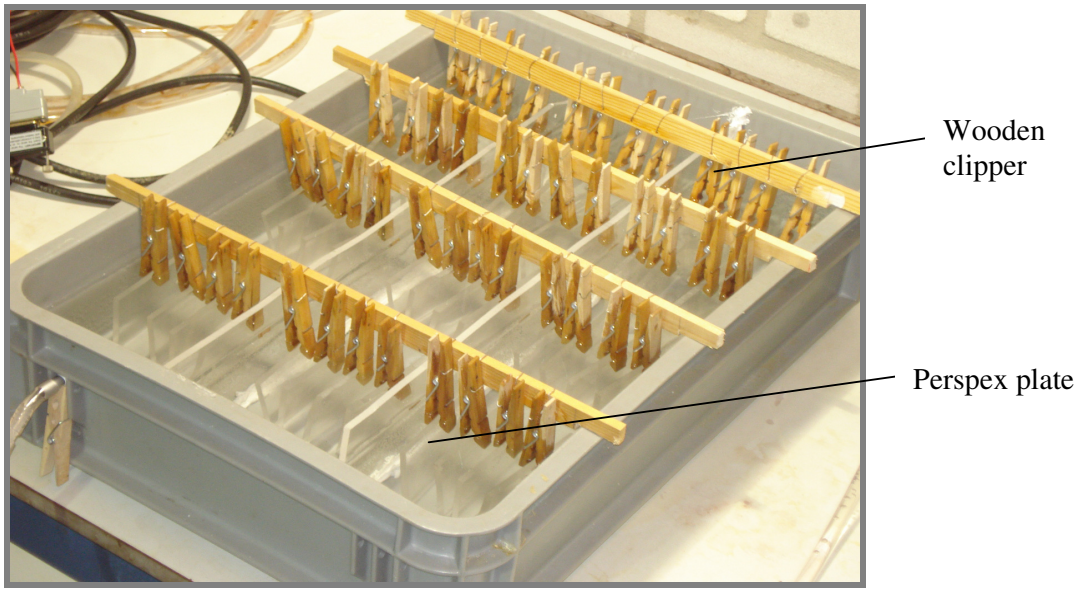


Fig. 3.1. A picture showing the arrangement of the 48 plates for biofilm attachment

The ponds shared the same influent tank and each had one pump pumping influent to the inlet up to a depth of 8 cm. A second pump was used to re-circulate the bulk liquid

from the outlet point back to the inlet in order to operate the ponds as mixed systems. This would allow for more uniform conditions throughout the ponds and reduce variability in biofilm characteristics. The characteristics and design of the ponds is shown in more detail in table 3.1 and figure3.2. Pumping rates were calibrated to give flow rates ranging for 2-3.5ml/min giving HRT of 3-5 days. The recirculation pumps speeds were 11.33 and 17 ml/min respectively for the light and dark ponds respectively.

The volume of effluent collected was measured daily together with recordings of the time period of collection. These reading were used to calculate the hydraulic retention times of the ponds as follows:

$$HRT = \frac{\text{flow rate at the outlet}}{\text{Volume of pond}}$$

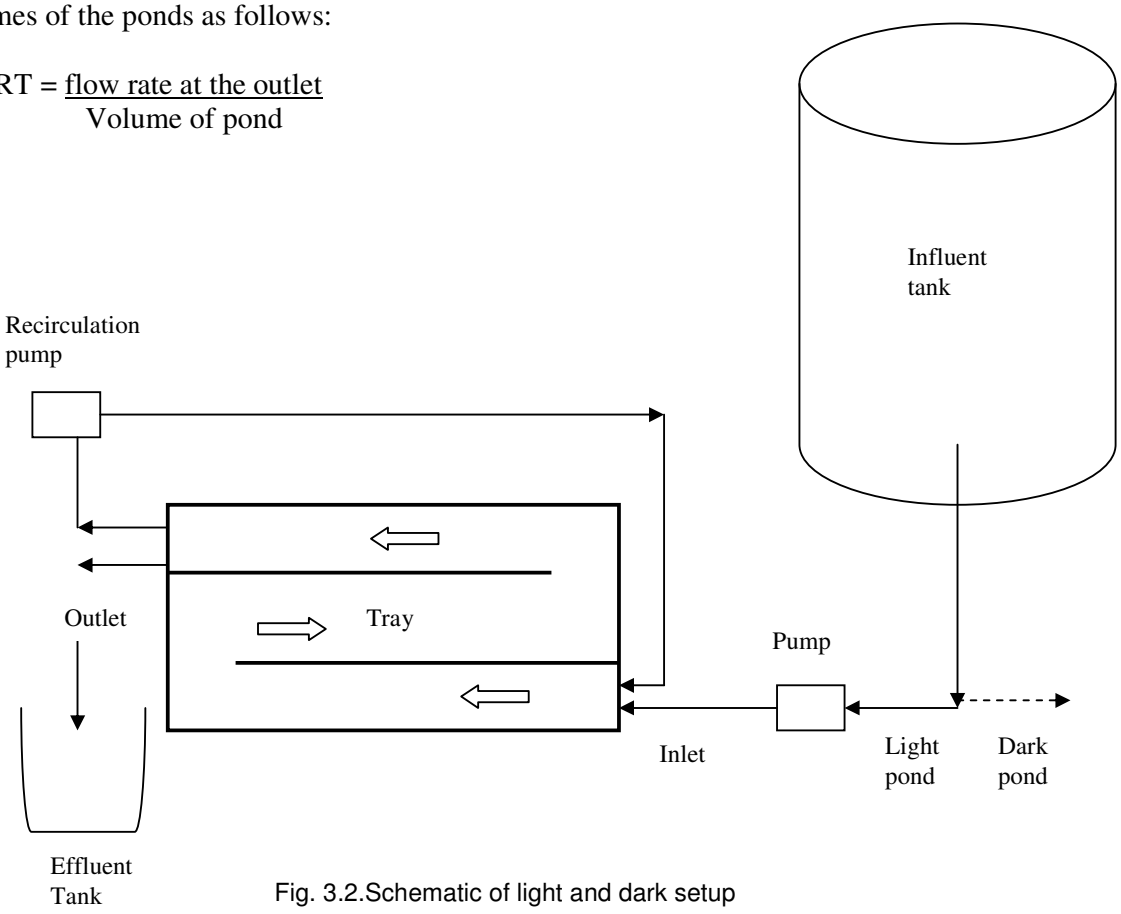


Fig. 3.2.Schematic of light and dark setup

Inoculation

Light pond:
The algae-based pond was seeded with 8 L of canal water from Delft and left to stand for 1-2 days to allow for the algae and microorganisms to take root. The canal water was inoculated with 10 ml of activated sludge and a concentrated algae culture. The algal species in the concentrate were *Anaebena cylindrica, Anaebena variabilis, Spirulina platensis, Scenedesmus quadricauda and Chlorococcus.* The activated sludge was obtained from Haenaschpolden treatment plant in Den Hoorn.

After 31 and 37 days of operation the nitrogen removal remained below 20%. Reinoculation was done using a concentrated nitrifying biomass acquired from TU Delft

University. The biomass contained *Nitrospira and Nitrobacter* species. The dissolved oxygen remained lower than expected for algal ponds so algae inoculum was also added.

Dark pond:
The lab scale pond was seeded with 8 L of canal water from Delft which was inoculated with 5 ml of the TU Delft nitrifying biomass and 5ml of activated sludge from Harnaschpolder.

During the course of nitrification development other inventions were taken to increase the nitrification rates of the pond. These included increasing the alkalinity by addition on 2.75g of $NaHCO_3$, changing pumps to reduce the flow rate and raising the pH of the influent which ranged from 6.3-6.9 after preparation. The pH was raised by adding NaOH.

Table 3.1.Summary of pond operating conditions

	Light pond	**Dark pond**
Inlet flow rate:	2-3.5 ml/min	2-3.5ml/min
Recirculation flow rate:	11.33 ml/min	17 ml/min
Illumination:	72.9µE/m^{-2}s	Dark
Days of operation:	93	48
Commissioning:	5 December 2007	19 January 2008
Decommissioning:	7 March 2008	7 March 2008

a) b)

Fig. 3.3. The continuous flow setup a) Picture of light pond b) Picture of dark pond

3.1.2 Operation

At first the artificial wastewater was prepared using demineralised water on a weekly basis. The composition of the artificial wastewater (influent solution) is given in table 3.2 for the influent composition. After observing a rapid decline in DO of the influent tank, the artificial wastewater was prepared twice a week using millicule water. This was done to prevent culturing of undesirable heterotrophic bacteria which could interfere with the pond microbial population dynamics

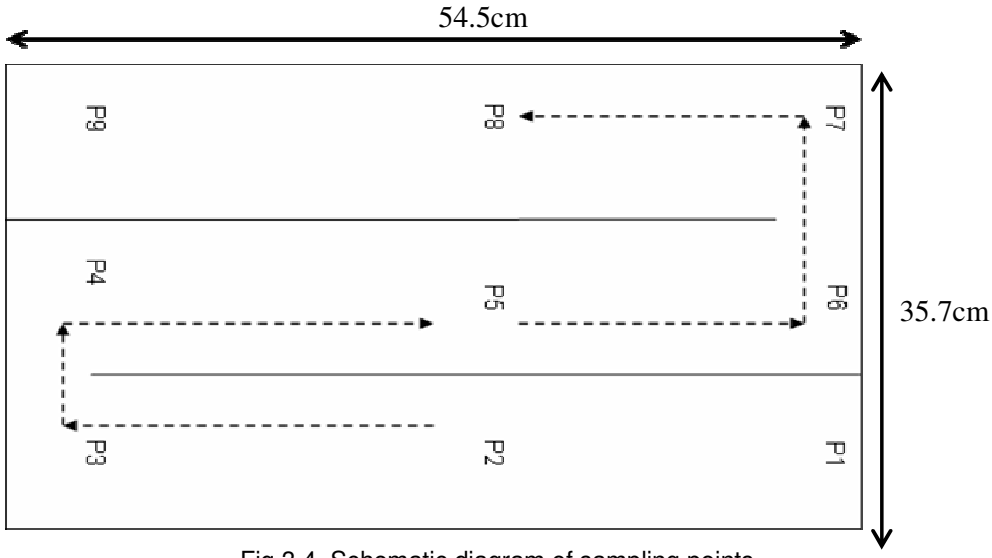


Fig.3.4. Schematic diagram of sampling points

3.1.3 Parameter for monitoring

pH, DO and temperature

Readings for pH, DO and temperature were recorded daily. To get representatives readings and monitor the effectiveness of the recirculation pump, measurements were taken first at 7 points: P1=Inlet, P2= Middle of 1st R, P3= Exit1, P5= Middle of 2nd R, P6= Exit2, P8= Middle of 3rd R and P9= Outlet. Later the sampling points were reduced to 5 points: P1, P2, P5, P8 and P9. Readings for the influent tank and tap water were taken as a quality check.

Nitrification

The levels of nitrification were monitored on a weekly basis by analysing for ammonia, nitrite and nitrate. Samples were taken from: P1, P5, P8, influent and effluent tank.

Table 3.2. Composition of synthetic wastewater

Macronutrients	**Conc. (mg/L)**	**Micro nutrients solution**	**Conc. (g/L)**
CH_3COONH_4	93.75	EDTA-disodium salt	10
NH_4Cl	87.70	$FeCl_3.6H_2O$	1.5
$NaH_2PO_4.H_2O$	26.70	H_3BO_3	0.15
$MgSO_4.7H_2O$	90.00	$CuSO_4.2H_2O$	0.03
$CaCl_2.2H_2O$	4.72	KI	0.18
KCl	36.00	$MnCl_2.4H_2O$	0.12
Micronutrient solution	0.6(ml/L)	$Na_2MoO_4.2H_2O$	0.06
$NaHCO_3$	2.75	$ZnSO_4.7H2O$	0.12
		$CoCl_2.6H_2O$	0.15
40mg/L NH4-N and 96mg/L COD			

[*Source*: adapted from Babu, 2007]

3.2 Batch experiments

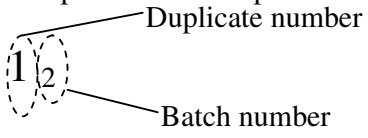
Batch experiments are generally preferred as they offer a simpler and more convenient method for interpretation of reaction kinetics, avoiding possible interference of reactor hydraulics (Sozen *et al*., 1996 as cited by Kubare, 2007). Batch tests were carried out to study the effect of different pH's on biofilm nitrification rate and to determine nitrification rates for biofilm grown in the absence of algae. The trial batch experiments were run first at 7 hours. Due to the low biofilm activity, experiments were then run for 24 hours and 51 hours. 15 ml samples were taken at hourly intervals and analysed for ammonia, nitrite and nitrate. When samples were not analysed immediately, they were filtered and stored at 4 ^oC. The batch tests conducted are summarised in table 3.3 below and discussed in more details in the proceeding chapters.

Table 3.3. Summary of batch experiments conducted

Batch	**Condition**	**Running time (hours)**	**Biofilm age in days**
1	pH 6, pH7, pH 7 control, pH 7 dark, pH 8, bulk.	7	58
2	pH: $7(1_2)$, $7(2_2)$, 7control, $8(1_2)$, $8(2_2)$, 8control	24	65
3	pH: 5, 5control, 6(1), 6(2), 6control, $7(1_3)$, $7(2_3)$, 7control, $8(1_3)$, $8(2_3)$, 8control, 9, 9control	57	80
4	Dark: $D1_4$, $D2_4$, Biomass	27	39
5	Dark&bulk: $D1_5$, $D2_5$, Dcontrol, Bulk1, Bulk2	31	46
6	Denitrification: Ana, Aer1, Aer3, D, Control	41.8	

NB: biomass and bulk taken from the light pond

Example of definition of symbols: D= plate from dark pond



3.2.1 Experimental set up

Biofilm plates were selected from the continuous flow system and immersed in plastic containers of a 450ml volume. The tests were conducted with 380ml of a buffered influent solution, aeration and continuous light conditions. The specific rectangular containers were selected in order to reduce the *volume: biofilm surface area ratio*.

3.2.1.1 pH

Batch influent solution (table 3.2) was buffered by a 20-30mM sodium phosphate buffer. The base and acid were mixed in appropriate ratios via titration, while monitoring the pH with a pH meter. Millicule water was used. The dark batches and bulk solution was buffered at pH 8. The batch influent solution had the same composition as the continuous flow system (refer to table 3.4) except for NH_4-N which was equivalent to 30mg/ L and a COD equivalent of 72 mg/L.

Table 3.4 Changes made in batch influent composition

Macronutrients	**Concentration (mg/L)**
CH_3COONH_4	70.31
NH_4Cl	65.78
30mg/L NH4-N and 72mg/L COD	

Buffer Preparation

A phosphate buffer was chosen because it had a buffering capacity close to the time range of pH's required with a pKa of 7.21. This buffer was also chosen since phosphate is already found in natural systems and required for growth although it would be present in high concentrations. The acid-conjugate base combination was that of 1M monosodium phosphate monohydrate ($NaH_2PO_4.H_2O$) and 0.5M disodium phosphate dihydrate ($Na_2HPO_4.2H_2O$) respectively. (Note 0.5M solution of $Na_2HPO_4.2H_2O$ was prepared because the 1M solution was too concentrated and precipitated out of solution). The buffer worked well from pH6 to 8 but not as well at pH 9. This meant NaOH had to be added to the system to bring it up. Fig. 3.5 shows the set up during the buffer preparation.

The titration was carried out as follows:
An x5 strength batch influent solution was prepared i.e. 100ml of influent had to be diluted to 500ml to get the normal strength. The required volume of influent was measure into a beaker, acid was added while stirring with a magnetic stirrer. The base was then titrated into the beaker until the desired pH was reached. Buffer tables were used to estimate the volume of acid required, monosodium phosphate monohydrate, in order to get final buffering strength of 20-30mM. The buffered influent was transferred to a volumetric flask and diluted to the required final influent volume. Tables summarising the titrations made can be found in appendix 3.

Nitrifying bacteria grow at an optimum pH range of 7.5-8.5 refer to section 2.5.1. The effect of pH was studied for pH values: 5, 6, 7, 8 and 9. pH, temperature and DO were monitored regularly throughout the experiments. pH adjustments were made using 0.1M NaCl or HCl if the pH fluctuated by more than 0.05.

Fig. 3.5 Buffer preparations

Fig. 3.6. a) Picture of 2nd batch tests b) Picture of 3rd batch tests

3.2.1.2 Setup of denitrification

The composition of the denitrification growth media had the composition as the influent solution in table 3.2. The different was that there was no ammonia acetate, (CH_3COONH_4) and no ammonium chloride, (NH_4Cl) added. The following reagents were added to the denitrification growth media:

1. 91.07 mg/L, sodium nitrate ($NaNO_3$) equivalent to 15mg/L of NO_3-N
2. 70.31 mg/L, sodium acetate.
3. 0.01 mg/L, of nitrification inhibitor, 2-Chloro-6-(trichloromethyl) pyridine (TCMP). Caution was taken while handling TCMP as is a harmful chemical.

5 batches (0.5-1L glass bottles) were run for 41 hours summarised in table 3.3 and shown in fig.3.7b. The batches were prepared as follows:

- Ana- anaerobic batch: 6.36g wet weight of light pond biomass was placed into 200ml of the growth media. Nitrogen gas was bubbled into the bottle to remove oxygen. The DO checked then addition bubbling was done to get rid of any

residual oxygen. After this, the DO was not checked but the bottle was immediately closed to prevent reaeration.

- Aer1, Aer2- aerobic batches 1 and 2: These two batches were duplicates and were treated the same. 6.36g wet weight of light pond biomass was placed into 200ml of the growth media. Aerators were placed inside the bottles to provide oxygen.
- D- Dark biofilm batch: 3 plates from the dark pond were scrapes of biofilm. The suspended cells were also placed in 200ml of growth media under continuous aeration.
- Control-the control batch: 200ml of growth media was placed inside the bottle with continuous aeration.

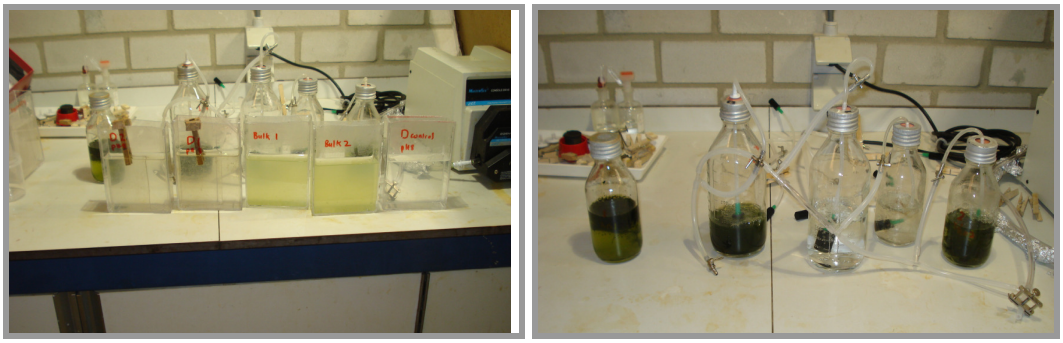


Fig. 3.7. a) Picture of 4th batch tests (bulk and dark plates.). b) Picture of 6th batch tests (denitrification)

3.3 Analytical methods

3.3.1 pH

The pH was measured using a WTW pH340i glass electrode pH meter calibrated once a week.

3.3.2 DO and temperature

The DO and temperature was measured using a WTW Oxi 340 oxygen meter calibrated once a week. The 24 and 48 hrs DO profiles readings were measured using a Hach LDO HQ10 meter.

3.3.3 Ammonia

Ammonia (NH_4-N) was determined in accordance to Standard methods (1992)/NEN 6472 using the salicylate method. Measurements were done using a UV-VIS spectrometer model Perkin Elmer, Lambda 20.

3.3.4 Nitrite

Nitrite (NO_2-N) was determined by a colorimetric method in accordance to Standard methods (1992). Measurements were also done using a UV-VIS spectrometer model Perkin Elmer, Lambda 20.

3.3.5 Nitrate

Nitrate (NO_3-N) was determined using the Ion chromatograph model Dionex ICS-1000 and autosampler series Dionex ASI-100.

3.3.6 Dry weight measurements

Calculations of dry weight measurement carried out as follows:

DW-S = Dry SFC – Clean FC

Where
DW-S -Dry weight of sample
Dry SFC -Dry sample filter cup
Clean -FC Clean filter cup

3.4 Statistical methodology

Mean values were expressed ± standard error of the mean (S.E.).

$SE = sd/\sqrt{n}$

Where
S.E. -standard error of the means
sd - standard deviation
n -sample size

Light and dark

The following statistical analysis was conducted to assess if there was a significant difference in the nitrification rates of biofilm grown under light and dark conditions:

An independent t-test was used to analyse the data at the 95% confidence interval. A one-tailed t-test was used based on our theoretical background i.e. a directional change (increase) from dark to light treatments was expected. Histograms were then constructed.

SPSS was used to check for normality by checking the skewness and kurtosis values, Kolmogorov-Smirnov and Shapiro-Wilk, histograms and normal Q-Q plots. As the dataset was not large, n=4, no transformations were made (the test is a rigorous test). Homogeneity of variances was checked using the F- test, comparing the p-value of 0.05. The t-test p-value was compared to α (0.05).

3.5 Rates

According to Aslan and Kapdan (2006), the initial substrate removal rate R_i for batch kinetics is used for calculating kinetic coefficients. The final substrate concentration used to calculate this rate is at time t, which is the time at which the substrate concentration does not change significantly. In the case of the batch tests conducted,

there was no observed point at which there was a significant reduction in the substrate removal. It was therefore assumed that the rates calculated are close to the initial removal rates. The nitrification rates of the batch tests were calculated as follows:

Correction for losses in volume

The ammonia concentrations (mg/L) were corrected for hourly changes in volume as 28% of the batch volume was lost due to sampling.

[NH_4^+-N] (mg/L) * volume(L) = NH_4^+-N (mg) (14)

Correction for ammonia volatilization

The net ammonia-nitrogen (mg) calculated above, was plotted against time and represented by a linear trend line together with the equation of the line. The gradient of the line was used to obtain the rate (mg/h).

Gradient of line: y = mx + c (15)

The total amount of ammonia removed for each batch was calculated by multiplying the gradient or rate (mg/h), by the duration, (hours), of the experiment.

Rate (mg/h) * time (hours) = total NH_4^+-N removed (g) (16)

The amount of ammonia lost in the control samples was assumed to be due to ammonia volatilisation and was used for correction of the corresponding treatments. This result gave the amount of ammonia removed due to nitrification. The nitrification rates (mg/h) were then calculated by dividing the corrected amounts by the duration (hours) of the experiments.

$$\textbf{Nitrification rate (mg/h)} = \frac{\textbf{NH}_4^+\textbf{-N - NH}_4^+\textbf{-Ncontrol (g)}}{\textbf{Time (hours)}} \quad (17)$$

The final nitrification rates were calculated as shown below:

$$\textbf{Rate (mgNH}_4\textbf{-N/gDW/h)} = \frac{\textbf{Rate (mgNH}_4\textbf{-N/h)}}{\textbf{DW-S (g)}} \quad (18)$$

$$\textbf{Rate (mgNH}_4\textbf{-N/m}^2\textbf{/h)} = \frac{\textbf{Rate (mgNH}_4\textbf{-N/h)}}{\textbf{Surface area of plate (m}^2\textbf{)}} \quad (19)$$

Where,
surface area of plate = 0.01232 m^2
DW-S = dry weight of biofilm

3.6 Mass Balance

3.6.1 Algae-based continuous flow system

In order to account for the ammonia removal in the continuous flow system, a mass balance was conducted. The system boundary includes the influent tank, pond and effluent tank. The mass balance is based of the basic equation:

Accumulation = Input – Output + Generation + Consumption (20)

The ammonia balance can be calculated as follows:

Ammonia Input

Input = ($Q^* [NH_4^+\text{-}N]_{in}$) (21)

where,
Q = flow rate
$[NH_4^+\text{-}N]_{in}$ = concentration of ammonia nitrogen in influent

The system volume can be assumed constant therefore the influent flow rate is equal to the effluent flow rate.

Ammonia Output

Output = ($Q^* [NH_4^+\text{-}N]_{out}$) (22)

where,
Q = flow rate
$[NH_4^+\text{-}N]_{in}$ = concentration of ammonia nitrogen in effluent

Ammonia production processes can be considered to be negligible

Ammonia consumption and transformation process can be attributed to nitrification volatilisation, algal uptake, denitrification and sedimentation.

The model equation Zimmo et. al (2004) developed for ammonia volatilization in pond systems is as follows:

$Y = 3.3 x + 4.90$ (23)

where,
Y is the ammonia volatilisation in $mg\text{-}Nm^{-2}d^{-1}$,
X is the calculated NH_3 ($mgNl^{-1}$) as a function of pH, water temperature and ammonium concentration in pond water (equation 3).

3.6.2 Batch reactors

The amount of ammonia volatilized in the batch reactors will be determined experimentally. These batch reactors will be the controls and will be run in the absence

of biofilm plates. The mass balance for the batch reactor can be calculated as shown below.

Nitrogen removal by biofilms shall be calculated from an equation adapted from Zimmo, (2004):

$$\mathbf{(Ni - N_f) = N_s + N_v + N_{den}} \quad (24)$$

where,

$(Ni - N_f)$ = initial total nitrogen minus the final total nitrogen content of wastewater in containers after 8 hours. ($KjN + NO_3^- + NO_2^-$)

N_s = N sedimentation which will be considered to be zero as there are not suspended cells in the wastewater.

N_v = ammonia volatilization

N_{den} = N-removal via denitrification

The equation with be further expanded as follows for a batch reactor:

$$\mathbf{V_{reactor} \times \frac{d[\ KjN]}{dt} = (R_{overall} \times V_{reactor}) + U_{vol}} \quad (25)$$

$$\mathbf{R_{overall} = (R^{biofilm} \times A^{biofilm}) / V^{reactor}} \quad (26)$$

where,

[KjN] = Kjeldahl nitrogen (g/m^3)

$R_{overall}$ = Overall nitrification rate

$R^{biofilm}$ = Nitrification rate in the biofilm ($g/m^3/d$)

$A^{biofilm}$ = biofilm area (m^2)

U_{vol} = Ammonia volatilization (g/d)

$V^{reactor}$ =Total volume of batch reactor

where

Total Kjeldahl Nitrogen (TKN/ KjN) = orgN $+NH_3 + NH_4^+$ since artificial wastewater is used, organic nitrogen is zero.

Total Nitrogen = $[KjN] + [NO_3] + [NO_2]$ ($mg\text{-}Nl^{-1}$)

4 Results

4.1 Operation of Continuous flow system

4.1.1 Pond hydraulic retention times

The mean pump flow rates for the light and dark ponds were 2.86 ± 0.21 ml/min and 2.46 ± 0.42 ml/min respectively during the development of the biofilm. The first pump installed for the light system could not achieve the target 5 days HRT. On the 10 January 2008, the first pump was replaced by a smaller pump able to obtain a lower range of flow rates. The biofilm formed was a thin algal-bacteria layer in the order of 0.5mm thick. The operation HRT are summarised in table 4.1.

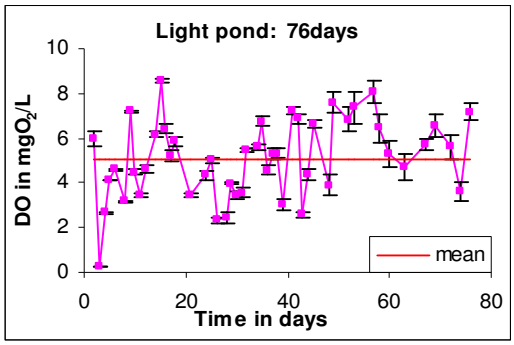
Table 4.1 Hydraulic retention times during the operation of light and dark ponds

	DATE	HRT in days		DATE	HRT in days
Light pond			**2008**		
2007					
1	05-06 Dec	1.52	22	05-07 Jan	3.67
2	06-07 Dec	3.01	23	07-08 Jan	3.67
3	07-08 Dec	3.16	24	08-09 Jan	3.59
4	08-09 Dec	3.06	25	09-10 Jan	3.20
5	09-10 Dec	3.33		**New Pump**	
6	10-11 Dec	3.13			
7	11-12 Dec	3.11	26	10-11 Jan	7.04
8	12-13 Dec	3.16	27	11-12 Jan	7.74
9	13-14 Dec	3.42	28	12-14 Jan	6.96
10	14-15 Dec	3.42	29	14-15 Jan	6.49
11	15-17 Dec	4.02	30	16-17 Jan	4.42
12	18-19 Dec	4.90	31	17-18 Jan	5.17
13	19-20 Dec	4.38	32	18-19 Jan	2.35
14	21-24 Dec	4.37	33	24-25 Jan	3.45
15	24-27 Dec	5.62	34	14-15 Feb	3.66
16	27-28 Dec	4.01	35	15-16 Feb	4.30
17	28-29 Dec	3.05	**Dark pond**		
18	29-31Dec	3.04			
19	31-02 Dec	4.44	1	11-12 Feb	3.91
2008			2	14-15 Feb	3.27
20	02-03 Jan	3.61	3	15-16 Feb	3.34
21	03-04 Jan	4.12			

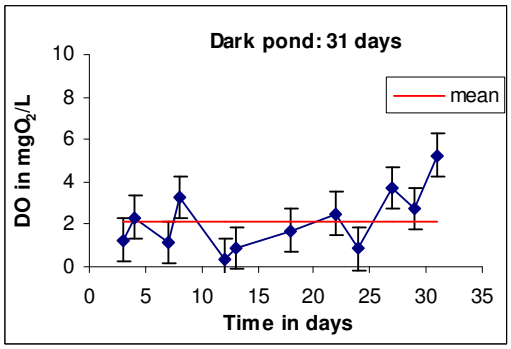
4.1.2 Physical-chemical parameters

DO, pH and temperature measurements were taken at 5-7 points along the length of the ponds as described in section 3.1.3 and fig. 3.4. The DO and temperature values were then averaged. The first day of operation for the dark pond corresponds to 46 days of operation of the light pond refer to table 3.1.

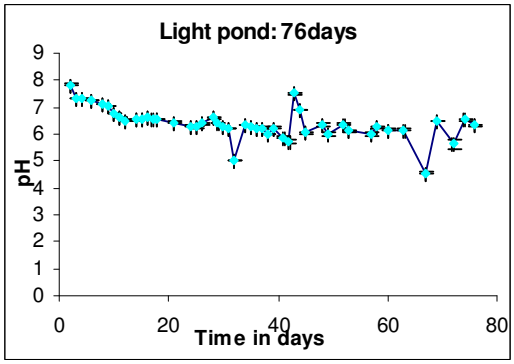
There was greater variation in the readings of DO along the length of the plugflow system in comparison to readings for pH and temperature as shown by the standard error (fig. 4.1). The ponds operated closer to completely mixed systems than to that of plug flow systems due to the recirculation pump. The presence of algae in the light pond, fig.4a), contributed to the higher DO levels recorded in contrast to the lower DO levels in the dark pond which was run in the absence of algae. The mean temperature of the light pond was also higher than in the dark pond. This was the reverse for the pH with pH ranges for the dark pond being higher than the light pond. The temperatures in the light pond were higher than for the dark due to the heat given off by the fluorescent lamp.



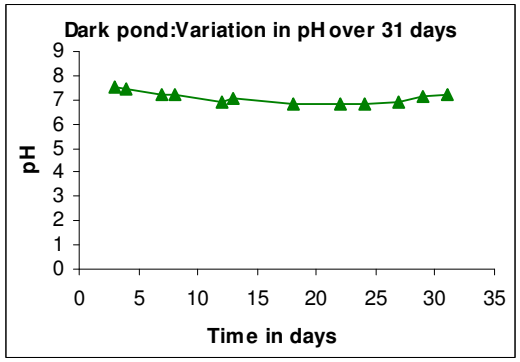
a) Mean DO: 5.08 ± 0.26 mgO_2/L



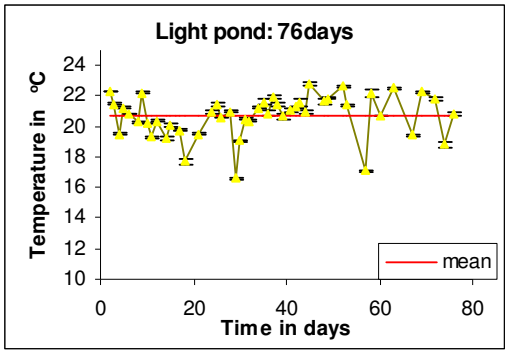
b) Mean DO: 1.43 ± 0.41 mgO_2/L



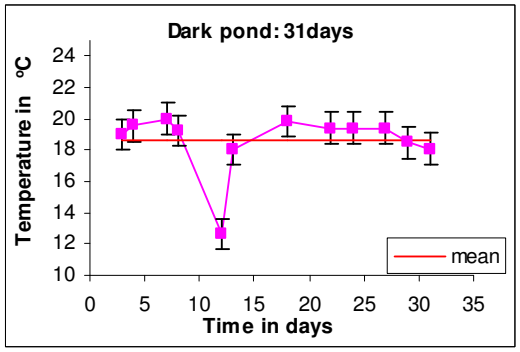
c)



d)



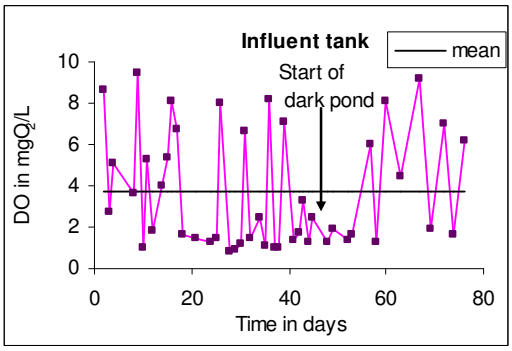
e) Mean temperature: 20.7 ± 0.2 °C



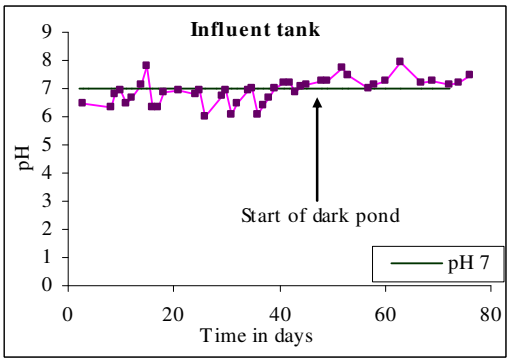
f) Mean temperature: 18.6 ± 0.6 °C

Fig. 4.1 Variation in a) & b) dissolved oxygen, c) & d) pH and e) & f) temperature during the operation of the light and dark continuous flow systems. Each bar represents the mean +/- standard error (SE=sd/√n) for a four to seven separate measurements.

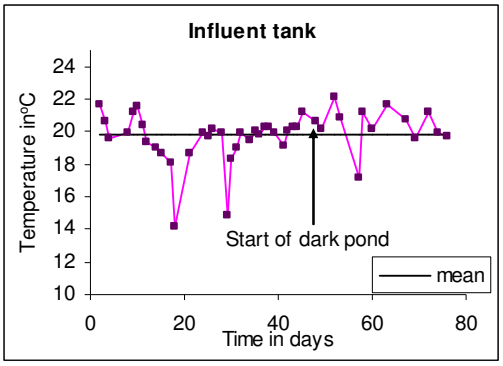
The pH of the influent solution remained within a range of 6.1-8, fig 4.2. There were large variations in the daily DO readings of the influent solution ranging between 1-9.5 mgO_2/L. The DO of the influent was affected the age of synthetic wastewater which decreased with age from an initial DO of ±8mgO_2/L, to levels below 1$mg/O_2/L$ within a period of 4-5 days. The pH and DO of the influent tank influenced the conditions of pH and DO in the dark pond fig 4.1 b) and d). This was not the case for the light pond fig. 4.1a) and c). Although there were differences in mean temperatures for the influent, dark and light ponds, temperature was primarily affected by the time of day the readings were taken refer to appendix 1.



a) Mean DO: 3.68 ± 0.41 mgO_2/L



b)



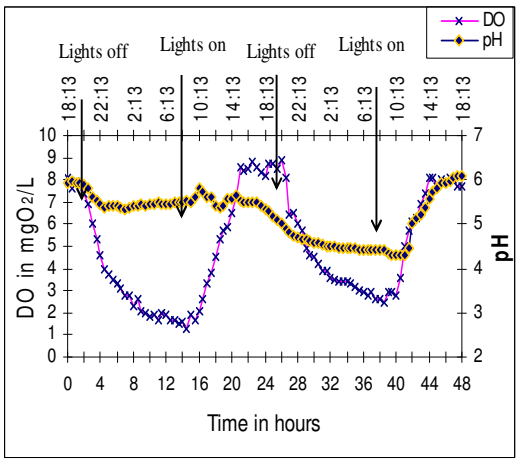
c) Mean temperature: 19.8 ± 0.2 °C

Fig. 4.2 Variation in a) dissolved oxygen, b) pH and c) temperature during the operation of influent tank

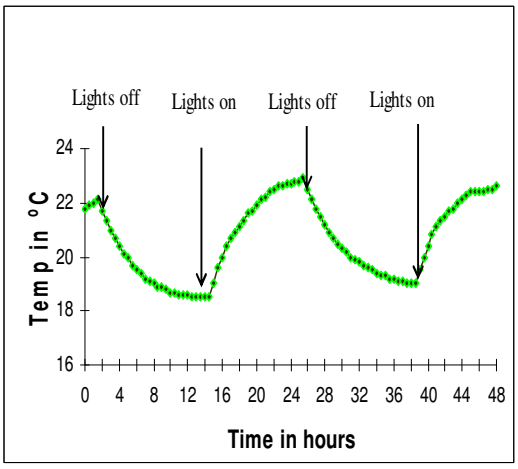
4.1.3 Profile for light pond

The DO profile shown in fig.4.3a) shows the maximum recorded DO to be 8.9 mgO_2/L, equivalent to a 97.8 % DO saturation. The pond system remained aerobic even when photosynthesis stopped at night time with the lowest value at 1.3 mgO_2/L. The diurnal variation in the pH, fig. 4.3a) was not as pronounced in the first 24 hrs, pH ranging from 5.35-5.94 in contrast, to pH ranges of 4.31-6.07 in the last 24 hrs. The lower pH values occurred during the night when photosynthesis had stopped.

The temperature profile in fig. 4.3b) shows that the peak temperatures occurred during the day at between 22.1-22.9 °C. The lowest temperatures recorded occurred during the night and ranged from 18.5-19 °C. At 19:51 the lights went off, marking the start of 12hrs of night and came back on at 7:51 marking the beginning of 12 hrs of daylight.



a) DO and pH profile



b) Temperature

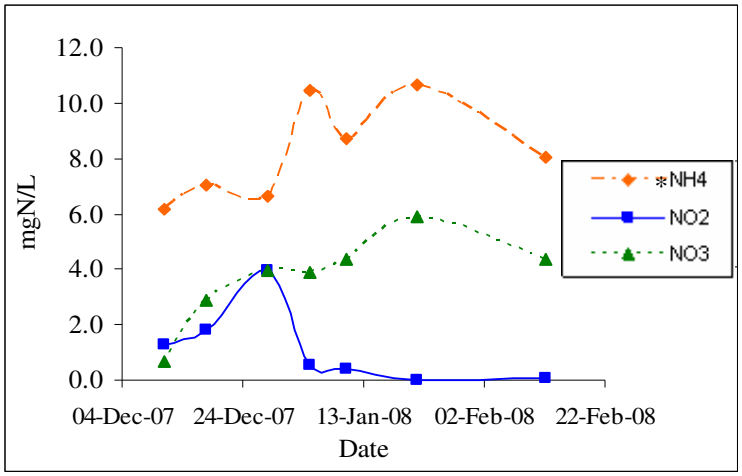
Fig. 4.3. The DO, pH and temperature profile of the light pond showing the diurnal variation over 48 hrs

4.1.4 Ammonia removal in ponds/continuous flow system

Light pond

Fig 4.4 illustrates the progression of ammonia removal and nitrite/nitrate levels over a period of 61 days in the light pond. The concentration of ammonia removed increased from 6 to 11mgNH_4-N/L over the duration of the observations. Ammonia removal efficiencies are summarised in table 4.2. The net nitrate levels measured increased from 0.7 to 5.9 mgNO_3-N/L. There was not much change in the nitrate concentration after 31 days of monitoring

Nitrite accumulation occurred over the first 17 days of monitoring with a maximum of 1.8 mgNO_2-N/L. After this there was a gradual decline in the nitrite levels. These nitrogen results were used to give an indication of the presence and development of the nitrifying bacteria.



*NH_4 is the decrease in ammonia (Δ NH_4)

Fig 4.4 Development of ammonia removal over time in light pond. Note that *NH_4 is the decrease in ammonia (Δ NH_4)

Table 4.2 gives the overall summary of the nitrogen removal efficiency for fig. 4.4. It can be seen that the efficiencies almost doubled over the 61 days from 15 to 29%.

Table 4.2. Light pond: Nitrogen removal efficiencies

Date	Influent NH_4^+ [mgNH_4-N/L]	ΔNH_4^+ [mgNH_4-N/L]	% removal
11-Dec-07	39.97	6.19	15.48
18-Dec-07	38.45	7.02	18.25
28-Dec-07	39.43	6.63	16.81
04-Jan-08	47.27	10.45	22.11
10-Jan-08	41.81	8.70	20.82
22-Jan-08	36.84	10.63	28.87
12-Feb-08	30.18	8.05	26.68

Increasing N-removal efficiency ↓

Dark pond

After 24 days of running the dark continuous flow system, shown in fig. 4.5., ammonia removal and nitrite/nitrate levels were monitored over 9.5 hours. The light pond fig.4.4 had higher levels of ammonia removal than the dark pond which had a mean efficiency of 11% and a range of 9.5-12% over the 9.5 hours. The maximum nitrate was 3 mgNO_3-N/L with no nitrite accumulation.

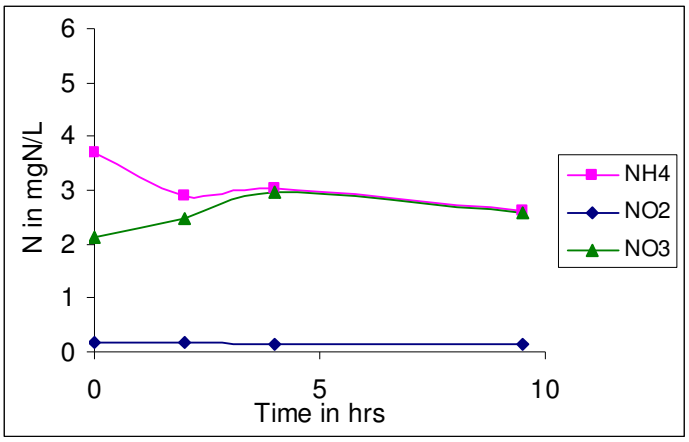


Fig 4.5 Nitrogen profile for the dark pond run over 9.5hrs after 24 days of operation

4.2 Batch Reactions

Effect of pH on biofilm nitrification rates

Three batch experiments 1-3 were conducted to study the effect of pH on biofilm nitrification rates expressed in terms of ammonia-nitrogen depletion. Concentrations of nitrate and nitrite were also measured. The pH tests were only conducted using plates from the light pond i.e. the algae-based pond. A summary of all nitrification rates for batch 1-3 are shown in table 4.7. The batch reactors were buffered and carried under continuous illumination and aeration as described in section 3.2 and summarised in table 3.3.

It should be noted that after batch 2, biofilm sloughing occurred and was not completely covering the baffles see fig. 5.2. The mean dry weight for batch 2 experiments was 0.057 ± 0.002 g and that of batch 3, 0.036 ± 0.003 g. This represented a 37% mean loss of dry weight caused by the sloughing of the algae-bacterial biofilm.

4.2.1 Trial Batch 1

No clear trends in the ammonia removal could be seen in the 7 hours of running the experiment, see fig. 4.6. Result for nitrate and nitrite can be found in appendix 8.

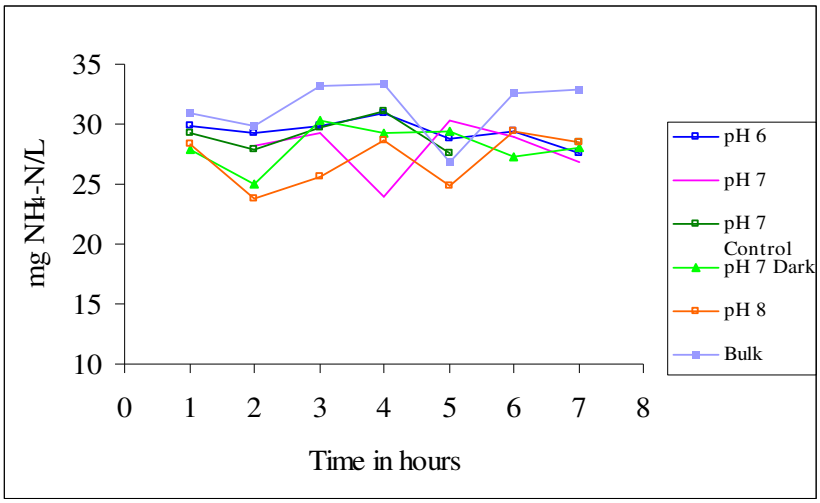


Fig. 4.6 Effect of different treatments on nitrogen removal rates

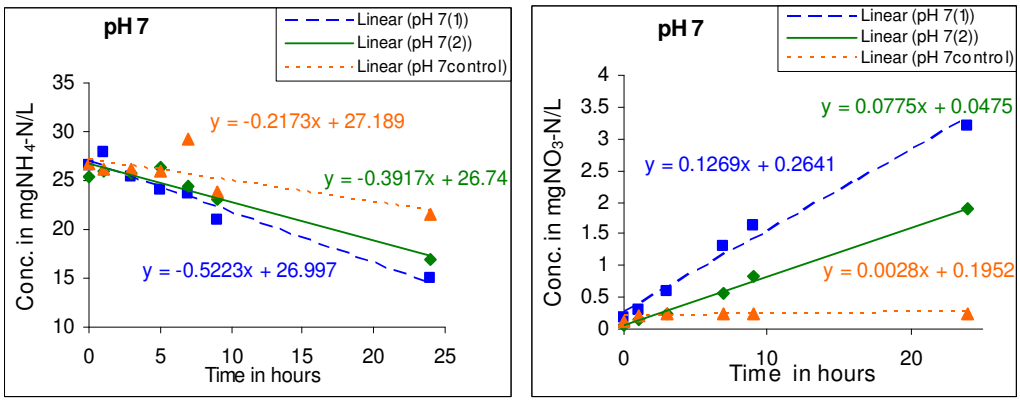
4.2.2 Batch 2

A second round of pH batch experiments were conducted in duplicate for pH 7 and 8. The experiments lasted 24 hrs. The results are showing the decrease in ammonia and increase in nitrate concentrations are shown in fig. 4.7 below.

The maximum rate of nitrification occurred at pH 7, x1.7 times faster than the rate at pH 8. The mean rate of nitrification expressed in terms of ammonia depletion was 1.28 and 0.76 mgNH_4-N/gDW/h for pH 7 and 8 respectively.

Similar results were found for rates expressed in terms of net nitrate production whereby the mean rate at pH 7 also x1.7 times faster than pH 8: 608.9 ±170.9 and 357.6 ±73.0 µgNO_3-N/gDW/h respectively.

As expected the nitrogen volatilisation rate as indicated by the controls was high for pH 8 than for pH 7.



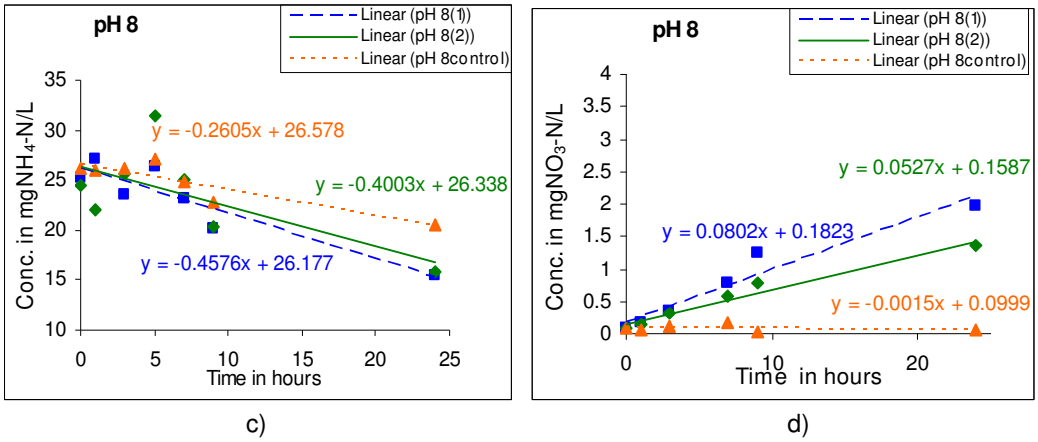


Fig. 4.7 Batch Experiment 2: Results for the effect of pH on the nitrification rates for pH a) & b) 7 and c) & d) 8.

Fig. 4.8 shows the nitrite concentrations were higher for pH 8 than for pH 7 with maximum recorded values of 1.27 and 0.75 mg/L. There was no nitrite production in the controls.

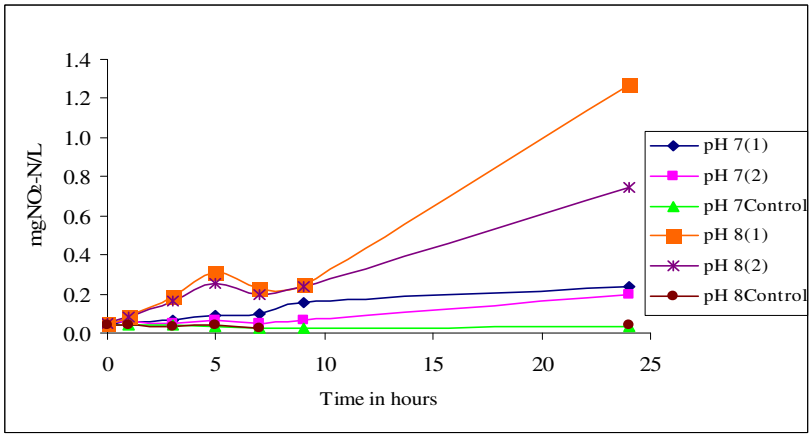


Fig 4.8 Batch Experiment 2: Nitrite concentration

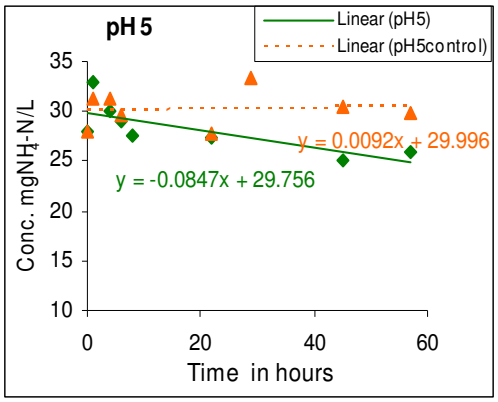
Table 4.3 is an example of the physical-chemical conditions during the running of the batch 2 experiment. At the beginning the experiment temperatures had not yet reached reaction temperatures of ± 23 °C and averaged 13 °C. DO was above the limiting concentrations with values above 8 mgO_2/L. The pH was monitored and adjustments were made as described in section 3.2.

Table 4.3. DO, temperature pH checks and adjustments

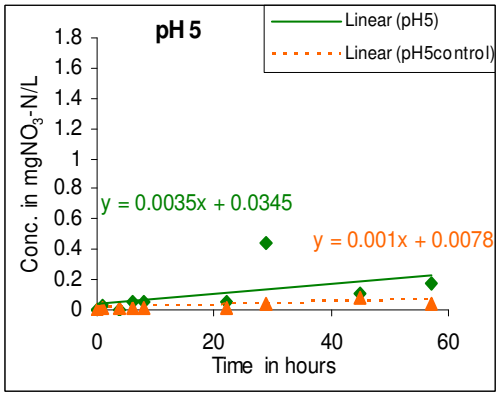
	0 hrs			**5 hrs**			**24 hrs**		
All batches were aerated	pH	DO mg/L	Temp °C	pH	DO mg/L	Temp °C	pH	DO mg/L	Temp °C
pH 7 (1)	7.14→7.01	11.1	13.6	6.92→7.01	8.68	23.3	7.02	8.35	22.8
pH 7 (2)	7.09→7.09	10.8	14.7	6.96→7.03	8.42	23.8	7.05	8.46	23.4
pH 7 Control	7.07→7.01	11.3	12.8	6.95→7.01	8.49	23.6	7.09	8.3	22.9
pH 8(1)	8.04→8.02	11.1	13	7.92→8.01	8.44	23.9	7.97	8.63	23.4
pH 8(2)	8.03→8.02	11.2	12.9	7.88→8.02	8.72	24.1	8.01	8.58	23.9
pH 8 Control	8.03→8.00	11.1	13.1	7.88→8.02	8.75	24.3	8.02	8.4	23.8

4.2.3 Batch 3

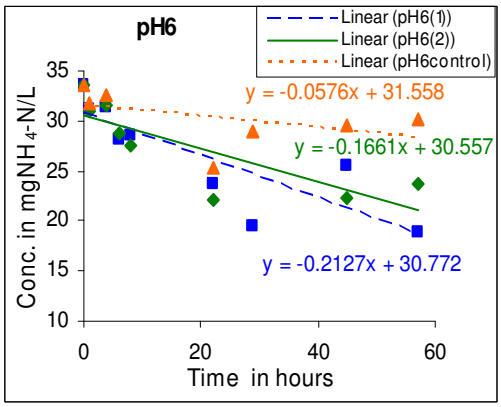
The results of batch 3 experiments confirm the results found in batch 2 where the maximum rate of nitrification also occurred at pH 7. The variations in ammonia nitrogen and nitrate nitrogen concentrations over are shown in fig. 4.9 and nitrite nitrogen in fig. 4.10 and were run for 57 hours.



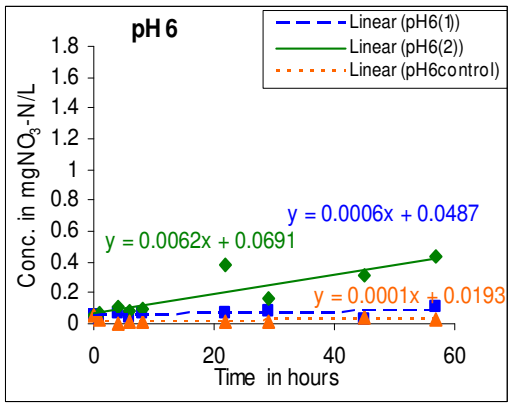
a)



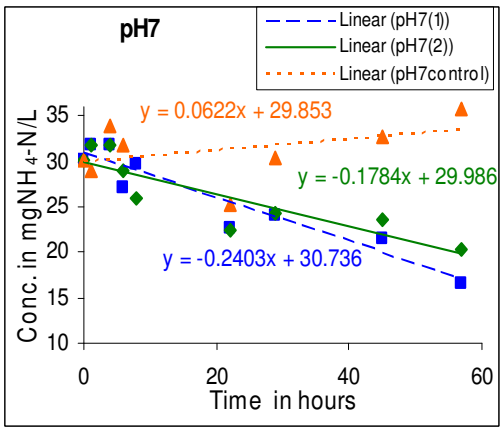
b)



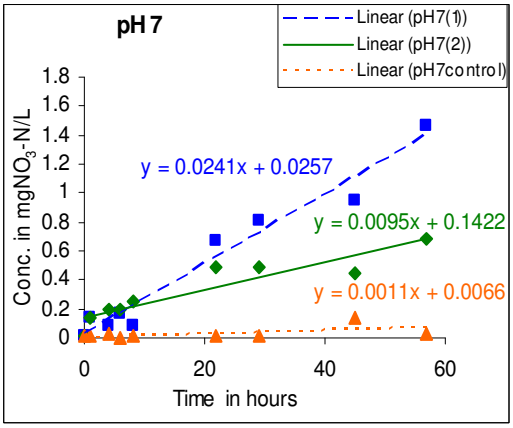
c)



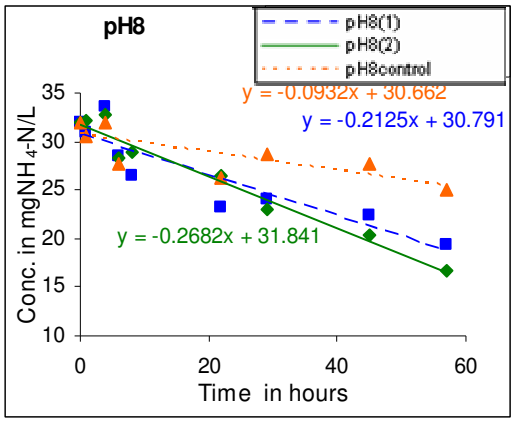
d)



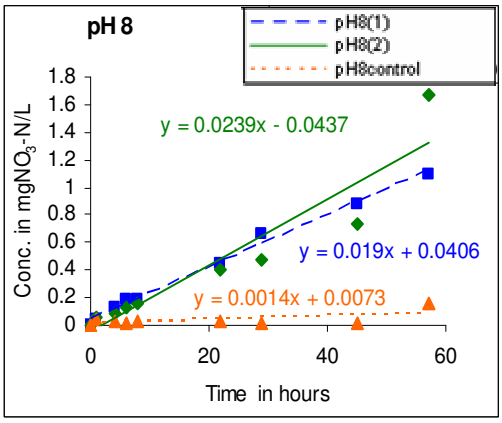
e)



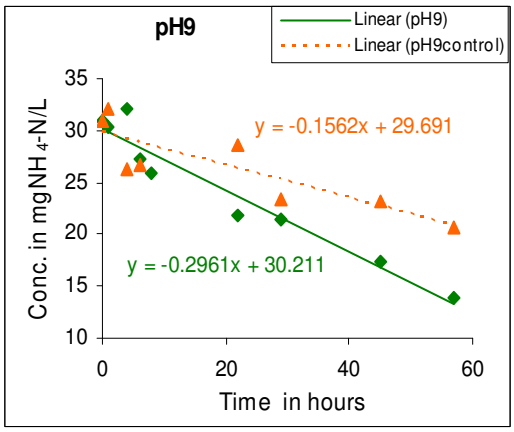
f)



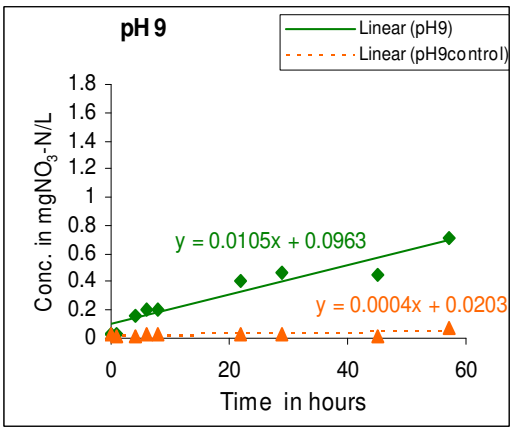
g)



h)



i)



j)

Fig. 4.9 Batch Experiment 3: Variation of ammonia nitrogen and nitrate-nitrogen over 57 hours for pH 5 a) & b); pH 6 c) & d); pH 7 e) & f); pH 8 g) & h); pH 9 i) & j).

Some nitrite accumulation was evident for pH 9 and to lesser extent for pH 8.

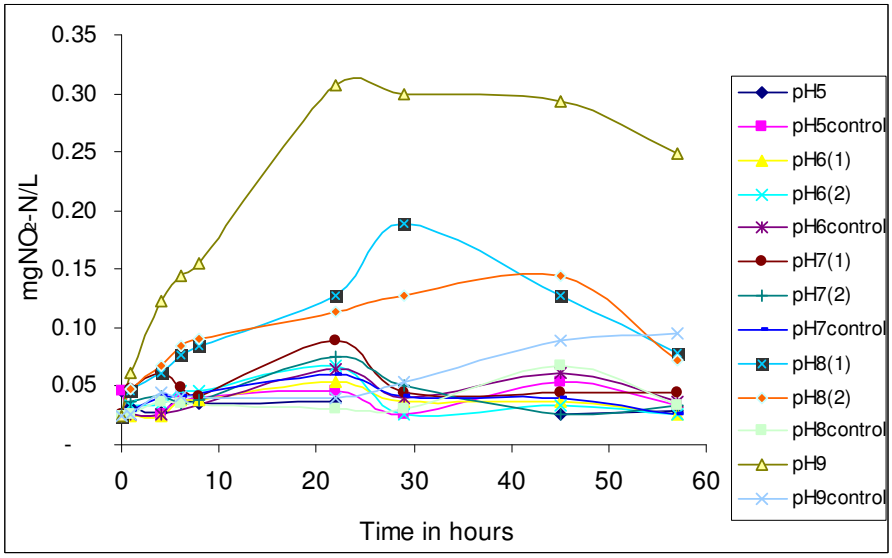


Fig. 4.9 Batch Experiment 3: Variation of nitrite-nitrogen concentration with time

The results of batch 3 experiments given in fig. 4.9 are summarised in fig. 4.10 below. The mean nitrification rates, ($mgNH_4$-$N/m^2/h$), corrected for changes in volume and ammonia volatilisation were calculated as described in section 3.5. The variation of nitrification rates with pH expressed per unit dry weight were comparable with those expressed in terms of the surface area. The highest nitrification rate was recorded at pH 7 and the lowest pH at pH 5.

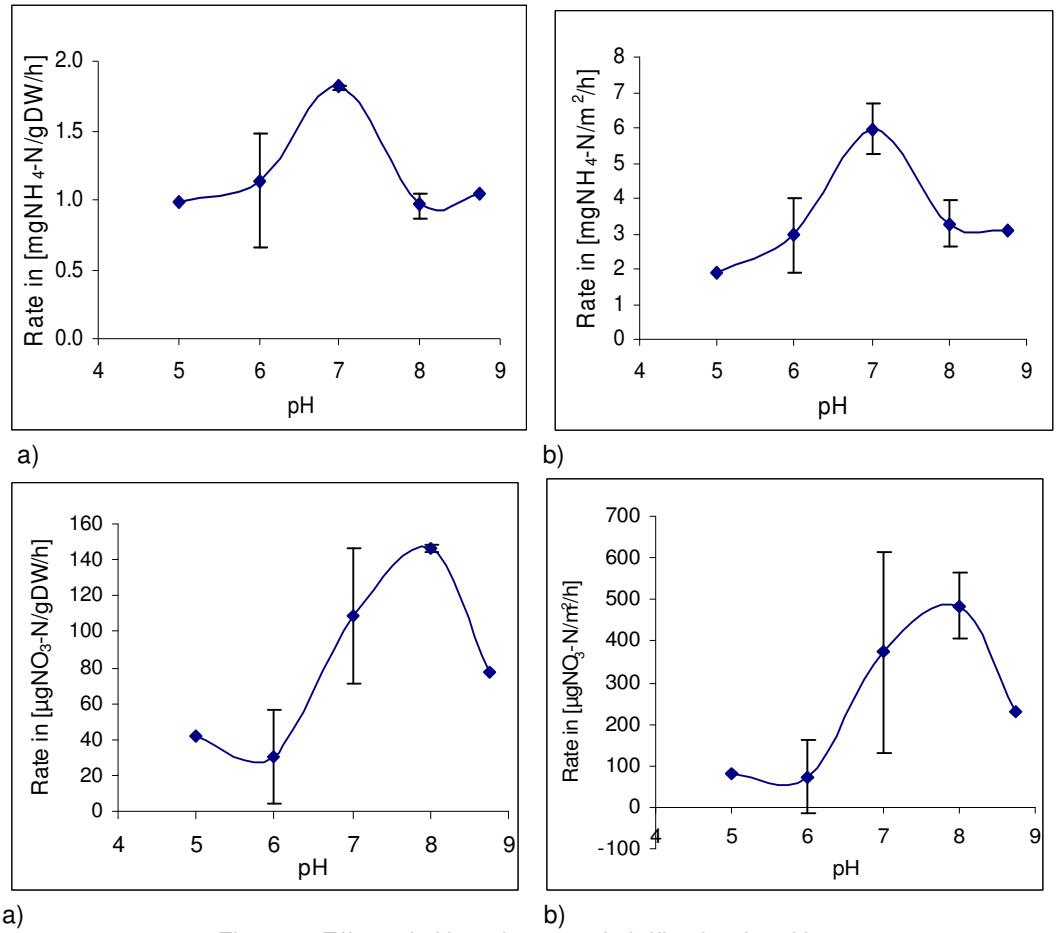
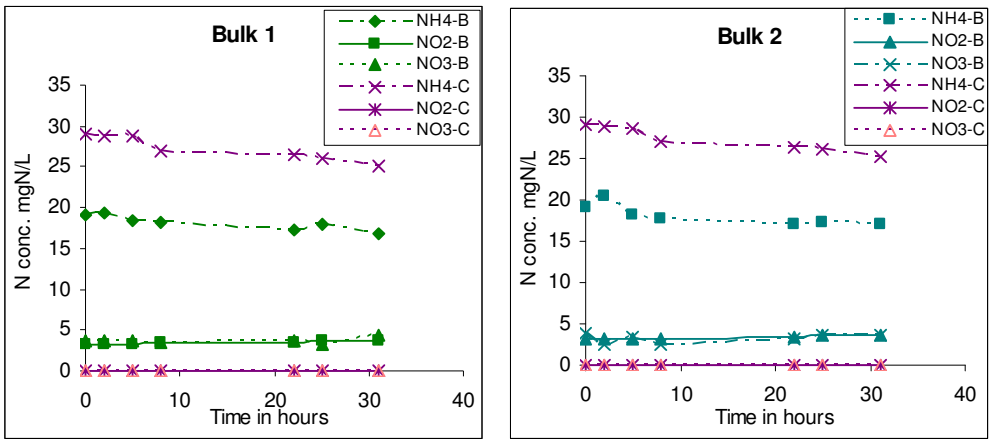


Fig. 4.10 Effect of pH on the rate of nitrification for pH 5-9

4.2.4. Nitrification in Bulk liquid

Batch 5 experiments were carried out to study the rate of bulk nitrification at pH 8. The ammonia removal in the bulk was less than the rate in the pH 8 control. The bulk nitrification rates were -1.09 and -0.81 mgNH_4-N/gDW/h for bulk 1 and 2 respectively. Thus it can be concluded that there was no nitrification occurring in the bulk. This is further substantiated by the constant levels of nitrate and nitrite over the 31 hours in the bulk.



a) b)

Fig. 4.11 Variation in NH_4^+, NO_2^- and NO_3^- concentration over 31 hrs for a) bulk1 and b) bulk2. C is the control at pH 8.

4.2.5. Ammonia volatilisation

There was an increase in the rate of ammonia volatilisation with pH with the exception of pH 7 for batch 3, see table 4.4. This is in agreement with equation 3 there the concentration of unionised ammonia (NH_3) is expected to increase with increase in pH, increasing the rate of ammonia volatilizated.

Table 4.4 Rate of ammonia volatilization in mgNH_4-N/h

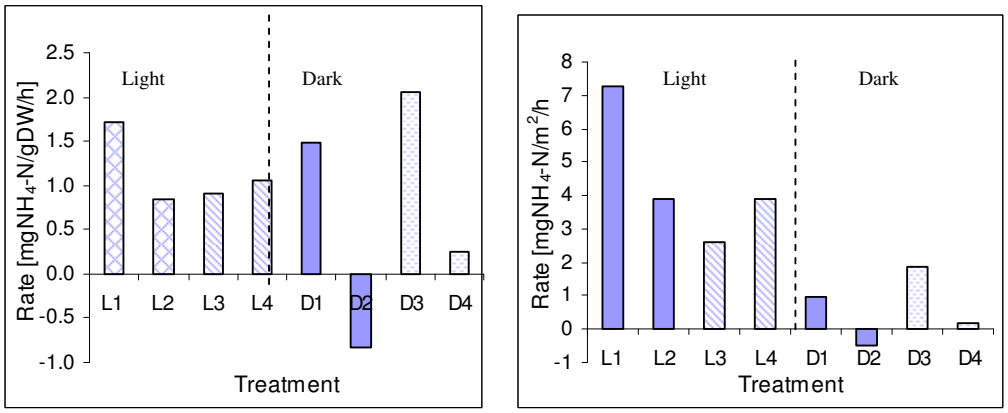
Experiment	pH 5	pH 6	pH 7	pH 8	pH 9
Batch 2			0.1548	0.1664	
Batch 3	0.0526	0.0785	0.0399	0.0834	0.0989
Batch 5				0.1115	

4.2.6 Effect of light of biofilm nitrification rates

A comparison of nitrification rates for light pond biofilm and dark pond biofilm at pH 8 is shown in fig. 4.12. All light and dark reactors where carried out at pH 8, under continuous light and aeration. The rate of nitrification for D2 can be considered to be zero as the ammonia removal lower than the rate of ammonia volatilisation in the pH 8 control resulting in a negative nitrification rate.

The nitrification rates, (mgNH_4-N/m^2/h), for biofilm grown under light conditions were significantly higher than the rates for biofilm grown under dark conditions ($p < 0.05$).

This was not the case for nitrification rates expressed in terms of mgNH_4-N/gDW/h where no significant difference was found between light and dark biofilm (p= 0.598). The mean dry weight for light plate biofilms was found to be higher than dark plate biofilms by a factor of 6.2. The mean dry weights were 0.0499 ± 0.0057and 0.0088 ± 0.0009 g respectively.



a) b)

Fig. 4.12. Effect of light on the rate of nitrification (ammonia) a) expressed in terms of dry weight b) expressed in terms of surface area. L-light and D-dark

It should be noted from table 4.5, the date and source of each of the batch experiments was carried out.

Table 4.5. Date of Batch Experiment

Date	**7 Feb**		**20 Feb**		**26 Feb***		**4 Mar**	
	Light				*Dark*			
Batch	2		3		4		5	
	L1	L2	L3	L4	D1	D2	D3	D4
Replicate	pH 8(1_2)	pH 8(2_2)	pH 8(1_3)	pH 8(2_3)	$D1_4$	$D2_4$	$D1_5$	$D2_5$

* Values correct for volatilisation with the mean volatilisation rate of controls at pH 8 as no control was run with this batch

It is important to also follow the net nitrate accumulation to correlate with the nitrification rates. The results in fig. 4.13 below show a higher rate of nitrate accumulation in the light back reactors in comparison to the dark batch reactors which is in agreement with he higher light reactor nitrification rates shown in fig. 4.12. A summary of the mean rates is given in table 4.6.

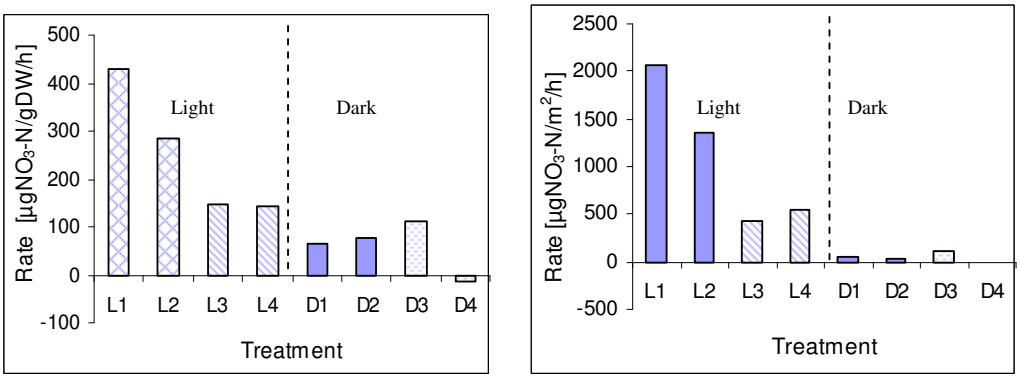


Fig. 4.13 Effect of light on the rate of nitrate accumulation a) expressed in terms of dry weight b) expressed in terms of surface area.

Table 4.6 summarises the mean nitrification and net nitrate accumulation rates shown in figures 4.12 and 4.13. In all cases the rate of nitrification and nitrate accumulation in the light reactors were almost all more than double the rates found in the dark ponds.

Table 4.6. Summary of nitrification and net nitrate accumulation rates for light and dark reactions at pH 8

	Treatment	**Mean rate**
R in mgNH_4-N/gDW/h	Light	1.13 ± 0.20
	Dark	0.73 ± 0.65
R in mgNH_4-N/m^2/h	Light	4.42 ± 0.99
	Dark	0.65 ± 0.51
R in µgNO_3-N/gDW/h	Light	252 ± 68
	Dark	59 ± 27
R in µgNO_3-N/m^2/h	Light	1100 ± 385
	Dark	44 ± 23

4.2.7 Summary on nitrification rates

Table 4.7 summarises the nitrification rates of the batch experiments discussed in the previous sections. Biofilm sloughing occurred as mentioned at the beginning of section 4.2 . This is also indicated in the table.

Table 4.7. Overall Nitrification rate for batch 2-5 experiments

Batch	Treatment	Rate in $mgNH_4$-N/gDW/h	Rate in $mgNH_4$-N/m^2/h
2	pH 7(1_2)	1.72	7.26
	pH 7(2_2)	0.84	3.90
	pH 8(1_2)	0.94	4.50
	pH 8(2_2)	0.58	2.78
After biofilm sloughing			
3	pH 5	0.99	1.91
	pH 6(1)	1.47	4.01
	pH 6(2)	0.80	1.89
	pH 7(1_3)	1.80	6.67
	pH 7(2_3)	1.83	5.25
	pH 8(1_3)	0.90	2.61
	pH 8(2_3)	1.05	3.92
	pH 9	1.05	3.11
4	$D1_4$	1.48	0.99
	$D2_4$	-0.84	-0.47
	Biomass	0.01	
5	$D1_5$	2.05	1.87
	$D2_5$	0.26	0.19
	Bulk1	-1.09	
	Bulk2	-0.82	

4.2.8 Denitrification batch tests

Preliminary denitrification tests were conducted to try and detect if any nitrification was occurring. It can be seen for table 4.8 that under aerobic conditions, some denitrification occurs. Biomass (biomass 1+2) was scraped from the surface of the light pond i.e. light continuous flow system. It is therefore difficult to quantify how much denitrification was occurring per surface area of the baffles. The results however show that there was no denitrification occurring in the dark pond baffles as the dark reactor had biofilm scraped off from 4 dark pond baffles.

Table 4.8. Denitrification rates

	DW-S in g	$mgNO_3$-N/h	$mgNO_3$-N/gDW/h	$mgNO_3$-N/m^2/h
Anoxic	0.35	19.1	54.5	
Biomass1	0.35	7.9	22.7	
Biomass2	0.35	2.4	6.8	
Dark		-14.6		-395.5
Control		25.2		

4.2.9 Mass Balance

A simplified mass balance was applied to estimate the main nitrogen transformation processes for the light and dark ponds. For the light pond, nitrogen removals for 28 Dec 2007-22 January 2008 were averaged refer to fig.4.4 and table 4.2.The mass balance for the light pond in given in fig.4.14 below. The nitrogen removal for the 3 days under consideration was 24%. Nitrification was the major process for nitrogen removal and transformation accounting for 13% of the ammonia removed. Ammonia volatilisation was estimated from equations 3-6 using a mean temperature of 20 ^{o}C and a pH of 6.5. The rate of ammonia volatilisation was found to be 0.5mgN/d (2.58 mgN/m^2/d) accounting for 0.3% of the ammonia removed. The remaining processes for ammonia removal are denitrification which is difficult to quantify with the preliminary results in section 4.2.9; algal and bacterial uptake (Org-N) and sedimentation.

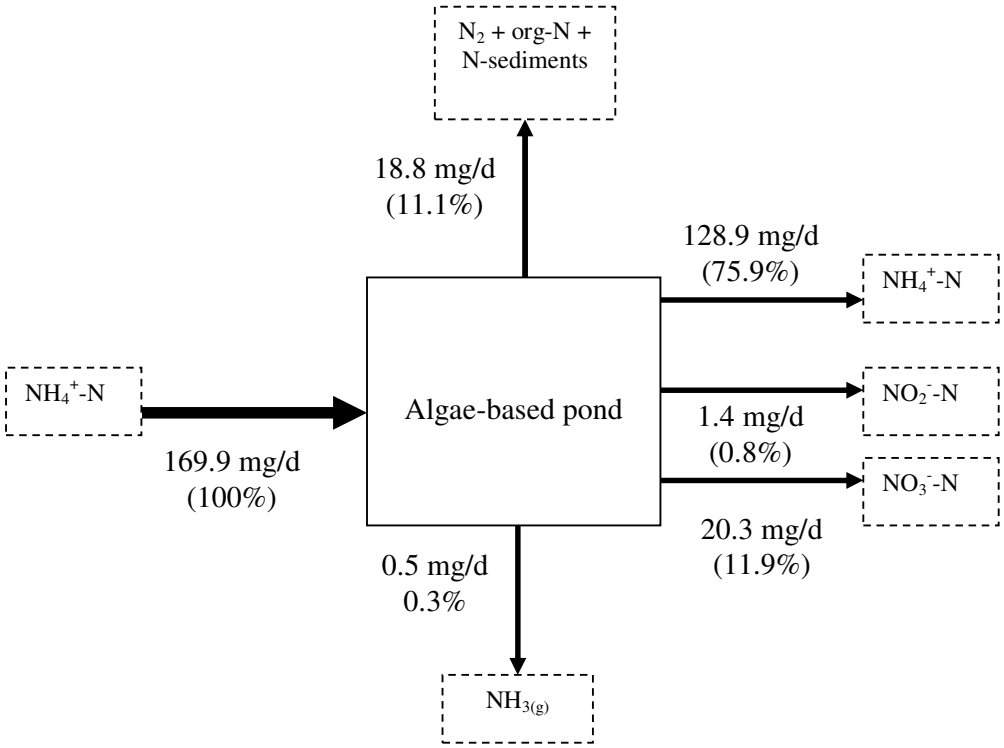


Fig. 4.14 Ammonia mass balance for light pond.

The mass balance of the dark is shown in fig 4.15. The mass balanced was calculated based on mean values of the 9.5 hours measurements of ammonia, nitrite and nitrate carried out in fig. 4.5. The nitrogen removal rate is about 11% as was found in section 4.1.4. Ammonia volatilisation was also calculated using equation 3-6 at a mean temperature of 19.4 oC, pH of 6.8. Nitrogen removal due to volatilisation was found to be 0.73mg/d (3.73 mgN/m^2/d), which is equivalent to 0.6%. This was higher than that calculated for the light pond as the pH was higher in the dark pond. Nitrification was found to be the main ammonia removal mechanism. As no denitrification was detected for dark pond plates it is likely that denitrification is negligible and therefore does not to nitrogen removal. This conclusion can be disputed as denitrification experiments were

carried out under aerated conditions which is different from the dark pond DO levels in the fig. 4.1a, averaging 1.43 mgO_2/L.

Algal uptake is a significant mechanism for ammonia removal as is evidenced by the Assuming that denitrification is the same,
N-sediments.

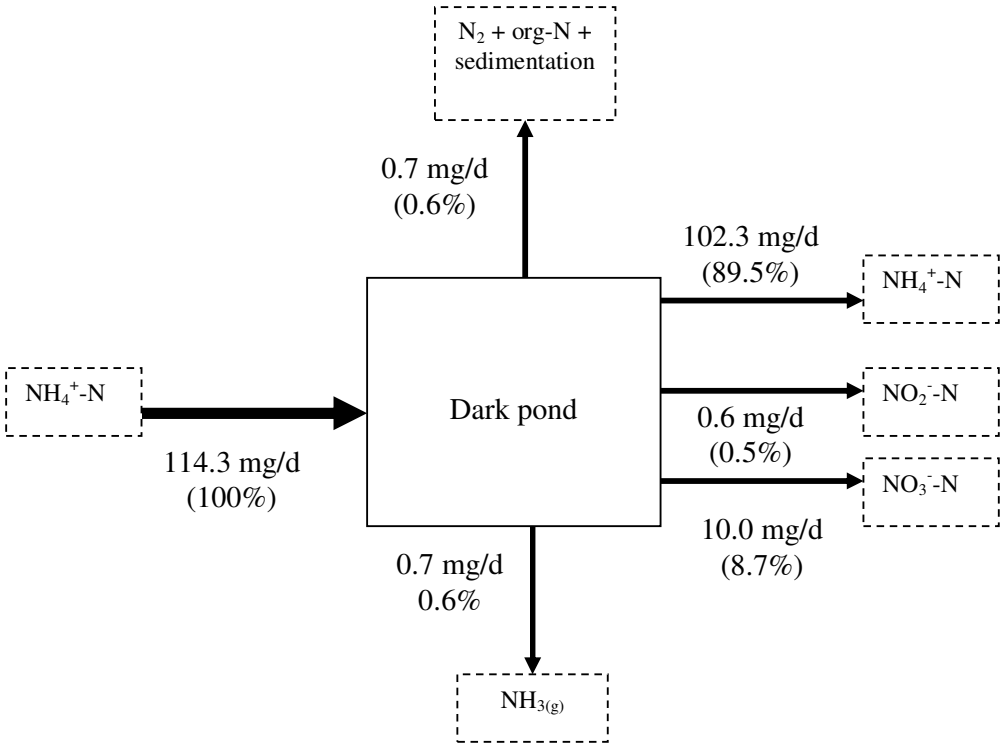
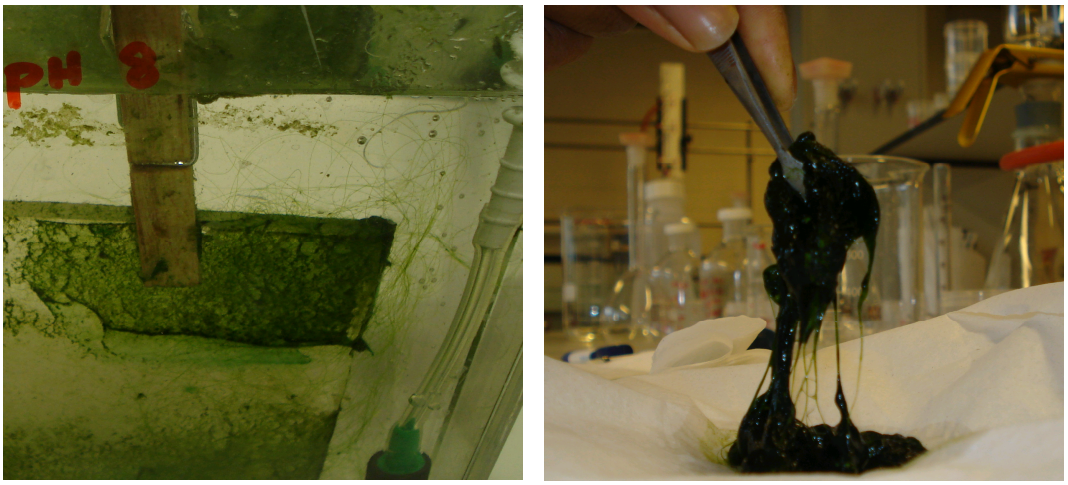


Fig. 4.15 Ammonia mass balance for dark pond.

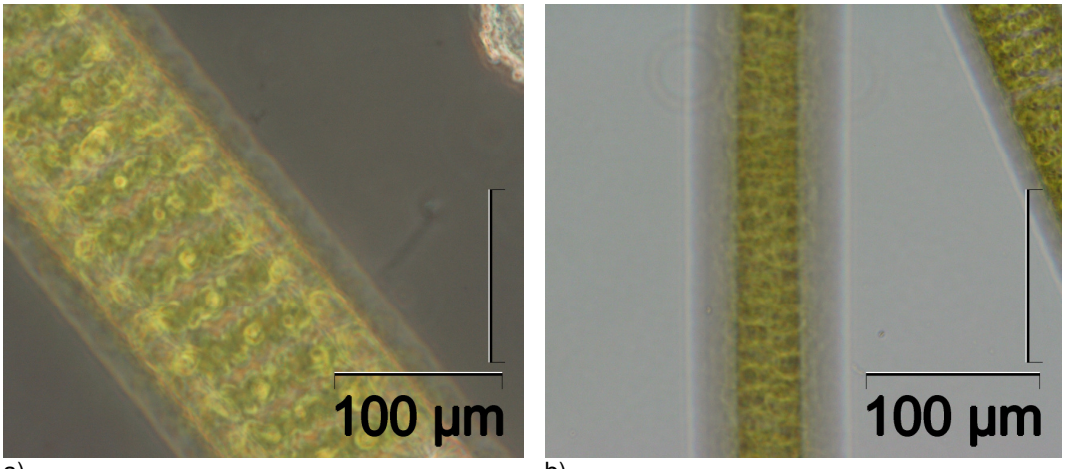
4.2.11 Filamentous algae in light pond

Various planktonic or microalgae species that include *Anaebena cylindrica, Anaebena variabilis, Spirulina platensis, Scenedesmus quadricauda and Chlorococcus* were inoculated into the light pond but as the pond aged, it was observed that a filamentous stringy alga began to dominate the pond. Later on a green scum formed at the surface on the pond. Figure 4.16 and 4.17 show photographs taken of the green algae with was identified to be from the genus *Spirogyra* due to its stringy and silky appearance fig. 4.16. Under the light microscope the algal cells were identified to be unbranched and filamentous fig. 4.17. The spiral arrangement of the chloroplast was not clearly visible in the image from the microscope.



a) b)

Fig. 4.16 Photographs of *Spirogyra* a) Stringy algae suspended in water. b) Silky appearance of stringy algae



a) b)

Fig. 4.17 Light microscope photographs of *Spirogyra* a) X400 and b) X100.

5 Discussion

Batch experiments

The overall objective of the study was to better understand the environmental factors affecting algae-bacteria biofilm nitrification rates in facultative waste stabilisation ponds using laboratory scale batch experiments. The study focused on the effect of pH and algae.

Effect of pH

The maximum rate of nitrification for a pH range of 5-9 occurred at pH 7. This finding is different from literature values for studies done with pure cultures of suspended cells by Blackburne *et al.,(*2007) and Grunditz and Dalhammar (2001). Blackburne *et al.*, (2007) reported optimum pH ranges for *Nitrospira* being in the range of 8.0-8.3. Grunditz and Dalhammar (2001) determined the optimum pH for pure cultures of *Nitrosomonas* to be 8.1 and *Nitrobacter,* 7.9 as shown in fig. 5.1. Biofilm systems are affected more by spatial gradients than suspended cells. Studies have shown biofilm pH profiles that differ from the pH of the bulk liquid. It is not likely that the biofilm and suspended cells have different optimum pH values, although further research in enzymatic proteins could be carried out to ascertain this. The physical process of diffusion could be the possible mechanism. The boundary layer described in section 2.6 acts as an interface between the biofilm and bulk liquid. The boundary layer has a different concentration to that in the bulk liquid. Substrate diffusion limitations may be responsible for a possible difference in pH between the biofilm and bulk liquid. As one moves deeper into the biofilm layer, the dissolved oxygen diminishes and the anoxic layer is formed where possible reduction of nitrate and nitrite may occur resulting in an anoxic interior layer (Wiesmann *et al.*, 2007).

Algae-bacteria biofilm differs from bacterial biofilm because of the additional source of oxygen from algal photosynthesis. The aerobic layer of the biofilm contains algae but as one moves deeper into the biofilm carbon dioxide becomes limiting and light to some extent although in the case of the lab-scale experiments, the biofilm plates were transparent and the biofilm was not very dense. It is possible that the presence of algae increases the net pH of the biofilm in comparison to the bulk liquid. The presence of the algae could serve to buffer the biofilm system from losses in alkalinity caused by the uptake of bicarbonate ions by nitrifying organisms. Another contributing factor to a higher pH in the biofilm microenvironment could be the activity of denitrifiers which are found in the deeper anoxic/anaerobic layers of the biofilm. During denitrification, the alkalinity increases, causing a subsequently increase in pH. Substrate limitation to denitrification may occur due to consumption of acetate by heterotrophs at the surface of the biofilm. This explicitly implies e.g. that pH 7 and 8 inside the bulk could be measuring closer to pH 8 and 9 respectively in the biofilm.

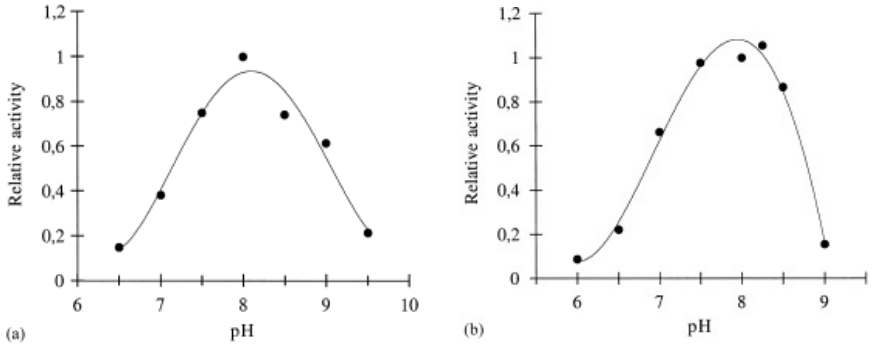


Fig. 5.1. a) Effect of pH on the activity of *Nitrosomonas*. The activity at pH 8.0 was used as the reference activity. b) Effect of pH on the activity of *Nitrobacter*. The activity at pH 8.0 was used as the reference activity. (Source: Grunditz and Dalhammar, 2001).

Nitrite accumulation was higher at pH 8 and 9 fig.4.8 and 4.9. This is likely to be caused by an increase in unionised ammonia (NH_3) at higher pH values. NH_3 has an inhibitory effect on *Nitrobacter* reducing the nitrite oxidation (Characklis and Marshall, 1990).

The rates were expressed both in terms of dry weight and surface area to counter the effects of differences in biofilm densities and heterogeneity in bacterial-algal species respectively. Both methods showed gave similar trends in the effect of pH on nitrification. The nitrification rates observed in the present study were lower than those observed by Babu, (2007) as shown in table 5.1. Only the mean for duplicates with the maximum nitrification rates are shown in the table. A possible explanation for the lower nitrification rate was the low pH i.e. below pH 7, observed in the light pond and a possibly low alkalinity limiting the rate of biofilm growth.

Table 5.1 Summary of nitrification results

Experiment	**Nitrification rate $mgNH_4$-N/m^2/d**	**Source**	
pH 7_2	133.93	Batch 2	
pH 8_2	87.47	Batch 2	*Refer to table 4.7
pH 7_3	144.90	Batch 3	
pH 8_3	79.29	Batch 3	
R biofilm (7 days)	1495	(Babu, 2007)	
R biofilm (7 hrs)	2125	(Babu, 2007)	
R biofilm (glass plates)	1652	(Babu, 2007)	
Fixed-film reactors	1000 at 20 ^oC	(Antonie 1976 as cited by Gray, 2004)	

*Refer to table 4.7 for identification batch 2 and 3 individual values of selected duplicates

Significance of adding a biofilm attachment surface

There was no nitrification detected in the bulk batch reactors. The ammonia removal being less than that recorded for control samples fig. 4.12. The concentrations of nitrite nitrogen and nitrate nitrogen remained constant which further supports that there were no nitrifiers present. Studies conducted by Babu (2007) and McLean, (2000) both confirmed that bulk nitrification rates were lower than for algae-bacterial biofilms. The lack of nitrifiers can be attributed to washout due to pump adjustments operation and maintenance of the system.

Performance of Continuous flow system

After 9 days of operation, the pH values for the light pond were found to be lower than expected ranging between 6-6.8. During the day carbon dioxide consumption via algal photosynthesis is expected to raise the pH to alkaline values. The low pH's could be explained in part by the consumption of bicarbonate (HCO_3^-) by nitrifying bacteria resulting in the loss of alkalinity and therefore the reduction of pH. According to Celenza (2000), an estimated 7.07 mg of alkalinity is reduced per mg of nitrogen nitrified. Numerous physical-chemical and biological processes occur that affect the pH algae-based systems. Another possibility, according to Metcalf and Eddy (1991), is the precipitation of carbonate in the presence of a high concentration of calcium ions of which 4.75 mg/L of $CaCl_2.2H_2O$ was added to the synthetic wastewater. This precipitation which increases as the pH rises (increasing CO_3^{2-}), would keep the pH from continuing to increase. An increase in algal biomass would also cause an increase in the amount of degrading organic matter with subsequent increase in production of carbon dioxide. Although these observations were made for full-scale WSPs, similar processes also occur in labscale ponds.

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

These low pH levels were not observed in the dark pond with pH ranging from 6.8-7.6. There was a small decline in pH between from the influent tank to the pond which could be due to nitrification.

The mean temperature and DO of the light system was higher than that of the dark system.

It could be argued that the low rate of nitrogen removal by the dark pond was due the shorter operating period of 48 days in comparison to the 93 days of the light pond. According to the experimental results, the light pond had reached over 15% nitrogen removal within the first 8 days of operation whereas the maximum recorded removal for the dark pond after 24 days was below 13%. According Gerardi (2006), maximum rates of nitrification are achieved at $3mgO_2/L$. The mean DO for the dark tank was 2.17mg/L at which the rate of nitrification can be said to be sustained but not optimum. The low DO and subsequently low nitrogen removal can be attributed to the absence of algal-photosynthesis

The lower rates of nitrification observed in the batch experiments in comparisons to previous studies could have been affected by slow biofilm growth due non-optimum operating conditions of pH or DO in the ponds. It is possible that the presence of the algae provides increased biofilm attachment.

Denitrification

The observed rate of denitrification was found to be higher that expected for batch reactions under aerobic conditions. Biomass was scraped from the light pond which could contain more denitrifying bacteria than that attached to the plates themselves. Morrison, 1984 as cited by McLean *et. al.* (2000), reported that nitrified waters were observed to undergo a degree of denitrification even in more aerobic ponds. This was inferred to take place in anoxic parts of the sediments, where high concentrations of denitrifying organisms are present.

Unlike nitrifying bacteria, denitrifying bacteria reproduce quickly with a generation time of ± 15-30 minutes (Gerardi, 2006).

Mass Balance

Continuous flow system: Light and dark pond

The main mechanism for ammonia removal in the baffled labscale ponds was found to be due to nitrification accounting for 53 % and 88 % of the total ammonia removed in the light and dark ponds respectively fig. 4.14 and 4.15. The contribution of denitrification to net nitrate concentrations was not considered.

Although the direct contribution of algae was not measured, there was a significant decrease in the ammonia removal in the absence of algae by a factor of 2 with maximum observed ammonia removal at 29% and 12% for the light pond and dark ponds respectively, fig. 4.4 and 4.5. Mass balances for the light and dark ponds showed that the contribution of sedimentation, denitrification and organic nitrogen to ammonia removal was 11.1 % for the light pond and 0.6% for the dark pond. Assuming the rate of denitrification and sedimentation are similar within the ponds. This result is agreement with the findings of Mara and Valero (2007a), that showed that algae uptake and not ammonia volatilisation was the main mechanism for ammonia removal in algae-based ponds (maturation) even under conditions that favoured volatilisation (high temperature and pH).

Batch experiments

The results from the batch experiments show that nitrification was not the only mechanism for ammonia removal. The results also point to the fact that there were several mechanisms for N removal, namely ammonia volatilisation, denitrification and algal uptake. Due to the continuous mixing of the batch reactions, sedimentation can be considered negligible.

The batch results showed that the quantity of ammonia removed from the batch reactors was not equivalent to the net nitrite and nitrate accumulation. One main mechanism for ammonia removal was ammonia volatilisation as indicated by the control samples which had rates ranging from 0.958-3.994 mgNH_4-N/d which corresponds to a removal of 20.4- 42% at retention times of 24 and 57 hours respectively. Zimmo *et al.* (2003b) observed that not more than 1.5% of the total ammonia nitrogen was lost due to ammonia volatilisation over a period of one and a half years. The higher volatilisation rates in batch samples can be attributed to the small size of the reactor and the continuous aeration in the reactor.

Algae

The growth of algae indicates excessive nutrients in combination to low alkalinity. Increasing concentrations of filamentous and planktonic (free floating) algae caused scum to form on top of the labscale ponds. This diminished the amount of light that could penetrate the pond. Light limitation can explain biofilm sloughing. It is likely the epiphytic algae began to die off due to these light limitations.

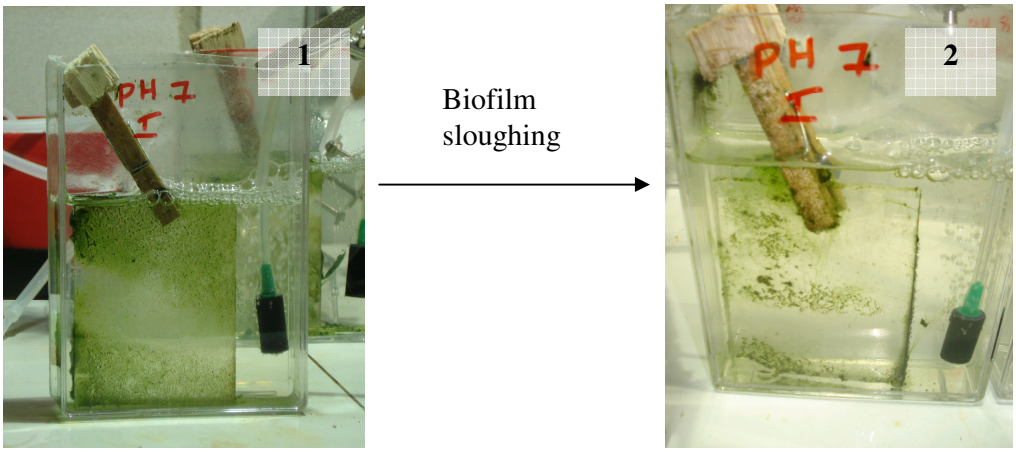


Fig 5.2 Biofilm sloughing. 1) is after 65 days of operation 2) after 80 days of operation

6 Conclusion and Recommendation

6.1 Conclusion

The effect on pH on algae-bacteria biofilms nitrification rates is different from that observed in suspended cells where maximum rate was observed at pH 7 in contrast to the pH 7.9-8.3 observed for suspended cells. It can be hypothesised that the net pH in the algae-biofilms is higher than the pH observed in the bulk liquid.

The absence of algae reduces the biofilm growth, subsequently reducing the nitrification rates of bacteria biofilm in comparison to algae-bacteria biofilms.

Bulk nitrification rates are significantly lower than algae-bacteria biofilms nitrification rates. No nitrification was observed in the bulk liquid during batch tests. The presence of biofilm attachment surfaces increases the nitrifying bacteria population and therefore the nitrification rates of labscale pond.

Nitrification followed possibly by algal uptake is the main mechanisms of ammonia removal in shallow baffled labscale ponds.

6.2 Recommendations for further research

1. FURTHER ALGAE-BIOFILM STUDIES

In order to be able to effectively apply algae-bacteria biofilm to waste stabilisation ponds, research should seek to integrate the top-down and bottom-up approaches which have seen other biofilm studies produce functional full scale system into operation in wastewater, drinking water, bioremediation and industrial processes (Characklis and Marshall, 1990). A bottom-up provides an understanding and defining of the chemical, physical and biological processes occurring at the molecular and cellular level. A top-down approach provides knowledge of the overall functioning and performance of the baffled waste stabilization ponds systems.

There has been great variation in the algae-bacteria biofilm nitrification rates for similar studies carried out by Babu, (2007) and Gunatilleke (2006) and the existing study. More work need to be done to optimize biofilm growth and make an inventory of the possible causes of this great variability. This will help to improve the operational control in designing the baffled WSP systems.

A model could be constructed to interpret batch reactor results in the context of full-scale systems. Full scale operation systems are usually continuous flow (open) systems which with a constant influx of reactants and outflow of products (Characklis and Marshall, 1990).

In order to quantify the nitrogen uptake of algae it is recommended that periodic analysis of algal concentration (chlorophyll a) be carried out.

In is likely that the alkalinity of the ponds was limiting. It is recommended that an initial determination or periodic tests for alkalinity be made for the ponds once in operation.

Demineralised and millicule water were used for this study. pH control of the system was poor even after elevating the pH of the influent to pH 7-7.5, the buffering capacity was poor and the system was below pH 7 during most days. A better alternative is the use of tapwater because although tapwater contains a residual nitrate concentrations, it has a better buffering capacity than demineralised and millicule water.

Further denitrification tests with shorter running times should be conducted to establish the progression of denitrification under aerobic conditions.

2. IMPLICATIONS FOR WASTE STABILISATION POND DESIGN

There is great variation in rates of nitrification between the current study and previous studies. This means there is need for better understanding of the system in order to reduce operational variability between ponds and improve on treatment efficiencies.

Such improvements can include choice of biofilm attachment material, algal species, species of nitrifying bacteria and age of the biofilm. These improvements would require further studies into the system and possibly the use of pure cultures to narrow down on possible causes of the variability.

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AppendicesAppendix 1: Physical-chemical parameters DO, pH and temperature

a) Daily Monitoring of Light Continuous Flow System

			pH										DO mg/L										Temperature °C									
Day	Date	Time	Tapwater	InfluentT	Inlet	Middle1	Exit1	Middle2	Exit2	Middle3	Outlet	Mean pH	Tapwater	InfluentT	Inlet	Middle1	Exit1	Middle2	Exit2	Middle3	Outlet	Mean DO	Tapwater	InfluentT	Inlet	Middle1	Exit1	Middle2	Exit2	Middle3	Outlet	Mean T
1	5-Dec-07	18:00																														
2	6-Dec-07	16:05	7.9	~	7.7	7.8	7.8	7.9	7.9	7.9	7.9	**7.8**	12	8.6	4.8	5	5.6	6.3	6.4	6.8	6.9	6	21	22	22	22	22	22	22	22	22	22.3
3	7-Dec-07	11:41	7.9	6.5	7.3	7.3	7.3	7.3	7.3	7.3	7.4	**7.3**	12	2.7	0.4	0.3	0.2	0.2	0.2	0.2	0.2	0.2	20	21	~	22	~	~	~	21	22	21.5
	7-Dec-07	16:02	7.9	7.3	7.9	7.9	7.9	7.9	7.9	7.9	7.9	**7.9**	13	5.6	7.6	7.6	7.7	7.8	7.7	7.6	7.3	7.6	20	21	22	22	22	22	22	22	22	21.9
4	8-Dec-07	10:40	7.7	~	7.4	7.3	7.3	7.3	7.3	7.3	~	**7.3**	12	5.1	2.8	2.7	2.5	2.5	2.6	2.7	2.8	2.7	18	20	20	20	20	20	20	20	20	19.5
	8-Dec-07	11:00	~	~	~	~	~	~	~	~	~		~	~	5.7	5.5	5.6	5.2	4.3	3.2	3.1	4.7	~	~	~	~	~	~	~	~	~	
5	9-Dec-07	12:44		~	~	~	~	~	~	~	~		~	1.1	4.1	4.1	4.1	4	4.2	4.1	4.2	4.1	~	~	21	21	21	~	21	~	21	21.2
6	10-Dec-07	10:56	7.8	~	7.2	7.3	7.3	7.2	7.2	7.3	7.3	**7.2**	11	~	4.6	4.7	4.5	4.5	4.4	4.7	4.7	4.6	20	~	21	21	21	21	21	21	21	20.8
	10-Dec-07	16:15	7.9	6.3	7.3	7.2	7.2	7.2	7.2	7.2	7.2	**7.2**	11	7.8	5.1	5	4.8	4.9	4.9	5	5	5	20	22	22	22	22	22	22	22	22	22.1
8	11-Dec-07	10:45	~	6.4	7.1	7.1	7.1	7.1	7.1	7.1	7.1	**7.1**	12	3.6	3.3	3.3	3	3.1	3.1	3.1	3.2	3.2	20	20	21	20	20	20	20	20	21	20.3
	11-Dec-07	17:18	7.9	~	7.1	7.1	7.1	7.1	7.1	7.1	7.1	**7.1**	12	1.7	4.6	4.6	4.6	4.6	4.5	4.7	4.8	4.6	17	20	22	22	22	22	22	22	22	21.8
9	12-Dec-07	19:06	7.8	6.8	7	7.1	7	7	7	7	7	**7**	12	9.5	7.1	7.2	7.2	7.1	7.2	7.3	7.3	7.2	22	21	22	22	22	22	22	22	22	22.2
10	13-Dec-07	11:02	7.8	7	6.7	6.8	6.8	6.7	6.7	6.7	6.7	**6.7**	11	1	4.3	4.2	4.1	4.5	4.7	4.9	4.8	4.5	21	22	20	20	20	20	20	20	20	20.2
	13-Dec-07	18:18	7.8	6.2	6.9	6.9	6.9	6.9	6.9	6.9	6.9	**6.9**	13	8	8	7.8	7.9	8.1	8.7	9	9	8.3	13	20	15	15	16	16	16	16	16	15.8
11	14-Dec-07	10:32	7.8	6.5	6.6	6.6	6.6	6.6	6.6	6.6	6.6	**6.6**	12	5.3	3.2	3.3	3.2	3.3	3.4	3.7	3.7	3.4	20	20	19	19	19	19	19	20	20	19.3
	14-Dec-07	17:50	7.3	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	**6.7**	11	8.6	5.7	5.6	5.8	6.2	7.1	7.2	7.4	6.4	21	21	22	22	22	21	21	22	22	21.6
12	15-Dec-07	12:00	7.8	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.5	**6.5**	11	1.8	4.2	4.1	4.2	4.5	5	5	5.2	4.6	20	19	20	21	21	21	20	20	21	20.4
14	17-Dec-07	12:00	~	7.1	6.7	6.6	6.6	6.5	6.5	6.5	6.4	**6.5**	10	4	5.6	5.9	6.2	6.2	6.6	6.5	6.1	6.2	19	19	19	20	19	19	19	19	19	19.2
15	18-Dec-07	14:20	7.2	7.8	6.6	6.6	6.6	6.5	6.6	6.6	6.5	**6.6**	11	5.4	8.4	8.4	8.5	8.5	8.8	8.9	8.7	8.6	19	19	20	20	20	20	20	20	20	20.1
16	19-Dec-07	16:28	~	6.3	6.6	6.6	6.6	6.6	6.6	6.6	6.6	**6.6**	12	8.1	5.7	5.8	6.1	6.3	6.7	7.1	7.1	6.4										
17	20-Dec-07	13:49	7.7	6.4	6.6	6.6	6.6	6.6	6.6	6.6	6.5	**6.6**	11	6.7	4.5	4.7	4.8	4.9	5.5	6	6.2	5.2	19	18	20	20	20	20	20	20	20	19.7
18	21-Dec-07	14:00	7.8	6.9	6.7	6.6	6.6	6.5	6.5	6.5	6.5	**6.6**	12	1.6	5.3	5.5	5.5	5.6	6.3	6.4	6.3	5.8	14	14	17	17	18	18	18	18	18	17.7
21	24-Dec-07	10:58	8.1	6.9	6.6	6.5	6.5	6.5	6.4	6.4	6.3	**6.4**	10	1.5	3.4	3.5	3.4	3.5	3.8	3.3	3.1	3.4	19	19	20	20	20	19	19	19	19	19.5
24	27-Dec-07	16:15	7.8	6.8	6.4	6.3	6.3	6.3	6.3	6.3	6.2	**6.3**	10	1.3	3.8	3.8	3.9	4.1	4.4	5.1	5.2	4.3	19	20	22	21	21	21	21	20	21	20.9
25	28-Dec-07	17:00	7.8	6.9	6.3	6.3	6.3	6.3	6.2	6.2	6.2	**6.3**	12	1.5	4.6	4.7	4.7	4.9	5.3	5.5	5.4	5	19	20	22	22	22	22	21	21	21	21.4

-continued- a) Daily Monitoring of Light Continuous Flow System

Day	Date	Time		InfluentT	Inlet	Middle1	Exit1	Middle2	Exit2	Middle3	Outlet	Mean pH	Tapwater	InfluentT	Inlet	Middle1	Exit1	Middle2	Exit2	Middle3	Outlet	Mean DO	Tapwater	InfluentT	Inlet	Middle1	Exit1	Middle2	Exit2	Middle3	Outlet	Mean T
26	29-Dec-07	12:12	7.9	6	6.4	6.4	6.4	6.4	6.4	6.4	6.4	**6.4**	11	8	2	1.9	2	2.4	2.7	2.7	2.7	2.3	20	20	21	21	21	20	21	21	21	20.6
28	31-Dec-07	13:15	6.9	~	6.7	6.6	6.6	6.6	6.6	6.6	6.5	**6.6**	10	0.8	1.8	1.8	2	2.3	2.8	3.2	3.3	2.5	19	20	21	21	21	21	21	21	21	21
29	2-Jan-08	14:05	8	6.7	6.5	6.4	6.4	6.4	6.4	6.4	6.3	**6.4**	10	0.9	3.8	3.9	3.9	4	3.9	4.1	4.2	4	18	15	17	17	17	16	17	17	17	16.6
30	3-Jan-08	13:28	8	6.9	6.3	6.3	6.3	6.3	6.3	6.2	6.2	**6.3**	10	1.2	2.9	2.9	3.1	3.5	3.7	3.9	3.9	3.4	19	18	19	19	19	19	19	19	19	19
	3-Jan-08	16:01	~	~	~	~	~	~	~	~	~		~	8.6	5.1	5	5.2	5.3	5.4	5.4	5.4	5.2		20	21	20	20	20	20	20	20	20.1
31	4-Jan-08	14:50	7.9	6.1	6.2	6.2	6.2	6.2	6.2	6.2	6.2	**6.2**	13	6.7	3	3	3.2	3.4	3.8	4.2	4.4	3.6	18	19	21	21	21	20	21	21	21	20.5
32	5-Jan-08	12:07	7.6	6.5	5.1	5	5	5	5	5	5	**5**	11	1.5	5.3	5.2	5.3	5.6	5.8	5.7	5.5	5.5	20	20	21	21	21	20	20	20	21	20.4
34	7-Jan-08	14:37	7.9	7	6.4	6.4	6.4	6.4	6.4	6.4	6.4	**6.4**	12	2.5	5.2	5.2	5.3	5.4	6	6	6.1	5.6	20	20	21	21	21	21	21	21	21	21.2
35	8-Jan-08	15:59	7.8	7	6.4	6.3	6.3	6.3	6.2	6.2	6.2	**6.3**	11	1.1	5.9	6.2	6.5	6.8	7.1	7.4	7.4	6.8	20	20	22	22	22	22	21	21	22	21.6
36	9-Jan-08	13:44	7.9	6.1	6.2	6.2	6.2	6.3	6.3	6.3	6.2	**6.2**	13	8.2	4	4.1	4.2	4.3	4.6	5.3	5.5	4.6	19	20	22	21	21	20	21	21	21	20.9
37	10-Jan-08	16:28	7.9	6.4	6.2	6.2	6.2	6.2	6.3	6.3	6.3	**6.2**	12	1	4.8	4.7	4.7	4.9	5.7	6.2	6.2	5.3	21	20	22	22	22	22	22	21	22	21.9
38	11-Jan-08	13:12	7.9	6.7	6	6	6	6	6	6	6	**6**	12	1	4.7	4.8	5	5.1	5.9	5.9	5.9	5.3	21	20	22	21	22	21	22	21	21	21.4
39	12-Jan-08	11:56	8	7	6.4	6.3	6.2	6.2	6.2	6.2	6.2	**6.2**	11	7.1	2.4	2.4	2.4	2.7	3.5	3.7	3.9	3	20	20	20	20	21	21	21	21	21	20.6
41	14-Jan-08	15:10	7.9	7.2	6.1	6	5.9	5.9	5.9	5.8	5.8	**5.9**	14	1.4	6.5	6.7	7	7.2	7.6	7.9	7.7	7.2	18	19	21	21	22	21	21	21	21	21.1
42	15-Jan-08	13:40	7.9	7.2	6	5.9	5.8	5.7	5.6	5.5	5.5	**5.7**	13	1.7	6.1	6.3	6.6	6.8	7.4	7.4	7.4	6.9	18	20	22	22	21	21	21	21	21	21.3
43	16-Jan-08	17:37	7.9	6.9	7.5	7.5	7.6	7.5	7.6	7.6	7.6	**7.5**	12	3.3	2.4	2.4	2.2	2.5	2.9	3	2.7	2.6	21	20	22	23	22	21	21	21	21	21.6
44	17-Jan-08	11:45	7.8	7.1	6.9	6.9	6.9	6.9	6.9	6.9	6.9	**6.9**	13	1.3	3.8	3.7	3.8	4.4	4.6	5	5.2	4.4	19	20	21	21	21	21	21	21	21	20.9
45	18-Jan-08	18:42	7.8	7.1	6.2	6.2	6.1	6	6	6	6	**6.1**	13	2.5	6	6.2	6.5	6.7	6.9	7	7.1	6.6	21	21	23	23	23	23	23	23	23	22.8
48	21-Jan-08	14:38	7.8	7.3	6.4	6.4		6.4		6.3	6.2	**6.3**	11	1.3	2.8	2.9		4		4.7	5	3.9	21	21	22	22		22		22	21	21.6
49	22-Jan-08	17:27	7.7	7.3	6.2	6.1		6		5.9	5.8	**6**	14	1.9	6.9	6.7		7.1		8.6	8.7	7.6	21	20	22	22		22			22	21.9
52	25-Jan-08	18:26	7.8	7.7	6.4	6.4		6.4		6.3	6.3	**6.4**	13	1.4	5.5	5.6		7		7.9	8.2	6.8	21	22	23	23		22		23	23	22.6
53	26-Jan-08	12:17	~	7.5	6.2	6.2		6.1		6.1	6	**6.1**	11	1.6	5.7	5.9		7.6		9.2	8.4	7.4	21	21	22	22		21		21	21	21.4
57	30-Jan-08	18:40	7.3	7	6.1	6.1		6		5.9	5.8	**6**	13	6	6.9	7		8.4		9	9.1	8.1	15	17	17	17		17		17	17	17.1
58	31-Jan-08	19:00	7.6	7.1	6.4	6.3		6.3		6.2	6.1	**6.3**	13	1.3	4.7	5.2		7.1		7.6	7.7	6.5	20	21	23	22		22		22	23	22.2
60	2-Feb-08	12:20	~	7.3	6.3	6.3		6.2		6.1	6	**6.2**		8.1	3.6	4.5		5.1		6.7	6.6	5.3		20	21	21		21		21	21	20.7
63	5-Feb-08	18:38	7.9	7.9	6.3	6.2		6.2		6.1	6.1	**6.2**	12	4.5	3.2	3.5		5		5.6	6.1	4.7	21	22	22	23		23		23	22	22.5
67	9-Feb-08	11:05	7.7	7.2	4.6	4.6		4.6		4.5	4.5	**4.6**	11	9.2	5.4	5.4		6.7		5.8	5.6	5.7	19	21	20	20		19		19	19	19.4
69	11-Feb-08	19:42	~	7.3	6.5	6.5		6.5		6.5	6.5	**6.5**	12	2	5.4	5.3		6.6		7.6	7.7	6.5	20	20	22	22		22		22	22	22.3
72	14-Feb-08	17:20	7.6	7.2	6.1	6		5.5		5.5	5.1	**5.6**	12	7	4.2	4.6		6		6.3	7	5.6	20	21	22	22		21		22	22	21.8
74	16-Feb-08	10:30	7.8	7.2	6.6	6.6		6.6		6.5	6.5	**6.5**	12	1.6	2.6	2.7		4.7		4	4	3.6	19	20		19		18		19	19	18.8
76	18-Feb-08	19:06	7.9	7.5	6.3	6.2		6.4		6.4	6.4	**6.3**	11	6.2	6.3	6.3		8		7.7	7.7	7.2	18	20	21	21		21		21	21	20.8

b) Daily Monitoring of Dark Continuous Flow System

			pH									DO mg/L							Temperature in °C							
Day	Date	Time	Tapwater	InfluentT	Inlet	Middle1	Middle2	Middle3	Outlet	Mean pH	Tapwater	InfluentT	Inlet	Middle1	Middle2	Middle3	Outlet	Mean DO	Tapwater	InfluentT	Inlet	Middle1	Middle2	Middle3	Outlet	Mean T
3	21-Jan-08	14:38	7.8	7.3	7.4	7.5	7.5	7.6	7.6	**7.5**	11	1.3	0.3	0.2	0.3	2.7	2.9	**1.3**	21	21	19	19	19	19	19	**19**
4	22-Jan-08	17:27	7.7	7.3	7.5	7.5	7.4	7.5	7.5	**7.5**	14	1.9	1.5	1.6	2.3	2.5	3.7	**2.3**	21	20	20	20	~	19	19	**20**
7	25-Jan-08	18:26	7.8	7.7	7.3	7.2	7.2	7.3	7.3	**7.3**	13	1.4	1	0.9	1.1	1.3	1.4	**1.1**	21	22	20	20	20	20	20	**20**
8	26-Jan-08	12:17		7.5	7.2	7.2	7.2	7.3	7.3	**7.2**	11	1.6	2.3	3	3.3	3.7	4.1	**3.3**	21	21	20	19	19	19	19	**19**
12	30-Jan-08	18:40	7.3	7	6.9	6.9	6.9	6.9	6.9	**6.9**	13	6	0.5	0.2	0.2	0.3	0.5	**0.3**	15	17	12	12	13	13	13	**13**
13	31-Jan-08	19:00	7.6	7.1	7.1	7.1	7.1	7	7.1	**7.1**	13	1.3	0.1	0.3	1.1	1.4	1.5	**0.9**	20	21	18	18	18	18	18	**18**
18	5-Feb-08	18:38	7.9	7.9	6.8	6.8	6.8	6.8	6.8	**6.8**	12	4.5	1.5	1.5	1.6	1.9	2	**1.7**	21	22	20	20	20	20	20	**20**
22	9-Feb-08	11:05	7.7	7.2	6.8	6.8	6.8	6.8	6.8	**6.8**	11	9.2	2.4	2.4	2.7	2.4	2.6	**2.5**	19	21	20	20	19	19	19	**19**
24	11-Feb-08	19:42		7.3	6.8	6.8	6.8	6.8	6.8	**6.8**	12	2	0.8	0.7	0.9	0.9	0.9	**0.8**	20	20	20	20	19	19	19	**19**
27	14-Feb-08	17:20	7.6	7.2	6.9	6.9	6.9	6.9	6.9	**6.9**	12	7	3.4	3.6	3.8	3.9	3.9	**3.7**	20	21	20	20	19	19	19	**19**
29	16-Feb-08	10:30	7.8	7.2	7.1	7.1	7.1	7.1	7.1	**7.1**	12	1.6	2.5	2.5	2.7	3	3	**2.8**	19	20	19	19	18	18	18	**18**
31	18-Feb-08	19:06	7.9	7.5	7.2	7.2	7.2	7.2	7.2	**7.2**	11	6.2	5.1	5.2	5.2	5.4	5.3	**5.2**	18	20	18	18	18	18	18	**18**

Appendix 2: Summary of Nitrogen removal in the light Continuous flow system

Date of sampling	Sample reference	Ammonia in mg/l	Nitrite in mg/l	Nitrate in mg/l	TN
11-Dec-07	Influent Tank	39.9	-	0.1	**40.0**
	Middle of 1st P	33.3	1.4	0.5	**35.2**
	Middle of 2nd P	34.5	1.2	0.7	**36.3**
	Middle of 3rd P	33.3	1.2	0.8	**35.3**
	Effluent	34.8	1.1	0.7	**36.6**
				0.7	
18-Dec-07	Influent Tank	38.3	0.1	0.1	**38.4**
	Middle of 1st P	31.8	1.8	3.0	**36.6**
	Middle of 2nd P	31.9	1.8	2.8	**36.5**
	Middle of 3rd P	30.1	1.8	2.9	**34.9**
	Effluent	34.1	1.9	3.1	**39.1**
28-Dec-07	Influent Tank	39.3	0.0	0.1	**39.4**
	Middle of 1st P	32.5	0.8	3.5	**36.8**
	Middle of 2nd P	30.6	0.8	4.2	**35.5**
	Middle of 3rd P	35.0	0.8	4.1	**39.9**
	Effluent	26.9	0.8	4.3	**32.0**
04-Jan-08	Influent Tank	47.2	0.0	0.1	**47.3**
	Middle of 1st P	37.4	0.5	4.1	**42.0**
	Middle of 2nd P	35.7	0.5	4.1	**40.3**
	Middle of 3rd P	37.1	0.5	3.5	**41.1**
	Effluent	35.5	0.7	4.8	**41.0**
4th Jan- further addition of nitrifying biomass					
10-Jan-08	Influent Tank	41.8	0.1	0.2	**42.0**
	Middle of 1st P	32.3	0.4	4.2	**37.0**
	Middle of 2nd P	33.4	0.4	4.4	**38.2**
	Middle of 3rd P	32.9	0.4	4.6	**37.9**
	Effluent	33.7	0.7	5.6	**40.0**
22-Jan-08	Influent Tank	36.8	0.01	0.1	**36.9**
	Middle of 1st P	26.2	0.02	5.7	**31.9**
	Middle of 2nd P	27.0	0.02	6.7	**33.7**
	Middle of 3rd P	25.5	0.02	5.3	**30.8**
	Effluent	27.1	0.03	6.6	**33.7**
12-Feb-08	Influent Tank	24.6	0.01	0.2	**24.7**
	Middle of 1st P	22.2	0.06	4.7	**26.9**
	Middle of 2nd P	22.6	0.05	4.4	**27.0**
	Middle of 3rd P	21.6	0.04	4.1	**25.7**

Appendix 3: Preparation of buffered influent

Example of buffer preparations:

Batch experiment 1: Trial Batch Experiment

	Influent pH	**$NaH_2PO_4.H_2O$ ml, pH 3.83**	**$Na_2HPO_4.2H_2O$ ml, pH 8.89**	**Final pH**	**Final vol L**
pH6	Approx 6.16	4.25ml x 2	<14.9		0.5
pH7i	6.02	2ml x 4	38.8	7	1
pH7ii	6.11	2ml x 2	13.6	7.01	0.5
pH7i+ii	-	-	-	7.05	1.5
pH8	6.06	0.425ml x 2	58.7	8	0.5

Batch experiment 2:

Table 3.5 Buffered influent preparations for batch 2 experiments

	Influent pH	**1M $NaH_2PO_4.H_2O$ pH 3.83**	**1M $Na_2HPO_4.2H_2O$ pH 8.89**	**Final pH**	**Final vol L**
pH6	6.16	8.5ml	14.9		0.5
pH7i	6.02	8ml	38.8	7	1
pH7ii	6.11	4ml	13.6	7.01	0.5
pH7i+ii	-	-	-	7.05	1.5
pH8	6.06	0.85ml	58.7	8	0.5

Appendix 4: Monitoring of pH, DO, temp during batch experiments

Batch1

pH	**Condition**
pH 6	Aeration
pH 7	Aeration
Control pH 7	No aeration
Dark pH 7	No aeration
pH 8	Aeration
Bulk	No aeration

Table 4: DO, temperature and pH Checks and adjustments

pH	**2 hrs**	**5 hrs**			**7hrs**
Batch 1	pH	pH	DO mg/L	Temp ^{o}C	pH
pH 6	5.98→ 6.01	6.02→6.01	8.1	24.9	6.10
pH 7	7.01	7.07→7.02	8	23.3	7.06
Control pH 7	6.99→7	7	6.6	23.7	7.02
Dark pH 7	7	7	2.5	24.3	7.03
pH 8	8.03	8.04→7.99	8.3	23.3	8.05
Bulk	6.08	6.20	10.1	24.1	6.30

	0 hrs			**5 hrs**			**24 hrs**		
All batches were aerated	pH	DO mg/L	Temp ^{o}C	pH	DO mg/L	Temp ^{o}C	pH	DO mg/L	Temp ^{o}C
Batch 2									
pH 7 (1)	7.14→7.01	11.1	13.6	6.92→7.01	8.68	23.3	7.02	8.35	22.8
pH 7 (2)	7.09→7.09	10.8	14.7	6.96→7.03	8.42	23.8	7.05	8.46	23.4
pH 7 Control	7.07→7.01	11.3	12.8	6.95→7.01	8.49	23.6	7.09	8.3	22.9
pH 8(1)	8.04→8.02	11.1	13	7.92→8.01	8.44	23.9	7.97	8.63	23.4
pH 8(2)	8.03→8.02	11.2	12.9	7.88→8.02	8.72	24.1	8.01	8.58	23.9
pH 8 Control	8.03→8.00	11.1	13.1	7.88→8.02	8.75	24.3	8.02	8.4	23.8

Start: 12:09	**0 hrs**			**23 hrs**			**55 hrs**		
All batches were aerated	pH	DO mg/L	Temp ^{o}C	pH	DO mg/L	Temp ^{o}C	pH	DO mg/L	Temp ^{o}C
Batch 3									
pH 5	5.03	8.34	28	5.28→5	6.75	22.8	5.03	7.43	23.7
pH 5 Control	5.03	8.34	28	5.16	7.75	22.7	5.34	8.54	23.7
pH 6 (1)	5.96	10	29.1	6.13→6.05	7.85	23.6	6.04	7.75	24
pH 6(2)	5.96	10	29.1	6.24→6.04	6.40	23.5	6	6.64	24.6
pH 6 Control	5.96	10	29.1	6.17→6.05	8.07	23	6.06	7.30	23.9
pH 7 (1)	7.05	10.58	28.5	7.15→7.04	7.18	23.6	7	8.4	24.9
pH 7 (2)	7.05	10.58	28.5	7.18→7.03	8.55	23.7	6.99	7.56	25
pH 7 Control	7.05	10.58	28.5	7.17→7.06	9.33	24	7.06	8.9	24.3
pH 8(1)	7.96	9.14	28.3	7.96→8.08	8.21	24.1	8.12	9.72	25.2
pH 8(2)	7.96	9.14	28.3	7.98→8.05	9.21	24.3	8	8.4	24.6
pH 8 Control	7.96	9.14	28.3	7.94→8.02	8	24.4	7.99	7.27	24.4
pH 9	8.94	8.38	27	8.52→9.02	9.3	22	8.68	7.71	23.4
pH 9 Control	8.94	8.38	27	8.31→9.07	7.78	21.8	8.51	7.62	23.4

Appendix 5: Raw data for ammonia, nitrite and nitrate

Batch 1	Time in hrs	pH 6	pH 7	pH 7 control	pH 7 Dark	pH 8	Bulk
mg NH_4^+-N/L	1	29.9		29.3	27.9	28.3	30.9
	2	29.3	28.2	27.8	25.0	23.8	29.9
	3	29.9	29.3	29.7	30.3	25.7	33.2
	4	30.9	24.0	31.0	29.3	28.6	33.4
	5	28.8	30.4	27.6	29.4	24.9	26.9
	6	29.3	28.9		27.2	29.4	32.6
	7	27.6	26.7	28.4	28.0	28.4	33.0
mg NO_2^--N/L	1	0.05	0.07	0.05	0.06	0.09	0.11
	2	0.05	0.09	0.05	0.06	0.10	0.11
	3	0.05	0.09	0.05	0.06	0.13	0.12
	4	0.05	0.11	0.05	0.07	0.15	0.11
	5	0.05	0.12	0.06	0.09	0.16	0.11
	6	0.05	0.14	0.05	0.10	0.17	0.12
	7	0.05	0.18	0.08	0.14	0.22	0.12
mg NO_3^--N/L	1	0.21	0.24	0.04	0.29	0.36	7.38
	2	0.28	0.37	0.22	0.45	0.64	7.70
	3	0.44	0.39	0.25	0.36	0.48	8.19
	4	0.19	0.48	0.10	0.44	0.69	7.24
	5	0.14	0.35	0.25	0.41	0.62	7.89
	6	0.37	0.57	0.09	0.60	0.59	8.15
	7	0.39	0.53	0.22	0.50	0.62	7.64

Batch 2	Time in hrs	pH 7(1)	pH 7(2)	pH 7 control	pH 8(1)	pH 8(2)	pH 8 control
mg NH_4^+-N/L	0	26.5	25.4	26.8	25.2	24.4	26.1
	1	27.9	25.9	26.1	27.1	22.1	25.9
	3	25.5	25.8	26.1	23.4	25.5	26.1
	5	24.0	26.4	26.1	26.4	31.5	27.1
	7	23.6	24.4	29.3	23.1	25.0	24.8
	9	20.9	23.1	23.8	20.1	20.4	22.7
	24	14.9	16.9	21.6	15.5	15.8	20.5
mg NO_2^--N/L	0	0.04	0.05	0.04	0.05	0.05	0.04
	1	0.06	0.05	0.04	0.09	0.08	0.04
	3	0.07	0.05	0.04	0.19	0.16	0.04
	5	0.09	0.06	0.04	0.31	0.25	0.04
	7	0.10	0.05	0.03	0.23	0.20	0.03
	9	0.15	0.06	0.03	0.24	0.24	
	24	0.24	0.19	0.04	1.27	0.75	0.04
mg NO_3^--N/L	0	0.17	0.05	0.13	0.08	0.08	0.09
	1	0.29	0.14	0.20	0.17	0.15	0.06
	3	0.59	0.22	0.24	0.36	0.32	0.11
	7	1.29	0.56	0.25	0.79	0.59	0.18
	9	1.64	0.84	0.23	1.25	0.78	0.03
	24	3.19	1.89	0.24	1.98	1.35	0.06

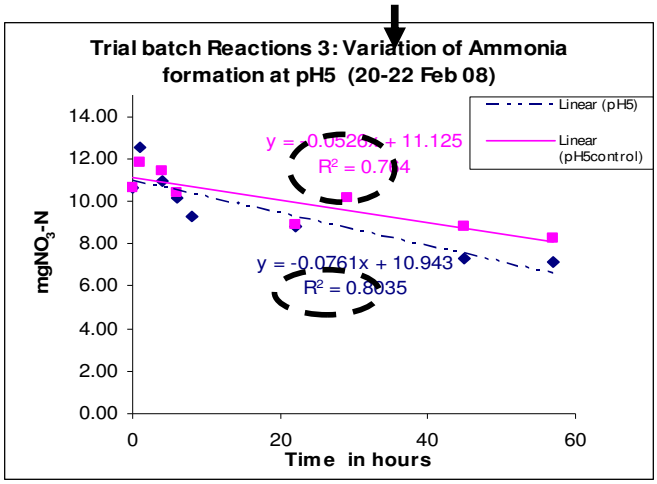
Batch 3	Time in hrs	pH 5	pH 5 control	pH 6(1)	pH 6(2)	pH 6 control	pH 7(1)ii	pH 7(2)ii	pH 7 control	pH 8(1)ii	pH 8(2)ii	pH 8cont	pH 9	pH 9 control
mg NH_4^+-N/L	0	28.0		33.5	33.5	33.5	30.2	30.2	30.2	32.1	32.1	32.1	30.9	30.9
	1	32.9	31.2	31.2	31.0	31.8	31.8	31.8	28.9	31.0	32.1	30.5	30.3	32.1
	4	30.0	31.3	31.3	31.6	32.6	31.7	31.7	33.9	33.5	32.7	32.1	32.0	26.3
	6	29.0	29.7	28.1	28.8			29.0	31.8	28.4	28.2	27.8	27.2	26.6
	8	27.6		28.6	27.6			26.0		26.5	29.0		26.0	
	22	27.4	27.7	23.8	22.1	25.3	22.8	22.4	25.3	23.2	26.4	26.3	21.8	28.7
	29		33.3	19.5		28.9	24.1	24.3	30.4	24.0	23.0	28.6	21.5	23.3
	45	25.2	30.4	25.6	22.3	29.6	21.5	23.6	32.7	22.4	20.3	27.7	17.4	23.2
	57	25.9	29.9	18.8	23.7	30.1	16.7	20.4	35.8	19.4	16.7	25.1	13.9	20.8
mg NO_2^--N/L	0	0.05	0.05	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02
	1	0.03	0.03	0.02	0.03	0.03	0.05	0.04	0.03	0.05	0.05	0.03	0.06	0.03
	4	0.03	0.03	0.02	0.04	0.03	0.06	0.04			0.07	0.04	0.12	0.04
	6	0.04	0.04	0.04	0.05		0.05	0.04	0.04	0.08	0.08	0.04	0.14	0.04
	8	0.04		0.04	0.05		0.04	0.04		0.08	0.09		0.15	
	22	0.04	0.05	0.05	0.07	0.06	0.09	0.08	0.06	0.13	0.11	0.03	0.31	0.04
	29	0.33	0.03	0.04	0.03	0.04	0.04	0.05	0.04	0.19	0.13	0.03	0.30	0.05
	45	0.03	0.05	0.04	0.03	0.06	0.04	0.03	0.04	0.13	0.14	0.07	0.29	0.09
	57	0.03	0.03	0.03	0.03	0.04	0.04	0.03	0.03	0.08	0.07	0.03	0.25	0.10
mg NO_3^--N/L	0	0.01	0.01	0.05	0.05	0.05	0.02	0.02	0.02	0.01	0.01	0.01	0.03	0.03
	1	0.02	0.01	0.02	0.07	0.02	0.14	0.14	0.01	0.04	0.05	0.04	0.04	0.02
	4	0.01	0.02	0.07	0.11	0.01	0.08	0.20	0.02	0.12	0.09	0.03	0.16	0.02
	6	0.05	0.01	0.04	0.08	0.01	0.17	0.19	0.01	0.19	0.13	0.01	0.20	0.03
	8	0.05	0.01	0.07	0.09	0.01	0.08	0.25	0.01	0.18	0.16	0.02	0.20	0.03
	22	0.05	0.01	0.07	0.38	0.01	0.67	0.49	0.01	0.44	0.40	0.02	0.41	0.03
	29	0.44	0.05	0.09	0.16	0.01	0.81	0.49	0.01	0.67	0.48	0.01	0.47	0.02
	45	0.10	0.09	0.03	0.31	0.04	0.94	0.45	0.14	0.88	0.73	0.01	0.45	0.01
	57	0.18	0.04	0.11	0.44	0.02	1.46	0.68	0.03	1.10	1.67	0.16	0.71	0.07

Batch 4	Time in hrs	D1	D2	Biomass
mg NH_4^+-N/L	0	30.0	30.0	30.0
	1	27.3	29.0	28.9
	3	29.3	30.2	30.5
	5	29.3	30.0	30.7
	7	30.0	30.7	30.9
	26	25.6	27.6	27.1
	27	25.6	27.6	25.9
mg NO_2^--N/L	0	0.04	0.04	0.04
	1	0.01	0.05	0.05
	3	0.02	0.01	0.01
	5	0.02	0.01	0.01
	7	0.05	0.05	0.05
	26	0.07	0.07	0.07
	27	0.07	0.03	0.03
mg NO_3^--N/L	0	0.01	0.01	0.01
	1	0.01	0.06	0.20
	3	0.03	0.06	0.56
	5	0.02	0.02	0.48
	7	0.01	0.03	0.29
	26	0.06	0.11	1.12
	27	0.06	0.05	1.37

Batch 5	Time in hrs	D1	D2	Dcontrol	Bulk1	Bulk2
mg NH_4^+-N/L	0	29.1	29.1	29.1	19.0	19.0
	2	30.0	28.9	28.9	19.4	20.4
	5	30.6	28.5	28.7	18.4	18.2
	8	27.4	28.1	26.9	18.1	17.7
	22	25.1	26.3	26.5	17.4	17.1
	25	25.1	25.2	26.0	18.0	17.3
	31	24.7	25.7	25.2	16.8	17.0
mg NO_2^--N/L	0	0.02	0.02	0.02	3.25	3.25
	2	0.07	0.04	0.02	3.18	3.19
	5	0.08	0.06	0.02	3.27	3.29
	8	0.12	0.04	0.06	3.35	3.26
	22	0.16	0.02	0.04	3.39	3.48
	25	0.20	0.03	0.04	3.69	3.69
	31	0.27	0.09	0.05	3.69	3.63
mg NO_3^--N/L	0	0.02	0.02	0.02	3.76	3.76
	2	0.07	0.03	0.02	3.72	2.55
	5	0.12	0.03	0.04	3.57	3.42
	8	0.11	0.05	0.10	3.39	2.43
	22	0.13	0.01	0.03	3.76	3.12
	25	0.04	0.01	0.03	3.30	3.55
	31	0.04	0.02	0.07	4.34	3.57

Appendix 6: Example of calculation of nitrification rates

Hours	pH 5	µg NH_4^+-N in 50ml	absorbance	mg NH_4^+-N/L	Vol in L	amount in mg
0	T0A	27.98	0.765	27.98	0.38	10.63
1	T1A	65.82	1.518	32.91	0.38	12.50
4	T4A	59.96	1.388	29.98	0.365	10.94
6	T6A	29.00	0.792	29.00	0.35	10.15
8	T8A	27.64	0.756	27.64	0.335	9.26
22	T22A	27.41	0.75	27.41	0.32	8.77
45	T45A	25.16	0.595	25.16	0.29	7.30
57	T57A	25.86	0.611	25.86	0.275	7.11



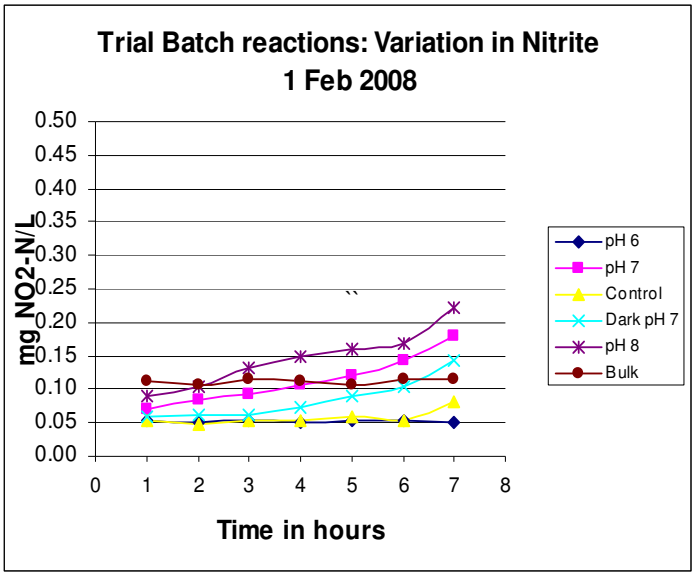
	Rate mg/h	Time	amount in mg	correct amount	Rate in mgNH_4-N/h
pH 5	-0.0761	57	-4.3377	-1.3395	0.0235
pH 5control	-0.0526	57	-2.9982		

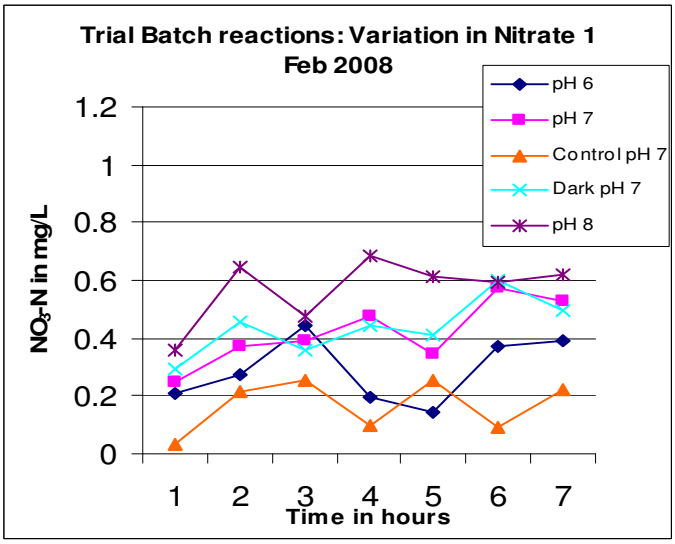
pH	Rate in mgNH_4-N/L/h	Rate in mgNH_4-N/h	DW-S in g	Rate in mgNH_4-N/gDW/h	Rate in mgNH_4-N/m^2/h
5		0.0235	0.0238	0.99	1.91

Appendix 7: Dry Weight measurements

Batch	Treatment	Clean FC [g]	Dry SFC [g]	DW-S [g]
1	pH 6	2.1776	2.2242	0.0466
	pH 7	2.1623	2.2142	0.0519
	pH 7 Dark	2.1642	2.2186	0.0544
	pH 8	2.1653	2.2267	0.0614
2	pH $7(1_2)$	2.1713	2.2232	0.0519
	pH $7(2_2)$	2.1688	2.2261	0.0573
	pH $8(1_2)$	2.1766	2.2359	0.0593
	pH $8(2_2)$	2.1698	2.2286	0.0588
3	pH 5	2.1684	2.1922	0.0238
	pH 6(1)	2.171	2.2046	0.0336
	pH 6(2)	2.1626	2.1918	0.0292
	pH $7(1_3)$	2.1791	2.2247	0.0456
	pH $7(2_3)$	2.1784	2.2138	0.0354
	pH $8(1_3)$	2.1747	2.2103	0.0356
	pH $8(2_3)$	2.164	2.2100	0.046
	pH 9	2.1641	2.2007	0.0366
4	$D1_4$	2.2714	2.2797	0.0083
	$D2_4$	2.1783	2.1852	0.0069
	Biomass			0.8231
5	$D1_5$	2.1731	2.1843	0.0112
	$D2_5$	2.1856	2.1945	0.0089
	Bulk1	2.1845	2.2242	0.0397
	Bulk2	2.1753	2.2207	0.0454

Appendix 8: Results from trial Batch experiment 1





Appendix 9: SPSS t-test statistics for light and dark batch experiments

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
R_gDW	Equal variances assumed	7.068	0.038	0.577	6	0.585	0.39	0.676
	Equal variances not assumed			0.577	3.59	0.598	0.39	0.676
R_m2	Equal variances assumed	1.091	0.336	3.386	6	0.015	3.7775	1.115
	Equal variances not assumed			3.386	4.46	0.023	3.7775	1.115