Effects of Carbon Dioxide Addition on Algae and Treatment Performance of High Rate Algal Ponds

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Abstract

Waste stabilisation ponds have been used for treating a great variety of wastewaters around the world for many decades. More advanced systems combine anaerobic or advanced facultative ponds with high rate algal ponds (HRAP) followed by a number of algae settling ponds and maturation ponds to achieve enhanced and more reliable removal of wastewater pollutants, while yielding possibly valuable by-products such as biogas and algal biomass. In recent years a growing number of scientists and engineers have proposed the use of HRAP treating domestic wastewater for carbon dioxide (CO_2) scrubbing from biogas and CO_2 sequestration. The experiments presented in this thesis sought to determine if the treatment performance of HRAP is affected by the addition of CO_2 and subsequent reduction of pond pH.

Experiments with algae cultures grown on domestic wastewater in laboratory microcosms, outside mesocosms and outside pilot-scale HRAP were conducted. Carbon dioxide addition to algae wastewater cultures restricted the maximum pH level to ~8. Key wastewater quality parameters of CO_2 added cultures, were compared to control cultures without CO_2 addition. The wastewater quality parameters monitored include temperature, pH, and concentrations of total suspended solids (TSS), ammoniacal-nitrogen (NH_4-N), dissolved reactive phosphorus (DRP), filtered biochemical oxygen demand ($fBOD_5$) and the faecal indicator *Escherichia coli* (*E. coli*).

Carbon dioxide addition to algae wastewater cultures was found to promote algal growth and increased the TSS concentrations. Over 8 day culture length CO_2 addition in laboratory and outside batch experiments increased algal growth (indicated by TSS) by up to 76% and 53%, respectively. During semi-continuous outside experiments CO_2 addition increased algal growth by ~20% in comparison to the control cultures. Despite enhancing algal growth (TSS), CO_2 addition appeared to have little effect on algae cell morphology, species composition and zooplankton activity in the algae wastewater cultures.

Monitoring of the key nutrients NH_4-N and DRP in cultures with and without CO_2 addition indicated that CO_2 addition can lead to an increase or a decrease in nutrient removal. Under culture conditions which allowed the control cultures to achieve high day-time pH levels CO_2 addition, and subsequent pH restriction, appeared to reduce overall nutrient removal. Only

slight changes or an increase in nutrient removal as a result of CO_2 addition were observed under culture conditions which allowed only for a moderate or small elevation of the control culture pH. However, the increases in algal biomass, observed in all CO_2 added cultures indicate a greater potential for the reclamation of potentially valuable wastewater nutrients in the form of algal biomass.

Monitoring of $fBOD_5$ levels during several outside experiments showed that CO_2 addition had no effect on the $fBOD_5$ removal by the algae wastewater cultures under those conditions.

During several outside batch experiments (of up to 8 day culture length) the removal of the faecal indicator bacteria *E. coli* was monitored. It was shown that CO_2 addition reduced *E. coli* removal by 1.4 to 4.9 log units compared to control cultures.

Basic modelling of carbon flows indicated that under New Zealand conditions the CO_2 volumes required for the changes described above would be available from the biogas produced in a wastewater pond system treating wastewater with a volatile solids (VS) concentration of ~ 500 mg/L. In systems treating weaker wastewaters additional CO_2 could be made available through the onsite combustion of biogas.

In summary, the obtained results suggest that CO_2 addition to a field-scale HRAP could increase algal biomass growth year-round and slightly enhance nutrient removal during winter, but might reduce nutrient removal during summer, and reduce *E. coli* removal year-round, while having no effect on $fBOD_5$ removal. The reduction in nutrient treatment performance during summer, and especially the losses in *E. coli* removal resulting form CO_2 addition may require more sophisticated downstream processing of the HRAP effluent, like increase retention times in maturation ponds. Such remedial measures have to be evaluated on a case by case basis, and are dependent on the given regulations and discharge regimes of the system.

This study indicates that in general HRAP can be employed for biogas purification and provide a useful sink for CO_2 rich waste streams. The beneficial effects of CO_2 addition to HRAP do not appear to allow for any design or management changes within the system, while it was indicated that most detrimental effects of CO_2 addition could be accommodated without major alternations, although in some cases significant remedial measures may be required for correcting the losses in disinfection and nutrient removal performance.

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1. Introduction

1.1 Waste Stabilization Ponds

For decades conventional pond-based wastewater treatment systems, commonly known as waste stabilization ponds, have provided communities and commercial enterprises around the globe with simple and low cost treatment of liquid wastes (Bratsch and Allum 1957; Gloyna and Espino 1969; Oswald 1991). These simple systems provide primary treatment (removal of settleable and floatable solids), secondary treatment (removal of dissolved organics and reduction in biochemical oxygen demand; BOD), as well as partial tertiary treatment (the removal of nutrients) and some disinfection (Bratsch and Allum 1957; Mara 1997; Mandeno 2003). Waste stabilisation ponds (WSP) can be classified as anaerobic ponds, aerobic maturation ponds and facultative ponds, depending on the dominant treatment processes that occur within them (Mara 1997).

1.1.1 Anaerobic Ponds

Anaerobic ponds settle and digest wastewater solids under conditions without oxygen. They are usually deep (2 to 5 m) to limit the surface area in contact with the atmosphere (Mara 1997). Anaerobic ponds treating municipal wastewater are designed with volumetric organic loading rates between 100 and 400 g $BOD_5/m^3/d$ depending on local pond temperatures which gives hydraulic retention times from 1 to 6 days (Mara 1997; Mara 2002). Anaerobic degradation of organic solids involves several stages and breaks the organic matter down to water, various mineral nutrients (e.g. ammoniacal-nitrogen and phosphates) and biogas (Metcalf and Eddy 1991; Green et al. 1995a; Hofmann et al. 2005). Further details of these degradation processes are available from Oswald and Golueke (1960b), Metcalf and Eddy (1991) and Hofmann et al. (2005). Anaerobic ponds are capable of excellent BOD removal, with 60% to 80% of the influent BOD_5 typically removed (Metcalf and Eddy 1991; Mara 1997). The biogas produced by anaerobic digestion consists generally of 50% to 70% methane (CH_4) and 30% to 50% carbon dioxide (CO_2) as well as some trace gases including: hydrogen sulphide (H_2S), hydrogen (H_2) and nitrogen (N_2) (Metcalf and Eddy 1991; Weiland 2003; Hofmann et al. 2005). Biogas can be utilised as an energy source, thereby reducing the overall energy costs of the wastewater treatment system (Green et al. 1995b; Benemann 2003).

1.1.2 Aerobic Maturation Ponds

Aerobic maturation ponds are mostly used as the last pond in a waste stabilization pond system (Mara 1997; Craggs 2002c). These ponds are designed primarily for the removal of pathogens and nutrients (Mara 1997), while aerobic microbiological degradation breaks down remaining dissolved organic matter. These ponds are usually shallow (~1.0 m), and designed based on the Marais' equation of temperature dependent faecal coliform removal, which leads to hydraulic retention times of 3 to 5 days, however in many cases several maturation ponds are employed in series (Mara 1997; Mara 2002). Aeration of these ponds is mostly provided by algal photosynthesis (see 1.3.1), which leads to elevated DO (dissolved oxygen) and pH levels in the surface layers of these unmixed ponds during the day-time (Pearson et al. 1987; Mara 1997). Under such conditions some nutrients like NH_4-N and phosphates are removed by volatilisation and precipitation respectively (see 1.3.2 and 1.3.3), and solar UV-radiation augmented by the elevated DO and pH levels reduces pathogen levels (Pearson et al. 1987; Oswald 1991; Gomez et al. 1995; Craggs et al. 2000; Davies-Colley 2005).

1.1.3 Facultative Ponds

Facultative ponds are the most common form of waste stabilisation ponds in New Zealand (Craggs 2002c). They are designed based on a surface loading rate of 84 kg BOD_5/ha/d and are usually 1m to 2m deep (Craggs 2002c). Facultative ponds typically stratify, forming 3 distinct zones: aerobic, facultative and anaerobic (Figure 1.1) (Metcalf and Eddy 1991; Mara 1997; Craggs 2002c).

The anaerobic zone is at the bottom of the pond, where settled wastewater solids are degraded anaerobically. The aerobic zone is at the surface of the pond where algal photosynthesis and some diffusion from the atmosphere provide oxygen for aerobic breakdown of organic matter to water (H_2O), carbon dioxide (CO_2) and nutrients (e.g. ammoniacal-nitrogen and phosphate). Under favourable aerobic conditions some NH_4-N may be oxidized to nitrate (NO_3^-) by nitrifying bacteria. Between these two zones is the facultative zone which is inhabited by micro organisms which can live in both anaerobic and aerobic conditions (Metcalf and Eddy 1991).

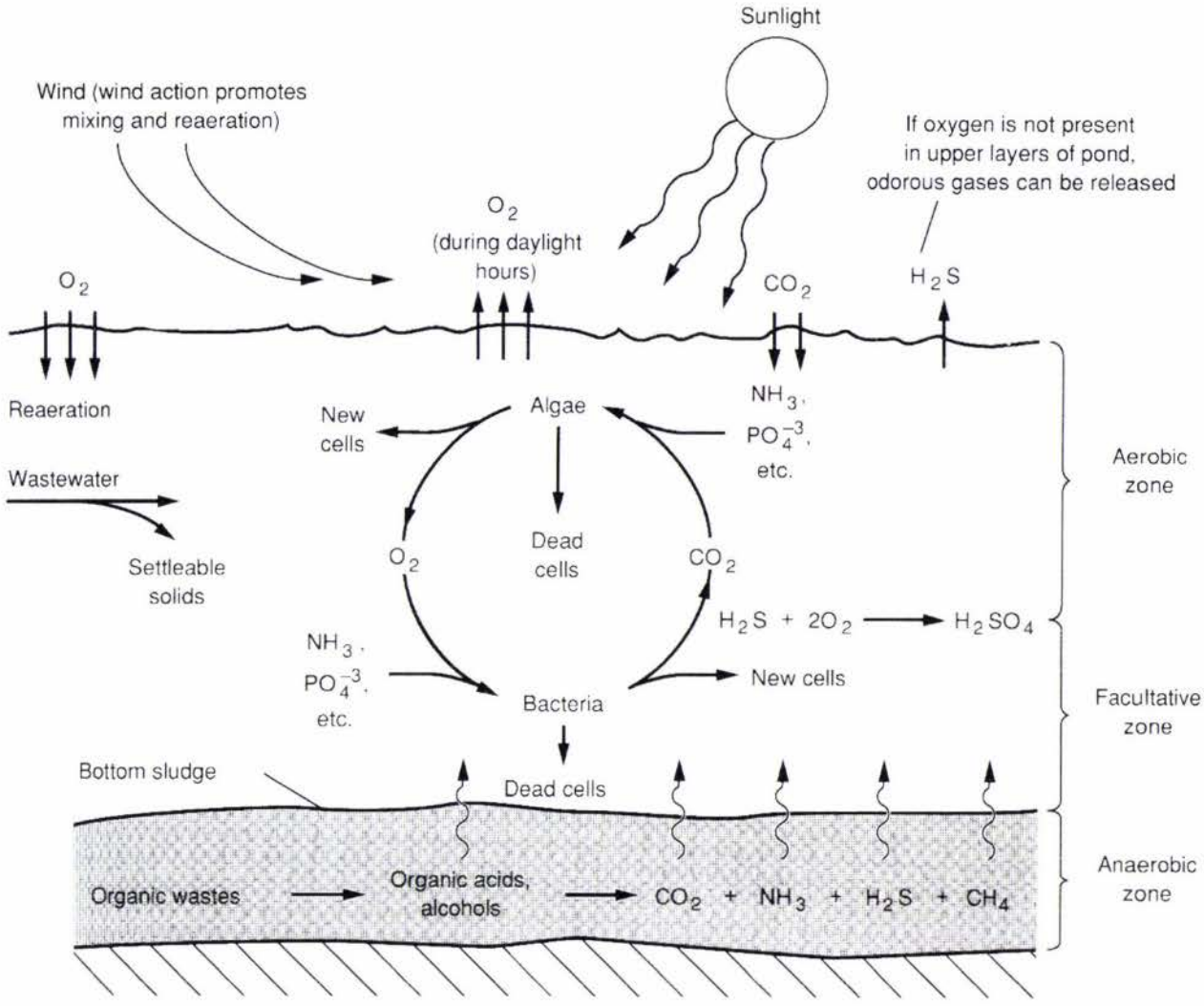

Figure 1.1: Degradation and transformation processes occurring in a facultative pond (from Metcalf and Eddy 1991).

The depths of these 3 zones within a facultative pond vary considerably depending on factors such as organic loading rate, local climate and season. Wind mixing can shift oxygen rich surface water to deeper regions of the pond, thereby expanding the facultative zone, as anaerobic bacteria can not live in the presence of oxygen (Green et al. 1995a).

The combination of aerobic and anaerobic wastewater treatment processes in a facultative pond has several advantages. Nuisance and odorous gases, like H_2S produced in the anaerobic zone have to pass through the aerobic surface layer where they are oxidised to less harmful and odorous products before reaching the atmosphere (Metcalf and Eddy 1991). The high exposure to sunlight and aerobic surface conditions in facultative ponds aid the removal of nutrients by assimilation into algal biomass or by pH mediated processes such as ammonia volatilisation and phosphate precipitation, and removal of pathogens by solar-UV disinfection (Pearson et al. 1987; Gomez et al. 1995; Craggs et al. 2000; Mara 2005).

1.2 Advanced Systems

Over decades scientists and engineers have tried to enhance the wastewater treatment performance of conventional waste stabilization ponds (in terms of solids, BOD and nutrient removal and improved disinfection) as well as to enable reclamation of valuable resources (nutrients, irrigation water and energy) from the wastewater during the treatment process (Abeliovich 1982; Oswald 1991; Benemann 2003).

1.2.1 Advanced Facultative Ponds

One of these improved designs is the "advanced facultative pond" (AFP) developed by Oswald and co-workers in California (Green et al. 1996). The key element of an AFP is the "in pond digester" or "fermentation pit" which is essentially a 3 to 4 m deep anaerobic pond within a 3 to 4 m deep facultative pond (Oswald 1991; Green et al. 1995a).

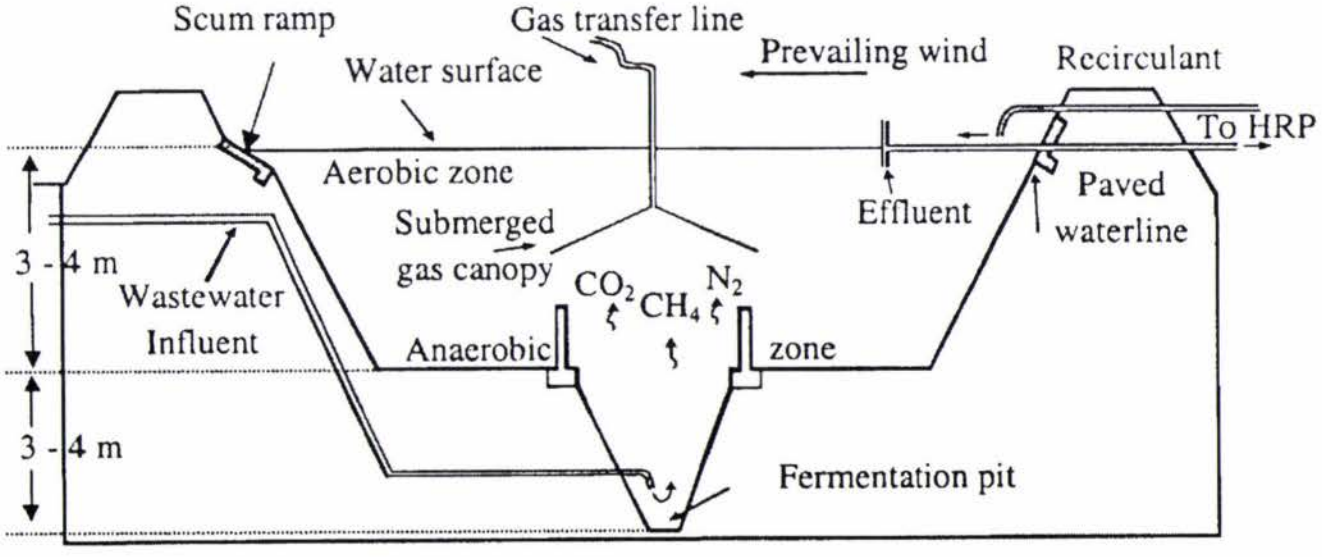

Figure 1.2: Schematic drawing of an advanced facultative pond (AFP) (after Craggs 2002c).

Anaerobic degradation of sewage solids in conventional facultative ponds can be negatively affected at times of strong wind mixing, when oxygenated surface waters are carried deep into the anaerobic zone (Green et al. 1996). Therefore the fermentation pit, which creates an isolated environment almost completely protected from the oxygen rich surface waters,

enables anaerobic processes to proceed without inhibition. Raw wastewater is introduced to the pond near the bottom of the fermentation pit so that the majority of suspended solids settle within the fermentation pit where they are subjected to intense anaerobic degradation. A sludge blanket is formed within the fermentation pit (as wastewater solids are re-suspended by gas bubble formation and then resettle when the gas bubbles break away). By passing through the sludge blanket at a low up-flow velocity (<2.5 m / day), the influent wastewater is brought into close contact with the anaerobic bacteria to enhance anaerobic treatment (Oswald 1991; Green et al. 1995a; Green et al. 1995b). The biogas yields from wastewater solids digested in AFP (up to 0.22 L CH_4 / g VS introduced, ~0.3 L biogas / g VS, Green et al. 1995b) are comparable to biogas yields obtained in anaerobic sludge digesters (Metcalf and Eddy 1991).

The relatively small surface area of a fermentation pit compared to a conventional facultative pond makes biogas capture much simpler and less expensive. This may be achieved using a submerged gas collector (Figure 1.2), which is not only protected from sun and wind by the overlaying water column, but also provides for some biogas purification (scrubbing) (Green et al. 1995a; Green et al. 1995b). While passing through the water column some of the CO_2 and trace gases in the biogas dissolve in the pond water, producing a biogas of more than 75% methane (CH_4), which may be used to generate electricity without further purification (Oswald 1991; Green et al. 1995a; Green et al. 1995b).

Several studies have shown that the composition of biogas from an advanced facultative pond differs from the biogas obtained from anaerobic mesophillic or thermopillic sludge digesters, especially in terms of the nitrogen gas concentration (Oswald 1988; Green et al. 1995a; Green et al. 1996). The increased level of nitrogen gas probably results from a process known as heterotrophic nitrification – denitrification in which organic nitrogen compounds are directly converted to nitrogen gas (N_2) thereby providing for some tertiary treatment, in terms of nitrogen removal (Green et al. 1995a; Green et al. 1996). Oswald (1988) states that in mature ponds 50% to 75% of the influent organic nitrogen concentration can be removed via this treatment mechanism.

1.2.2 High Rate Algal Ponds

High rate algal ponds (HRAP) were originally designed by Oswald and co-workers at the University of California at Berkeley (Moutin et al. 1992; Chen et al. 2002) This type of pond enhances algal growth and photosynthetic oxygen production for the aerobic wastewater treatment.

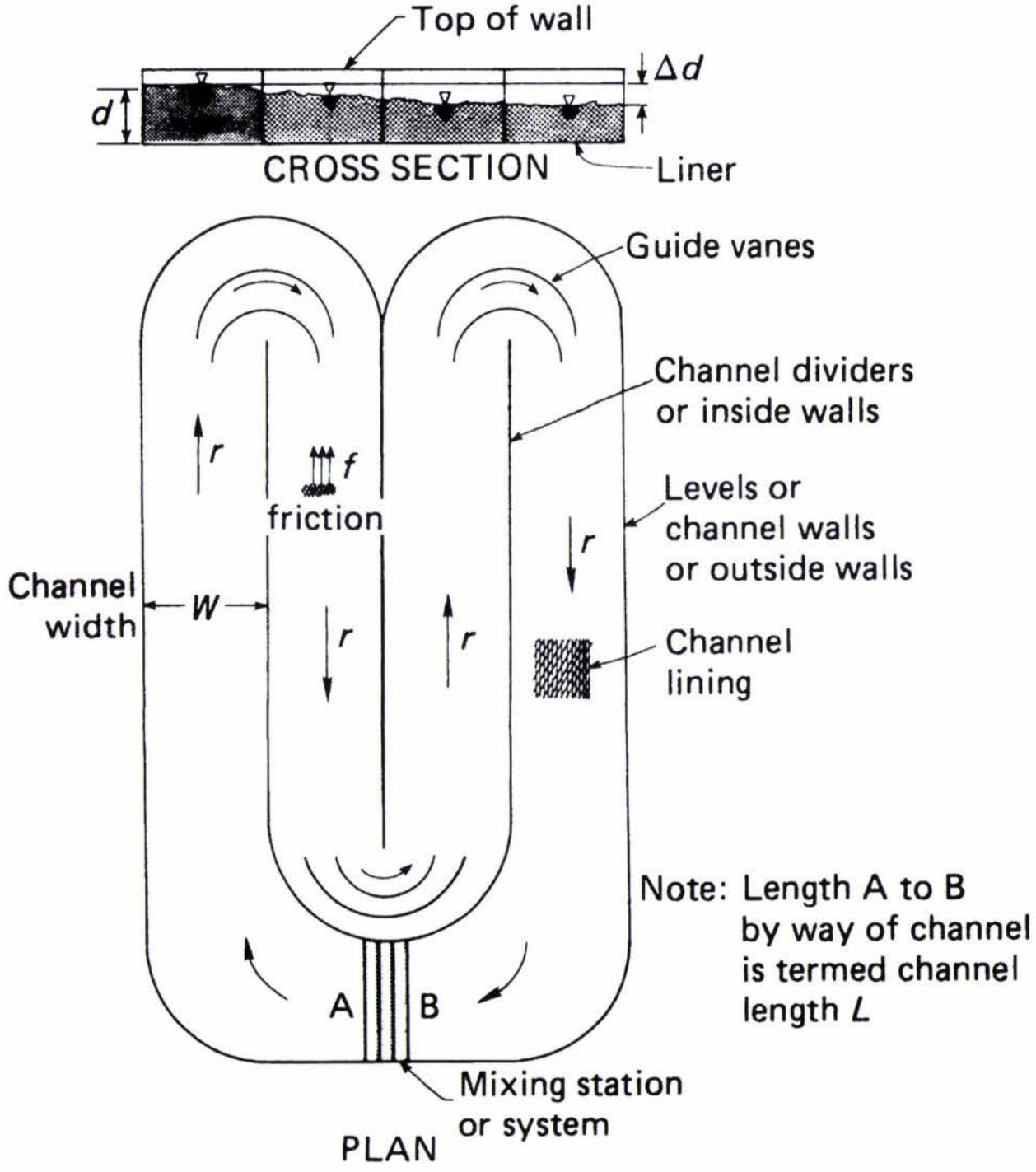

Figure 1.3: Schematic drawing of a high rate algal pond (HRAP) (from Oswald 1988).

The most common design for a HRAP is the meandering channel raceway (Figures: 1.3 and 1.4), around which the pond water is circulated at 15 to 20 cm / s by a paddlewheel (Oswald 1988; Craggs et al. 2002a). The paddlewheel promotes turbulent mixing to combine the influent wastewater (typically AFP effluent or primary settled sewage) with the algal laden pond water and prevent stratification. HRAP are shallow (20 to 80 cm deep, typically ~0.3 m for New Zealand applications) and have a short retention time (3 to 10 days, typically ~8 days for New Zealand applications), depending on the influent wastewater strength and environmental factors (solar insolation and temperature) that control algal growth (Oswald and Golueke 1960b; Moutin et al. 1992; Craggs et al. 2002b).

Figure 1.4: A large (~5 ha) paddle wheel mixed, meandering high rate algal pond treating municipal wastewater at Hollister, California (from Benemann 2003).

1.3. Processes in High Rate Algal Ponds

1.3.1 Photosynthetic Oxygenation and Algal Nutrient Uptake

The turbulent mixing in the paddlewheel mixed HRAP provides even exposure of the whole water column to the sunlight to enhance algal growth and photosynthesis (Oswald 1988). Enhanced oxygen production by algal photosynthesis during daylight hours (called photosynthetic oxygenation) drives aerobic breakdown of dissolved organic matter (Figure: 1.5) and is a cost effective method of achieving secondary treatment (Oswald 1991; Green et al. 1995b). Dissolved organic matter is broken down to CO_2 and nutrients including NH_4-N and phosphate, which are taken up by growing algae (Figure: 1.5).

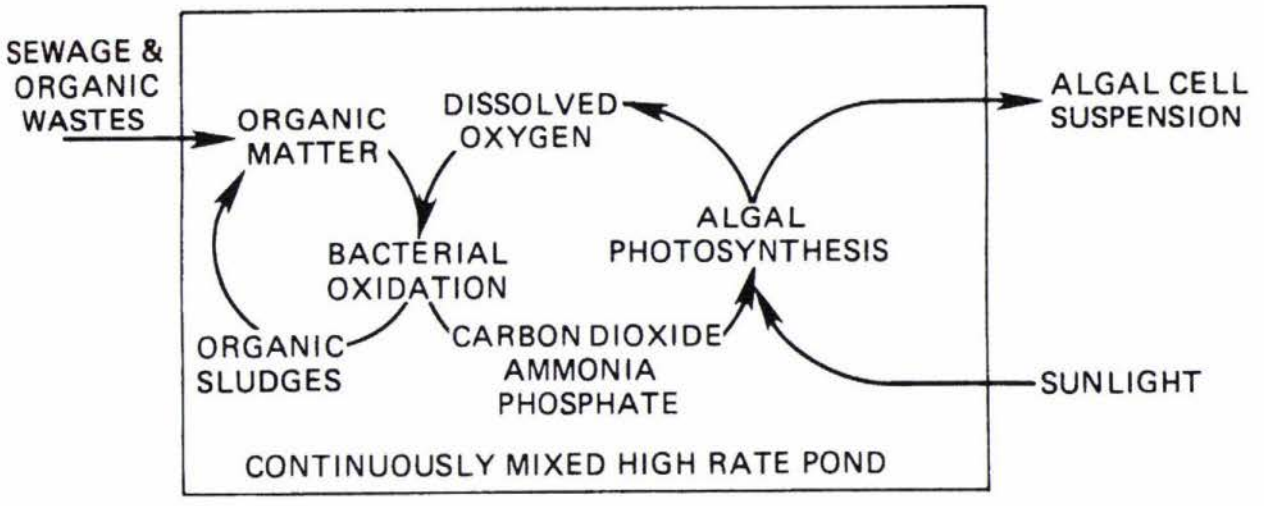

Figure 1.5: Schematic drawing of Photosynthetic oxygenation, the symbiotic relationships between algae and bacteria in a high rate algal pond (from Oswald 1988).

Nutrients are assimilated into algal biomass at an N:P ratio of ~7:1 which reflects the typical chemical composition of algal cells (e.g. 52.4% C, 29.7% O, 9.2% N, 7.4% H, and 1.3% P; Oswald 1988). Although some reports indicate that under special environmental conditions nutrient assimilation by algal biomass is more flexible, and phosphorus uptake in particular can be increased considerably, by more than a factor of 3 (Benemann 2003; Powell et al. 2005). Since the N:P ratio in municipal wastewater is typically ~5:1 (Metcalf and Eddy 1991), nitrogen is generally the nutrient that limits algal growth (as the relative supply of phosphorus is greater) and phosphate cannot be removed by algal assimilation alone, unless blue-green algae (which fix nitrogen from the atmosphere) are present (Fitzgerald and Rohlich 1964;

Benemann 2003). Algal growth (and associated nutrient uptake) varies widely with location and season. Goldman (1979) reports a wide yield range of wastewater grown algae between 9.1 and 27 gDM/m^2*d for systems in Israel and California. However under New Zealand conditions average yields are lower, ranging between 5 and 15 gDM/m^2*d (pers. comm. Rupert Craggs 2006).

Harvest of the algae from HRAP effluent provides tertiary treatment of the wastewater through removal of nutrients as algal biomass (Oswald et al. 1953; Oswald 1960a; Oswald 1988; Oswald 1991). This may be easily achieved using algae settling ponds, where the algal biomass is separated from the treated effluent by settling. Alternatively more sophisticated systems like dissolved air flotation, sand bed filtration or centrifuges can be employed (Oswald 1988). Algal biomass from wastewater treatment high rate algal ponds has many potential uses including: fertilizer (Oswald 1991; Green et al. 1996; Benemann 2003), feedstock for methane digestion (Oswald and Golueke 1960b; Samson and Le Duy 1982; Sanchez Hernandez and Travieso Cordoba 1993), or animal feed (Becker 1988; Green et al. 1996). High value chemicals (vitamins, dyes, and biodiesel) may also be extracted from algal biomass (Borowitzka 1988; Benemann 1994; Sheehan et al. 1998).

The algae in HRAP assimilate CO_2 from the pond water while photosynthesising, but during periods of intensive photosynthesis (high solar insolation and temperature) the algae require more CO_2 for photosynthesis than the aerobic bacteria can produce (Goldman et al. 1972; Brewer and Goldman 1976; Benemann 2003). When pond water CO_2 levels are depleted, the algae assimilate bicarbonate which causes a shift in the carbon dioxide - bicarbonate – carbonate equilibrium (Figure: 1.6) and leads to elevated pH levels in the pond (Goldman et al. 1972; Brewer and Goldman 1976; Goldman et al. 1982a; Oswald 1991; Green et al. 1996). This algal mediated pH elevation of the pond water contributes to several other processes that influence the wastewater treatment efficiency of HRAP.

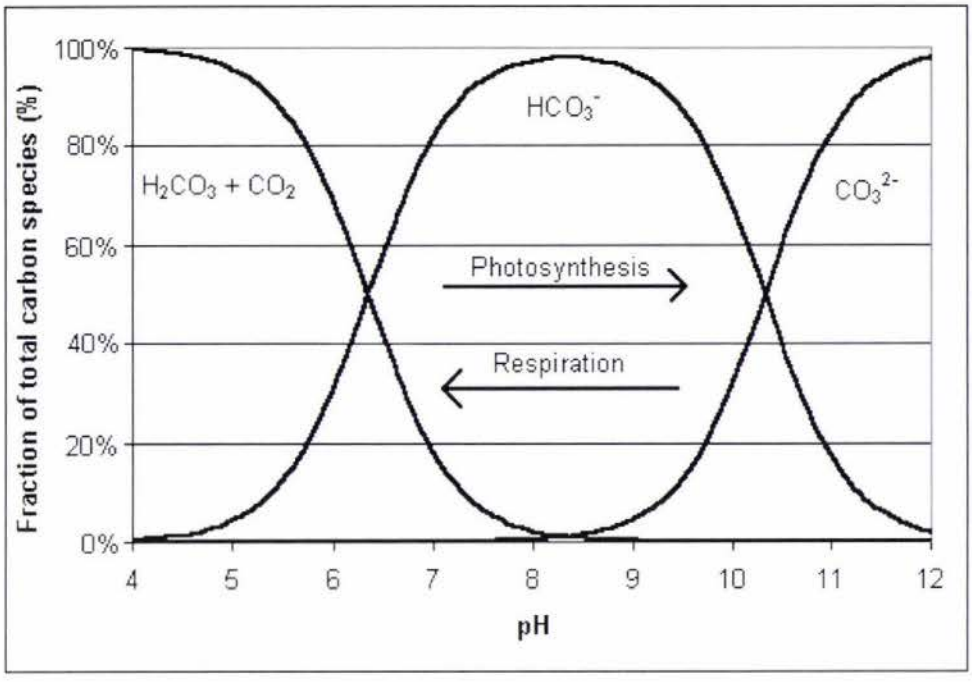

Figure 1.6: The relationship between the relative mole fractions of the inorganic carbon species in the carbon dioxide – bicarbonate – carbonate equilibrium and pH (after Goldman et al. 1972).

1.3.2 Ammonia Volatilisation

Ammonia volatilisation or ammonia stripping, the out gassing of NH_3 from the pond water to the atmosphere can occur at elevated pH levels as the NH_4^+ / NH_3 equilibrium is shifted in favour of free ammonia (NH_3). The amount of ammoniacal-nitrogen present as NH_3 is directly proportional to the pH and temperature of the pond water (Figure: 1.7). At 25°C the proportion of ammoniacal-nitrogen present as free ammonia increases from ~35% at pH 9 to ~85% at pH 10 (Konig et al. 1987), allowing for a greater quantities of NH_4-N to volatilise.

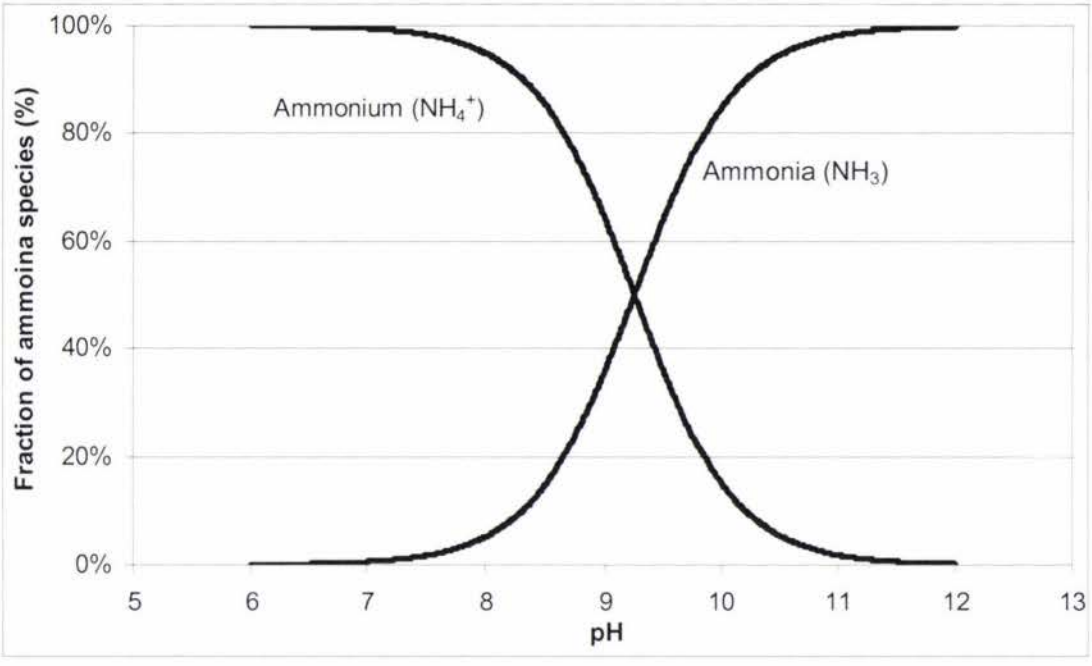

Figure 1.7: The relative proportions (%) of ammonia (NH_3) and ammonium (NH_4^+) in aqueous solution as a function of pH at 25°C (after Konig et al. 1987).

The relatively high pH and temperature of HRAP water combined with the large surface area and constant mixing of these ponds can enable significant nitrogen removal through NH_3 volatilisation (Gomez et al. 1995; Nurdogan and Oswald 1995; Green et al. 1996; Chen et al. 2002). However there is conflicting literature on the actual contribution of ammonia volatilisation to overall ammoniacal-nitrogen removal in HRAP. Green et al. (1996) found that volatilisation may only have accounted for 15% of the total NH_4-N removal from a Californian HRAP during the summer, while Chen et al. (2002) concluded that volatilisation contributed to most of the NH_4-N removal from a HRAP in China, especially when temperatures and solar insolation were high in summer. It is however noted that the contribution of ammonia volatilisation to overall NH_4-N removal will be lower during the colder winter months when pH levels in the pond water are much lower.

1.3.3 Phosphate Precipitation

Under high pH conditions phosphate reacts with metal cations such as Ca^{2+}, Mg^{2+} and Fe^{3+} to form relatively insoluble salts (Table: 1.1) which precipitate and accumulate on the pond bottom or coagulate with the algae cells (Nurdogan and Oswald 1995; Chen et al. 2002).

Table 1.1: Solubility products of calcium and magnesium salts at standard conditions (from Nurdogan and Oswald 1995).

Compound	Chemical Formula	pK_{so}
Phosphate	$CaNH_4PO_4$	115.0
	$Ca_5(OH)(PO_4)_3$	52.8
	$Ca_3(PO_4)_2$	28.1
	$Ca_2(OH)_2HPO_4$	27.3
	$CaHPO_4$	7.0
Hydroxide	$Mg(OH)_2$	10.5
Carbonate	$CaCO_3$	8.3
	$MgCO_3$	7.6
Sulphate	$CaSO_4$	4.6

Thus phosphorus precipitation in a HRAP is dependent upon the wastewater pH and cation concentration (Nurdogan and Oswald 1995). Precipitation generally occurs at pH greater than 8 (Fitzgerald and Rohlich 1964), although this is dependent upon the amount and ratios of cations available for precipitation (Moutin et al. 1992; Nurdogan and Oswald 1995). Some wastewaters may therefore require higher pH levels for a significant amount of phosphorus precipitation to occur. Night-time decline in HRAP pH to below pH 8 can result in resolution of some of the precipitates that were formed during the day (Fitzgerald and Rohlich 1964) and therefore reduce overall phosphorus removal efficiency (Bush et al. 1961; Hemens and Mason 1968; Oswald 1988; Garcia et al. 2006).

Increasing the pond water cation concentration, for example by addition of small amounts (e.g. 20 to 60 mg / L) of slaked lime (CaO) has been shown to an improve removal of phosphorus through precipitation (Nurdogan and Oswald 1995). Under these conditions polyphosphate and organic phosphorus may also be removed by adsorption to calcium carbonate ($CaCO_3$) crystals. Nurdogan and Oswald (1995) found that lime addition to HRAP with pH levels between 9 and 11 achieved ~100% removal of phosphate compared to ~50% removal in controls with similar pH. However, such high pH levels and therefore phosphorus precipitation are not always attainable in HRAP, especially during the winter months.

1.3.4 Disinfection

Much of the early literature discusses the importance of elevated pond water pH for disinfection in WSP (Pearson et al. 1987; Oswald 1991; Green et al. 1996). However, recent research on the mechanisms of disinfection for various indicator and pathogenic bacteria and viruses has shown that solar UV-radiation is the main cause of disinfection, with high pond water DO and pH levels augmenting the disinfection process for particular organisms (Davies-Colley et al. 2002; Davies-Colley 2005). Removal of many pathogens, particularly viruses, is unaffected by pond water pH, although the 'indicator of choice' for faecal contamination, *Escherichia coli* (*E. coli*) is susceptible to elevated pond water pH levels (Davies-Colley et al. 2002; Davies-Colley 2005).

Moreover, other pond disinfection processes such as protozoan grazing may be limited by the high pH environment of HRAP which is inhospitable to many protozoa. However, biological disinfection processes in particular are still poorly understood (Stott et al. 2005).

1.3.5 Ammonia Toxicity

Ammonia toxicity, due to high pond water NH_3 concentrations, can inhibit algal growth. Azov and Goldman (1982b) demonstrated, that the increase in free ammonia levels due to the shift in the NH_4^+ / NH_3 equilibrium at high pH (Figure: 1.7) can cause an almost complete inhibition of algal photosynthesis. For several marine and freshwater algae species tested at 25°C, the NH_4-N concentration required to achieve a 50% reduction in algal photosynthesis was 24 mg/L at pH 10.0, which was more than 15 times less than the concentration required at pH 8.0 (Azov and Goldman 1982b). However, Konig et al. (1987) observed that some algae species such as *Chlorella sp.* are inherently more tolerant of high free NH_3 levels than others e.g. *Euglena sp.*

Furthermore the productivity of *Scenedesmus sp.*, a dominant alga in HRAP, has been shown to be lower when grown at high pH with NH_4-N as a nitrogen source compared to nitrate-N, although the utilisation of nitrate-N is more energy intensive and should therefore lead to lower overall yields (Soeder and Hegewald 1988). Thus for HRAP treating wastewater with relatively high NH_4-N levels, pH control may be the only effective method of mitigating the effects of NH_3 toxicity on algal growth (Azov and Goldman 1982b).

1.3.6 pH Inhibition of Bacterial Growth

Aerobic bacteria play a vital role in wastewater treatment HRAP. They live in a symbiotic relationship with the algae, using photosynthetically derived oxygen to break down dissolved organic matter (providing efficient BOD removal) and release CO_2 and nutrients that are then assimilated by the algae (Oswald et al. 1953; Oswald 1960a; Oswald and Golueke 1960b). The optimum pH for the growth of aerobic bacteria in wastewater is around pH 8.3 and bacterial activity is increasingly inhibited at higher pH levels (Oswald et al. 1957; Oswald 1960a; Oswald 1988).

To prevent inhibition of bacterial growth at high pH levels Nurdogan and Oswald (1995) suggest to mix HRAP in a fashion which keeps algae in suspension, but allows for the establishment of a pH gradient between the pond surface (pH 9 to 11) and the pond bottom (pH 7 to 8). However this is not easily achieved in practise.

1.3.7 pH Inhibition and Carbon Limitation of Algae

Most green algae species grow optimally at around neutral pH (Bush et al. 1961; Goldman et al. 1982a; Soeder and Hegewald 1988). Thus, HRAP water pH may restrict algal growth by inhibiting cell metabolism and by nutrient limitation due to the precipitation of essential minerals (Goldman et al. 1972).

As discussed above, high pH levels are an indicator of low CO_2 concentrations in the pond water. At elevated pH levels bicarbonate becomes the major carbon source available for algal photosynthesis (Goldman et al. 1972; Goldman and Graham 1981; Goldman et al. 1982a,). Several algal species have been shown to have difficulty utilising bicarbonate as carbon source, resulting in reduced productivity in cultures with high pH (Goldman and Graham 1981; Azov1982a; Azov et al. 1982c).

1.4 Combining Ponds

Combining different pond types (each with particular dominant wastewater treatment processes) in series will increase the treatment performance and reliability of a pond system (Oswald 1991). One such combination is the Advanced Integrated Wastewater Pond System (AIWPS) consisting of an advanced facultative pond, a high rate algal pond, algae settling ponds and one or more maturation ponds for final polishing of the effluent (Figure: 1.8).

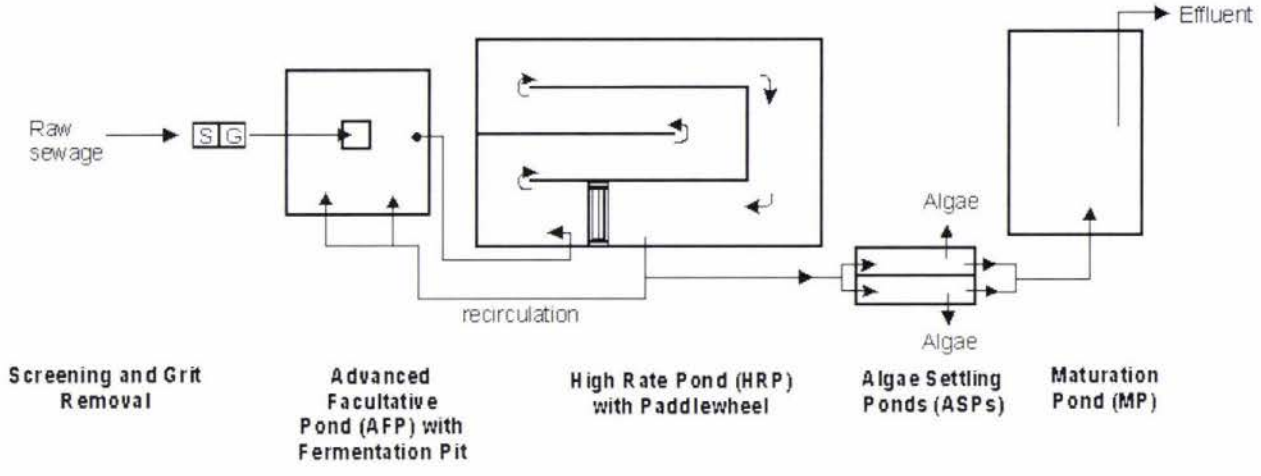

Figure 1.8: Schematic drawing of an Advanced Integrated Wastewater Pond System (from Craggs et al. 2000).

Linking an advanced facultative pond with a HRAP has many advantages (Oswald 1991; Green et al. 1995a):

- Pre-treatment of raw sewage in the AFP allows for effective reclamation of biogas energy, and provides the HRAP with an influent rich in readily available plant nutrients and low in BOD, which allows the HRAP to work with shorter retention times.
- High rate algal pond water, high in pH and rich in DO is re-circulated to the surface of the AFP were it provides for the improved control of odour emissions (Oswald 1991; Green et al. 1995a).
- An actively growing algae population on top of the AFP constantly re-seeds the HRAP with desirable algae, thereby stabilising the algae dominated ecosystem in the HRAP.
- Algal biomass harvested from the HRAP effluent e.g. in an algae settling pond, can be transferred back to the advanced facultative pond and utilised for biogas fermentation if nutrient removal is unimportant (Green et al. 1995a).

A further link between an advanced facultative pond or anaerobic pond and the high rate algal pond would be to use HRAP water to scrub the biogas released from the digestion of the wastewater. This concept was first discussed by Oswald and Golueke (1960b) who described three potential benefits. Firstly, a methane enriched biogas with favourable handling and utilisation characteristics would be obtained as most of the carbon dioxide, hydrogen sulphide and trace gas content would be absorbed into the HRAP water. Secondly, the transfer of CO_2 into the HRAP water would overcome carbon limitation of the algae at times of vigorous algal growth, and thereby increase algae productivity, nutrient assimilation and wastewater treatment. Thirdly the reduction in pond water pH to more neutral levels by CO_2 addition may further stimulate aerobic bacterial degradation of the waste (Oswald 1960a).

1.5 Biogas Scrubbing

1.5.1 The Need for Biogas Purification

Corrosive gases such as hydrogen sulphide (H_2S) can cause considerable damage to gas collection and handling equipment unless they are constructed from inert material (e.g. plastic or stainless steel). High carbon dioxide (CO_2) and nitrogen (N_2) levels dilute the energy value of biogas and increase storage and handling costs due to increased volume (Weiland 2003; Hofmann et al. 2005).

Biogas has many uses and each requires specific minimum purity levels. Raw biogas can be reliably used for heating purposes in boilers and gas burners but engine generators require more frequent oil changes and may have reduced life spans due to elevated H_2S concentrations. Most engine generator manufacturers recommend a maximum H_2S concentration between 200 and 250 ppm in the biogas for efficient and satisfactory operation, although modified engines with special oil additives can tolerate concentrations of up to 2000ppm (Weiland 2003).

Export of biogas to the public gas network requires that the impurity standards for transportable gas are met, which vary between countries (Hofmann et al. 2005). For example, in Austria these are: H_2S: <3.3 ppm, CO_2 <2% and N_2 <5 % (Ahrer 2005). Similar levels of purity are required if biogas is to be utilised as vehicle fuel as it has to be compressed with rather sophisticated machinery and stored at high pressure (Worley consultants 1986; Hofmann et al. 2005). Hydrogen sulphide has to be removed almost completely to prevent corrosion of the gas handling equipment and the vehicle. Carbon dioxide also has to be removed almost completely as it requires energy for compression and storage capacity without yielding any benefit and can cause freezing problems in parts of high pressure gas equipment where expansion takes place (Worley consultants 1986).

1.5.2 Conventional Biogas Purification Systems

Over the years various biogas purification systems have been developed employing chemical, physical and biological processes. Iron based filters are commonly used to scrub H_2S from small volumes of biogas, converting it to iron sulphides (FeS and Fe_2S_3) which may be subsequently oxidised to elemental sulphur (S) (Hofmann et al. 2005). Biochemical scrubbing methods include the injection of small amounts of air into a biogas stream. Microbes (often cultured in a dedicated scrubbing device) can be used to oxidise the H_2S to elemental sulphur (S) and subsequently sulphate (SO_4^{2-}) (Hofmann et al. 2005). Physical methods of H_2S and CO_2 removal are used where large biogas flows have to be purified and include processes such as: pressure swing absorption on zeolithes or silica gel; absorption to active carbon; membrane techniques; and pressure washing (Worley consultants 1986; Hofmann et al. 2005). Most of these processes only provide partial biogas purification, require significant capital investment and ongoing operation costs, and do not have secondary benefits.

1.5.3 Biogas Purification Using Algal Wastewater Cultures

Several studies indicate the potential to use the highly alkaline and oxygenated water from HRAP to completely scrub impurities from biogas. Conde et al. (1993) and Travieso et al. (1993) used an algal wastewater culture to scrub biogas in a laboratory-scale scrubbing device, achieving final methane concentrations of 97% and reducing H_2S levels from 1% to <0.5%, Algal productivity was also increased by 2 to 5 times. Green et al. (1995a) have demonstrated partial scrubbing of biogas within the alkaline and oxygenated surface water of a pilot-scale advanced facultative pond.

These reports do not indicate that the concentrations of H_2S contained in the biogas had negative effects on the algae cultures used, although other authors (Gloyna and Espino 1969) report about sulphide toxicity in algae wastewater cultures at low H_2S concentrations.

Mandeno (2003) showed that HRAP water could be used to reduce the CO_2 content of synthetic biogas from 40% to below 5% in a counter current scrubbing device. He also observed that the biogas took up oxygen from the HRAP water while being scrubbed, which could lead to improved combustion characteristics.

1.6 Benefits of Carbon Addition for Algae Cultures

1.6.1 Carbon Dioxide and Wastewater Algae Cultures

Enhancing algal production by addition of CO_2 to algae cultures has been subject of research dating from the earliest experiments on culturing algae. Laboratory algae wastewater cultures sparged with CO_2 were shown to have higher photosynthetic efficiencies and productivities compared to controls without CO_2 addition, especially at prolonged retention times (Oswald 1960a; Fitzgerald and Rohlich 1964).

Bush et al. (1961) concluded that maximum algal productivity in an experimental outside algae wastewater pond could only be achieved if additional CO_2 was added. Moreover Azov et al. (1982c) found that CO_2 addition to an outside pilot-scale HRAP in Israel more than doubled algae production compared to a control pond without CO_2 addition. Enhancement of algal production was also found to increase with longer retention times and reduced organic carbon content of the influent wastewater.

1.6.2 Carbon Dioxide Addition in Algae Farming Operations

The algae farming industry began during the 1950's when many scientists hoped to grow algal biomass as a protein source for humans and animals. Although this was never realised, the intensive research during this time laid the foundations for a highly sophisticated and specialised industry (Goldman 1979). Today the main products from algae farming industry are health foods and vitamins or high value chemicals (Borowitzka 1988; Oswald 1988) but the industry is far from reaching its potential, particularly as only a few of the several thousand known algal species are commercially produced Borowitzka (1988). Algal species that are commonly farmed include *Spirulina sp., Dunaliella sp.* and *Haematococcus sp.* (Benemann 2003).

Figure 1.9: Algae farming in high rate algal ponds at Cyanotech Corp., Kona, Hawaii (from Pedroni et al. 2001).

Most algae farming operations use HRAP to enhance algal production per hectare of pond area (Figure: 1.9). The algae are grown on specific nutrient media so that growth is only limited by light, temperature and CO_2 (Goldman 1979; Benemann 2003). Therefore, to further increase production, several farms have incorporated CO_2 addition to both reduce carbon limitation (increasing production following von Liebig's law of the minimum) and maintain the optimum pond water pH for algal growth (Goldman et al. 1972; Benemann 2003).

Several special devices are used to add CO_2 to the algal culture (Goldman 1979; Benemann 2003). For example, Cyanotech Corp. at Kona, Hawaii (Figure: 1.9) uses a counter current tower to add CO_2 from the exhaust stream of an engine generator to HRAP water which is subsequently distributed to the HRAP according to their carbon demand (Benemann 2003). The volumes of exhaust gas or flue gas contaminants like NO_x and SO_x transferred to the algae ponds through such operations, have been shown to have no negative effects on the algae population (Sheehan et al. 1998). In other cases industrial grade, bottled CO_2 is sparged into the HRAP, often using a counter current pit (Figure: 1.10)

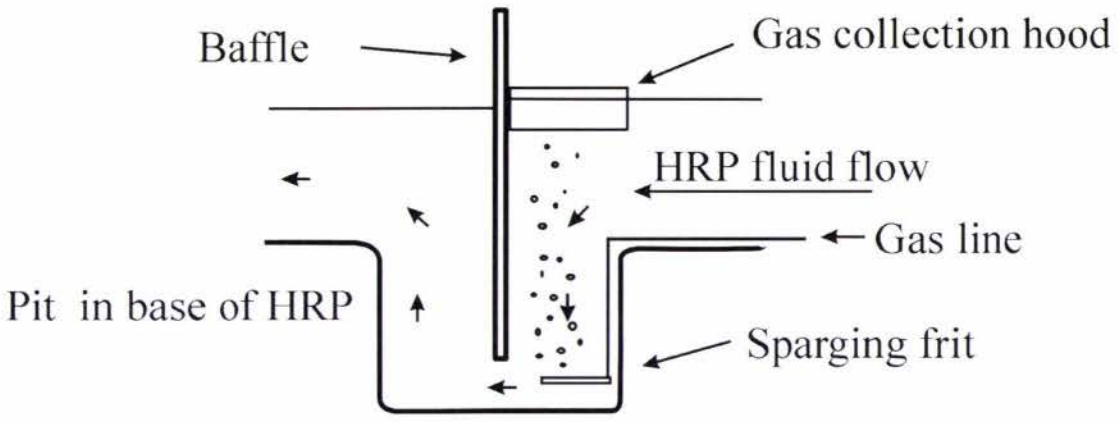

Figure 1.10: Schematic drawing of a counter current pit for carbonation and biogas purification (after Mandeno 2003).

Counter current pits (~1 m depth) are normally constructed across the HRAP channel and have a baffle in the centre that forces the HRAP water down into the pit on the upstream side of the baffle and back up into the pond on the downstream side. Carbon dioxide bubbles are sparged near the bottom of the pit on the upstream side of the baffle and have to rise against the down current of HRAP water. Thus, for a particular pit depth and pond water flow, decreasing the CO_2 bubble size will increase the time the bubble remains in the water column and the overall CO_2 transfer efficiency. Efficiencies as high as 70% have been obtained (Oswald 1988). The principle of a counter current pit with gas recapture has been suggested by Mandeno (2003) for biogas scrubbing.

Other CO_2 addition devices include pressurised CO_2 cushions floating on the pond surface and delivering the CO_2 into the pond by means of diffusion (Bush et al. 1961; Heussler et al. 1978). If pumps are used to mix the algae production ponds then the CO_2 can be directly injected into the pumped stream of pond water, or if an air-lift pump is used, CO_2 can be blended with the air required to achieve the hydraulic head (Goldman 1979).

1.7 Theoretical Calculation of HRAP CO_2 Assimilation Potential

To fully understand the potential to use wastewater treatment HRAP to assimilate CO_2 from the biogas produced by the anaerobic digestion of the raw wastewater and other CO_2 sources such as exhaust or flue gases, a simple model of the carbon mass balance in a wastewater HRAP has been used. This model should assist with interpreting the results from the small-scale experiments conducted in this thesis and extrapolating them to full-scale systems.

1.7.1 Simple Model of the Carbon Mass Balance in a Wastewater HRAP

Green et al. (1996) described the carbon mass balance for a hypothetical advanced integrated pond system treating municipal sewage. The authors assumed that the HRAP works as a carbon transformer exporting carbon in the pond effluent in a rough ratio of 10 organic carbon (~91%) (mainly algal biomass) to 1 inorganic carbon (~9%) (mainly bicarbonate alkalinity) (Figure: 1.11). To achieve these carbon exports a slightly higher amount of carbon (to make up for CO_2 losses from the HRAP during times without active algal growth) has to be assimilated from sources including the influent wastewater organic carbon (~40%) (mainly dissolved organic matter), influent wastewater inorganic carbon (~40%) (mainly bicarbonate alkalinity), and atmospheric carbon dioxide (~23%) harnessed through diffusion at times of high HRAP pH.

Using a very simplistic model where CO_2 addition continually adjusts the pH of the HRAP to near neutral (pH ~7), it can be assumed that carbon diffusion from the atmosphere into the pond water and the use of bicarbonate alkalinity by the algae are reduced to very low levels. In this case only the organic carbon contained in the wastewater influent, and added CO_2 originating from biogas scrubbing are carbon sources for the algae.

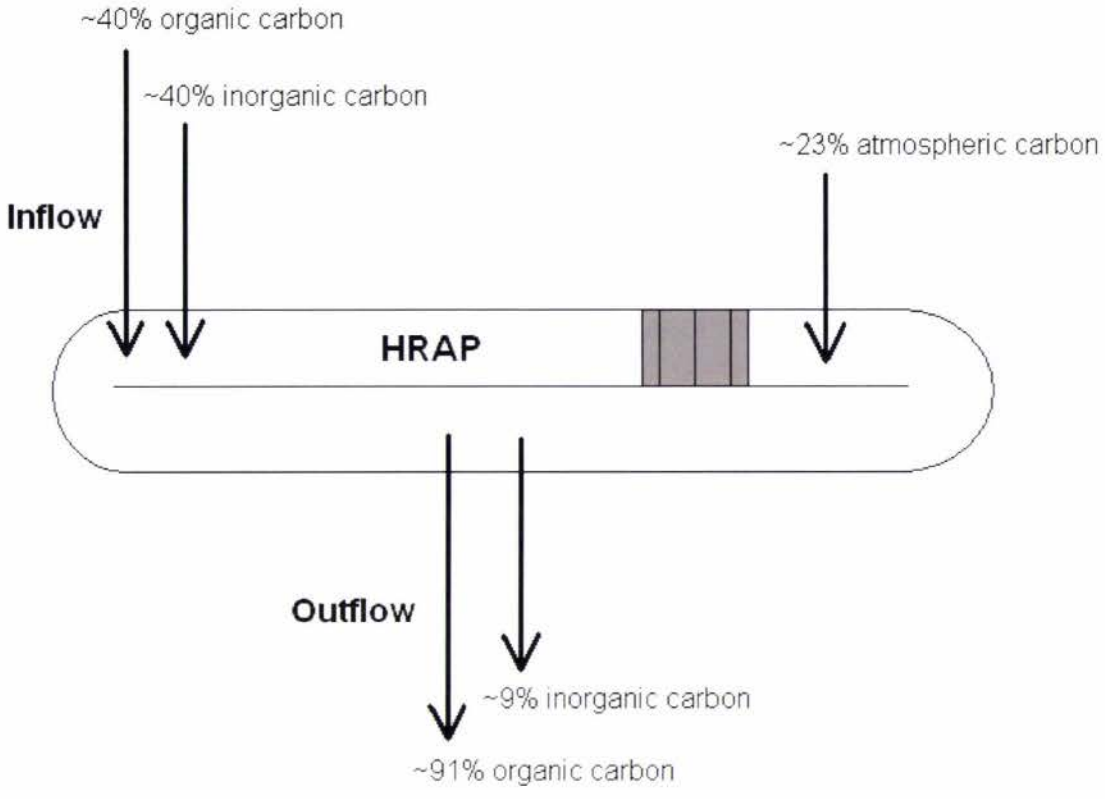

Figure 1.11: Schematic drawing of carbon transformations in a HRAP (based on Green et al. 1996).

1.7.2 Estimation of Carbon Assimilation

Based on the assumptions mentioned above, the maximum carbon assimilatory capacity ($C_{max.}$) of a HRAP can be calculated as:

$$C_{max} = C_{ass} + C_{assCO2} + C_{loss} \qquad \text{Equation (1)}$$

Where:

C_{ass} Carbon assimilated into algae biomass

C_{assCO2} Carbon assimilated into additional algae biomass as a result of CO_2 addition

C_{loss} Carbon losses through diffusion to the atmosphere due to night-time respiration

The total CO_2 diffusion loss (C_{loss}) was assumed to equal the pond water DO concentration at night fall. Dissolved oxygen losses to the atmosphere and atmospheric oxygen uptake by the pond water during the night were neglected, and it was assumed that anaerobic breakdown of organic matter does not occur in the HRAP during the night (Garcia et al. 2006).

The maximum summer-time CO_2 assimilation potential of a municipal wastewater treatment HRAP receiving digested wastewater from an anaerobic treatment stage, located in the upper North Island of New Zealand is calculated using data provided in Table 1.2. These assumptions are based on monitoring data from pilot-scale HRAP at the Ruakura experimental facility (Mandeno 2003) and literature on integrated wastewater treatment ponds and HRAP (Goldman 1979; Goldman et al. 1982a; Oswald 1988; Green et al. 1995a; Green et al. 1995b; Green et al. 1996).

Table 1.2: Assumptions for modelling the maximum summer-time CO_2 assimilation potential of a municipal wastewater treatment HRAP receiving digested wastewater from an anaerobic treatment stage, located in the upper North Island of New Zealand.

Parameter	Value	Units	Reference
Raw wastewater strength (VS)	250	mg/L	Mandeno (2003)
Carbon concentration in VS	50	%	Metcalf and Eddy (1991)
Biogas yield	0.3	L/g VS	Green et al. (1995b)
CO_2 concentration in biogas	35	%	Weiland (2003) (excluding the N_2 fraction found in AFP biogas)
Methane concentration in biogas	65	%	
HRAP retention time	8	days	Craggs et al. 2002a&b
HRAP depth	0.33	m	Craggs et al. 2002a&b
Algal growth			
Algae carbon content	50	%	Oswald (1988)
Daily algal growth	10.0	$g/m^2/d$	Based on Goldman (1979)
Daily carbon assimilation into algal biomass	5.0	$g/m^2/d$	
Additional algal growth due to CO_2 addition	3.0	$g/m^2/d$	Based on Goldman et al. (1982a)
Additional algal C assimilation due to CO_2 addition	1.5	$g/m^2/d$	
HRAP Influent organic C concentration (digested sewage)	3.5	$g/m^2/d$	
HRAP DO level at night fall	14	mg/L	Mandeno (2003)
Night-time CO_2 diffusion losses	1.7	$g/m^2/d$	

The maximum carbon load which can be assimilated by the HRAP can be calculated using Equation (1) (see Appendix A for detailed calculation):

$$C_{max} = 5.0gC/m^2/d + 1.5gC/m^2/d + 1.73gC/m^2/d = 8.23gC/m^2/d$$

1.7.3 Estimation of Biogas Production

Using the data given in Table 1.2, the biogas produced from the raw wastewater in an anaerobic treatment step preceding the HRAP can be calculated as 75 L biogas / m^3 of raw influent wastewater (see Appendix A for detailed calculation).

For the assumed CO_2 concentration in the biogas and the depth and retention time of the HRAP, this biogas production represents a CO_2-carbon supply of 0.58 g C / m^2 of HRAP / d.

Furthermore the digestion of wastewater solids leaves a fraction of organic carbon in the HRAP influent. This fraction was calculated to supply the HRAP with 3.50 gC/m^2/d of organic carbon (see Appendix A for detailed calculation).

1.7.4 Estimation of Biogas Scrubbing Potential

The maximum CO_2-carbon load (C_{maxut}) which can be assimilated by a HRAP with the given characteristics can be calculated using equation (2):

$$C_{maxut} = (C_{ass} + C_{assCO2} + C_{loss}) - C_{orgin} \qquad \text{Equation (2)}$$

Where:

C_{ass}	Carbon assimilated into algae biomass
C_{assCO2}	Carbon assimilated into additional algae biomass as a result of CO_2 addition
C_{loss}	Carbon losses through diffusion to the atmosphere due to night-time respiration
C_{orgin}	Organic carbon in the influent

For the data given in Table 1.2 the maximum CO_2 assimilation potential was determined (see Appendix A for detailed calculation) to be:

$$C_{maxut} = (5.0\ gC/m^2/d + 1.5\ gC/m^2/d + 1.73\ gC/m^2/d) - 3.5\ gC/m^2/d = 4.73\ gC/m^2/d$$

Assimilation of 4.73 $gC/m^2/d$ equates to 8.84 $L/m^2/d$ of CO_2 which is equivalent to scrubbing 25.25 $L/m^2/d$ of raw biogas (assuming a 35% CO_2 concentration and an uptake efficiency of 100%). Thus, the actual CO_2-carbon supply from the wastewater derived biogas (0.58 $gC/m^2/d$) will only satisfy 12% of the maximum CO_2-carbon sequestering (biogas scrubbing) potential of the HRAP and would therefore most likely have little influence on algal productivity.

This shortfall in CO_2-carbon supply from the wastewater derived biogas provides the potential to assimilate CO_2 from waste gases such as exhaust or flue gases. The data given in Table 1.2 indicates that the methane fraction of the biogas obtained during digestion of the raw wastewater in the anaerobic treatment step preceding the HRAP contains approximately twice as much carbon as the CO_2 fraction of the biogas. If the methane is utilised for energetic purposes on site, e.g. in an engine generator or boiler, the carbon fraction of the exhaust gases from the combustion of methane could also be assimilated in the HRAP. This would essentially triple the total amount of CO_2-carbon available to the HRAP to 35% of the maximum CO_2-carbon sequestering potential (see Appendix A for detailed calculation).

For the given case-study, this indicates that even if all carbon contained in the raw wastewater is assimilated within the HRAP a substantial (~65%) demand for carbon still exists. This shortfall could be met by using external CO_2 waste streams like flue gasses from coal or gas fired power plants (Benemann 1994; Pedroni et al. 2001; Benemann 2003).

1.7.5 Maximum Wastewater Strength

Treating higher strength wastewaters (industrial, agricultural or more concentrated municipal wastewaters) in a system consisting of an anaerobic digestion step followed by a HRAP would increase the carbon loading of the HRAP in two ways. Firstly, digestion of the higher strength wastewater would produce more biogas. This would increase the CO_2 supplied to the HRAP through biogas scrubbing. Secondly, the concentration of residual organic carbon in the HRAP influent would also be higher. High rate algal ponds treating high strength wastewaters usually have longer retention times and shallower depths than those treating municipal wastewater. However, in this simple example we calculate the maximum wastewater strength which would fully satisfy the carbon requirement of a HRAP assuming the dimensions of the HRAP remain the same (Table: 1.2).

Calculating the maximum wastewater strength is possible by modifying Equation (2):

$$C_{ass} + C_{assCO2} + C_{loss} = C_{biogasCO2} + C_{orgin} \qquad \text{Equation (3)}$$

Where:

C_{ass}	Carbon assimilated into algae biomass
C_{assCO2}	Carbon assimilated into additional algae biomass as a result of CO_2 addition
C_{loss}	Carbon losses through diffusion to the atmosphere due to night-time respiration
$C_{biogasCO2}$	Carbon fraction contained in the biogas CO_2.
C_{orgin}	Organic carbon in the influent

By assuming that C_{orgin} and $C_{biogasCO2}$ are directly proportional to each other, and using the data given in Table 1.2, the concentration of organic matter (VS) in the raw wastewater required to meet the total carbon demand of the HRAP was calculated to be 505 mgVS /L.

Some municipal wastewaters can be as concentrated as 500 mgVS/L or more (Metcalf and Eddy 1991; Mara 2002), whereas agricultural and industrial wastewaters are typically one to two orders of magnitude stronger (Metcalf and Eddy 1991; Craggs et al. 2002b, Mandeo 2003). This comparison clearly shows that biogas scrubbing and CO_2 sequestration in HRAP is feasible for municipal wastewater treatment systems only, as the CO_2 content of the large quantities of biogas produced through the digestion of agricultural and industrial wastewaters could not be assimilated by the subsequent HRAP.

1.8 Objectives

The preceding literature review and calculations indicate that biogas purification with HRAP water is both theoretically and practically possible. Although, extensive research has been carried out on enhancing algal production by CO_2 addition to HRAP, there is little information in the literature on how the addition of CO_2 from biogas scrubbing will influence the overall performance of HRAP used for wastewater treatment. Information on the consequences of CO_2 addition to HRAP water for all aspects of treatment performance (removal of BOD, nutrients and disinfection) is required before this further link between anaerobic and aerobic treatment stages in an integrated wastewater treatment system can be used at full-scale.

Therefore the objective of this thesis was to determine the influence of CO_2 addition on algal growth and treatment performance of HRAP treating municipal wastewater by monitoring algal productivity and species composition, the removal of BOD, nutrients (ammoniacal-N and DRP) and *Escherichia coli* (indicator bacteria of faecal pollution).

The specific aims of this study were:

1. To determine the effect of CO_2 addition and controlled culture pH on HRAP algal growth (indicated by TSS) and species composition in terms of changes in algal growth and dominant algal species or species morphology that would require alteration of pond operation.

2. To determine the effect of CO_2 addition and controlled culture pH on HRAP nutrient (NH_4-N and DRP) removal in terms of assimilation into algal biomass and the pH driven removal processes of ammonia volatilisation and phosphorus precipitation.

3. To determine the effect of CO_2 addition and controlled culture pH on HRAP BOD removal.

4. To determine the effect of CO_2 addition and controlled culture pH on HRAP disinfection in terms of removal of the indicator bacteria *E. coli*.

2. Materials and Methods

2.1 Analytical Methods

To evaluate the changes in treatment performance during our experiments, a range of chemical, physical and biological parameters were monitored in the algae wastewater cultures. This section gives details of the experimental procedures used.

2.1.1 Chemical Parameters

The analysis of all chemical parameters was carried out in the inorganic chemistry laboratory at NIWA Hamilton. All analyses followed standard laboratory methods given in the NIWA laboratory manual (Crump 2002) which is largely based on the Standard Methods for the Examination of Water and Wastewater (APHA 2000).

2.1.1.1 Total Suspended Solids (TSS)

Total Suspended Solids (TSS) concentrations provide a good estimate of the algal concentration in wastewater grown algal cultures (Fitzgerald and Rohlich 1964) and were used to estimate algal growth during these experiments. Measurement of TSS largely followed the APHA methods 2540D (APHA, 2000; Crump 2002).

2.1.1.2 Ammoniacal-Nitrogen

Ammoniacal-nitrogen (NH_4-N) typically makes up 75% of the total nitrogen concentration in Ruakura anaerobic digester effluent (NIWA 2002; Mandeno 2003) which was used as the algal culture growth medium and HRAP influent in these experiments. Since it is widely known that algae prefer to assimilate NH_4-N rather than other nitrogen forms (Goldman et al. 1982b; Green et al. 1996), NH_4-N analysis was used to measure nitrogen removal performance in these experiments.

Measurement of wastewater NH_4-N was by a manual phenol hypochlorite method, largely based on the APHA method 4500 D (APHA 2000). All analysis was carried out on samples that had been previously filtered through a glass fibre filter (0.45 µm Whatman GF/C) to remove suspended solids which interfere with this colorimetric test. Some filtered samples were frozen for extended periods of time before being analysed, which has been found to have no significant impact on the NH_4-N concentration of the sample (Crump 2002).

2.1.1.3 Dissolved Reactive Phosphorus (DRP)

Dissolved reactive phosphorus (DRP) is defined as the sum of orthophosphate plus the organically fixed phosphorus which passes through a 0.45 µm (Whatman GF/C) filter and is hydrolysed to orthophosphate under the conditions of the test. The latter fraction barely impacts the overall results in most cases (Crump 2002). Measurement of DRP largely followed the APHA ascorbic acid method 4500P (APHA 2000). Samples were filtered through a glass fibre filter (0.45 µm Whatman GF/C), and were often stored in a freezer before being analysed, which has been found to have no significant effect on the DRP concentration of the sample (Crump 2002).

2.1.1.4 Biochemical Oxygen Demand (BOD_5)

Biochemical oxygen demand (BOD_5) provides a measure of organic material which is biochemically degradable under aerobic conditions over a five day period at 20°C in the dark. Biochemical oxygen demand measurement of HRAP water samples generally leads to an overestimation of the measurement of easily degradable wastewater solids, as algae respiration under the dark conditions of the test also uses large amounts of oxygen. Therefore measurement of filtered BOD_5 ($fBOD_5$) is a more useful test, as much of the influent wastewater BOD is dissolved, and under field conditions much of the algal biomass would be removed from the HRAP effluent in subsequent algae settling ponds (Green et al. 1995a; Green et al. 1996).

Measurement of $fBOD_5$ largely followed the APHA method 5210B (APHA 2000). Samples were filtered through a glass fibre filter (0.45 µm Whatman GF/C) diluted with pure water where appropriate and reseeded with 0.5 or 1 ml of aerobic bacteria rich liquid (usually raw sewage). All tests were carried out without nitrification inhibition.

2.1.2 Biological Parameters

2.1.2.1. Faecal Indicator Bacteria - Escherichia coli

Escherichia coli is the preferred indicator for faecal contamination of freshwaters in New Zealand. *Escherichia coli* was measured using IDEXX Colilert test kits (IDEXX Laboratories Inc., Westbrook, ME, USA). The procedure involves the addition of "Colilert" reagent to a 100 ml sample or diluted sample, sealing the mixture into a Quanti– tray (IDEXX Laboratories Inc., Westbrook, ME, USA) and incubating it for 24 h at 35 °C. The *E. coli* concentration is calculated as a most probable number (MPN / 100ml) based on the number of positive cells on the Quanti-tray which are detected under UV light.

2.1.2.2 Algae and Zooplankton Abundance

The dominant algae and zooplankton species present in the algae cultures of each experiment were identified by microscopic analysis. Identification of algae and zooplankton species was carried out using algae identification guides (Moore 2000) and with the help of Dr. Rebecca Stott, Dr. Karl Safi and Dr. Rupert Craggs at NIWA Hamilton. Abundance was ranked using a relative scale from 0 (just detectable) to 5 (very dominant).

2.1.3 Physical Parameters

Temperature and pH measurements were made during laboratory and outside experiments using a pH / Conductivity / Salinity multimeter (TPS WP – 81, TPS Pty. Ltd., Springwood Australia). This meter was equipped with a pH electrode (Blue Line 25 pH and Blue Line 17 pH, Schott GmbH, Mainz, Germany) and a temperature probe. The pH electrode was calibrated on a weekly basis with a two point calibration using calibration solutions (pH 4.00 and pH 6.88, or pH 6.88 and pH 9.23, TPS Pty. Ltd., Springwood Australia). The accuracy of the temperature probe was regularly checked against a laboratory thermometer in NIWA's inorganic chemistry lab, which is calibrated daily by NIWA staff. The accuracy of the portable temperature probe did not change throughout the experimental period.

All pH and temperature measurements obtained during the experiments were intended to be taken at times of highest algal activity, to obtain the maximum values occurring each day. Measurements taken in the outside experiments were made in the early afternoon between 1pm and 3 pm which follows the peak in daily solar insolation and temperature. Previous experiments at Ruakura (Mandeno 2003) and observations by other authors (Nurdogan and Oswald 1995; Garcia et al. 2006) have shown that, in general, temperature and pH levels are at their daily maximum at this time of the day.

Measurements during the laboratory experiments (with constant artificial insolation from 7 am to 7 pm), were made towards the end of the illumination period between 5pm and 7 pm as laboratory experiments carried out by Oswald (1960a) showed that with constant artificial illumination, the highest pH level occurred during the final hours of the illumination period.

2.2 Experimental Methods

Indoor and outdoor experiments were conducted to evaluate the effects of CO_2 addition on algae growth and wastewater treatment. This section provides details of the experimental conditions and specific methods used.

2.2.1 Laboratory Experiments

Several initial experiments to examine the effects of CO_2 addition on algal growth and wastewater treatment under controlled laboratory conditions were carried out in microcosms in a constant temperature (CT) room.

2.2.1.1 Laboratory Microcosms

All laboratory experiments were carried out using 2 litre glass jars (18 cm high and 11.5 cm in diameter), which enabled the algae cultures to be illuminated from the sides as well as the surface. These microcosms were placed on magnetic stirrer plates (Model: N1471, Industrial Equipment & Control Pty. Ltd. Australia), and equipped with 30 mm long round magnetic spin bars, which at ~100 rpm provided constant mixing of the cultures.

2.2.1.2 Wastewater Source

The wastewater used in all laboratory experiments was effluent from the anaerobic digester, which feeds the pilot-scale HRAP at the Ruakura experimental wastewater treatment facility (see 2.2.3.1).

2.2.1.3 Algal Inoculum

High rate algal pond water from the Ruakura pilot-scale HRAP (see 2.2.3.1) was used as the algae inoculum for the laboratory experiments. The algae from the HRAP were already adapted to the wastewater used and, as some laboratory experiments were run in parallel with experiments conducted in the pilot-scale HRAP (see 2.2.3.2); it was also possible to source algae which had already been grown with CO_2 addition. The natural succession of algae species in the Ruakura HRAP over time allowed for some investigation of the effects of CO_2 addition on different algae species during various laboratory experiments.

2.2.1.4 Culture Conditions

The constant temperature (CT) room used for the laboratory experiments was located at NIWA's Ruakura research facility in Hamilton, New Zealand. The CT room air temperature was set at 20°C, and regular checks with a standard maximum – minimum thermometer showed that the temperature varied only within a margin of ± 1°C. Artificial illumination was provided by a light bank of fluorescent light bulbs (fL65SSBR/58 H6 Tri-Phosphor T8, 58 Watt, NEC, Japan and TDL 58W/840 New Generation, Philips, Holland), simulating the radiation spectrum of visible sunlight. These lights were operated on a 12 hour on 12 hour off cycle (from 7 am to 7 pm) throughout all experiments. At the height of the algal microcosms, the lights provided a total daily insolation of 7.4 mole photons per square metre and day (7.4 $mol/m^2/d$), which is about half the average winter illumination in the upper north island of New Zealand.

2.2.1.5 pH Controller

As CO_2 addition lowers the pH of the algal culture, CO_2 addition was measured in terms of the change in culture pH rather than as the volume or mass of CO_2 added. pH controllers were used to automate the CO_2 addition and to maintain the culture pH as close as possible to pH 8.0. This pH was chosen as it is a critical threshold for several processes that occur in HRAP. Phosphorus precipitation commences at pH levels above pH 8.0 (Fitzgerald and Rohlich 1964; Moutin et al. 1992) and ammonia volatilisation increases considerably at pH

levels above pH 8.0 (Konig et al. 1987). Oswald (1988) found that the optimum pH level for aerobic bacteria in a HRAP was ~8.3, while Goldman et al. (1982a) found that a pH level of 7.9 (achieved through CO_2 addition), to be optimal for the growth of several algae species which can dominate HRAP.

During the laboratory experiments an analogue pH controller was used (Model pH-40, New Brunswick Scientific Co. Inc., Edison, New Jersey, USA). This controller was fitted with a KCl pH probe (Blue Line 25 pH, Schott GmbH, Mainz, Germany).

2.2.1.6 Carbon Dioxide Addition Assembly

A CO_2 addition assembly was developed by the author to provide accurate and efficient CO_2 addition to the laboratory algal cultures (Figure: 2.1).

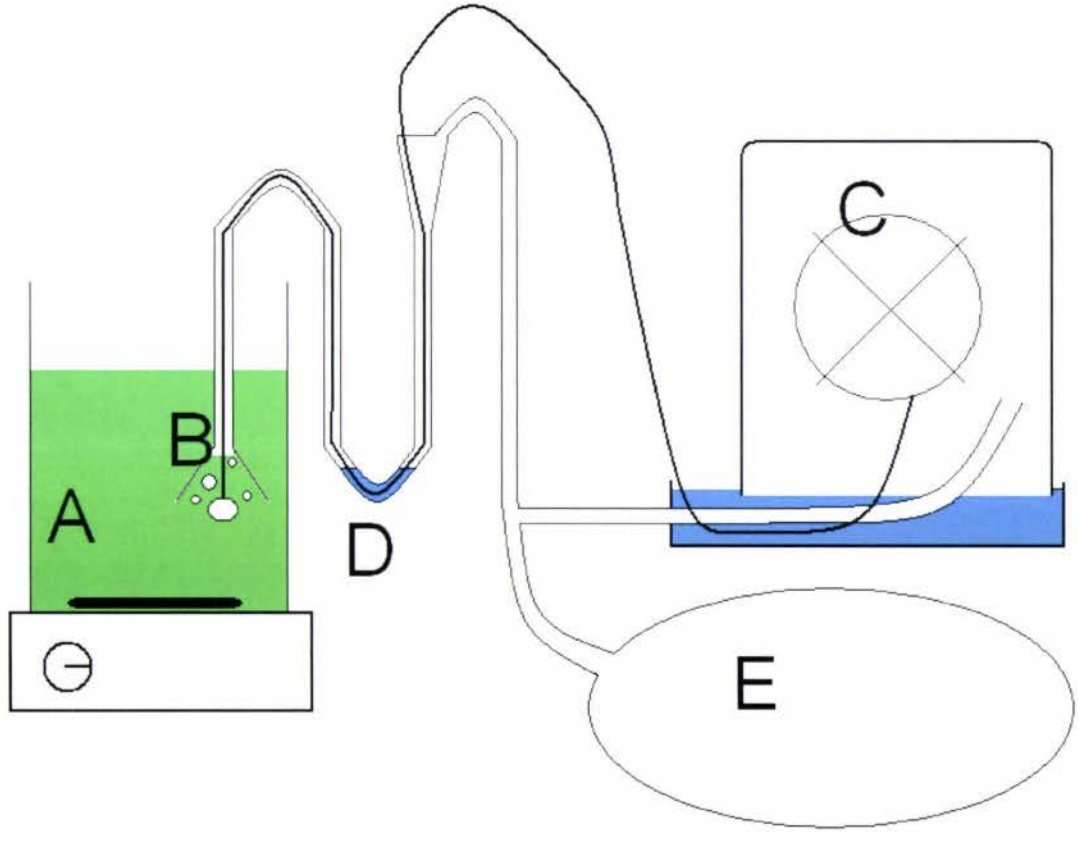

Figure 2.1: Schematic diagram of the CO_2 addition assembly used in laboratory and outside mesocosm experiments including: Spin bar mixed algae culture (A); CO_2 sparging and recapturing unit (B); CO_2 supply, and pH controller operated aquarium pump (C) housed in a water sealed glass jar; water stop valve (D) for gas diffusion control; flexible gas reservoir bag (E).

The set-up included a small aquarium air pump (Model: SP 402 SLR, Schwarzer, Germany) housed in a CO_2 filled 5 L glass jar placed upside down in a dilute acid bath (pH < 3). The

acid bath physically sealed the gas space within the jar and the acid prevented any diffusion of CO_2 from the gas space. The pump was controlled by the pH controller (see 2.2.1.5). When activated it pumped CO_2 through 4 mm standard aquarium tubing to an aquarium air stone submerged within the algae culture about 1 cm beneath an upside down funnel. Carbon dioxide was sparged into the algal culture through the air stone and any gas which did not dissolve was recollected in the funnel. The recaptured gas was piped back to the 5L glass jar using standard 13 mm aquarium tubing. A water filled depression in the return hose acted as a stop-valve which only opened when slight excess pressure (due to gas build-up) was applied on the funnel side of the return pipe. This stop-valve provided as a diffusion barrier and prevented uncontrolled diffusion of CO_2 from the gas reservoir around the pump into the algal culture. To maintain ambient pressure in the CO_2 sparging assembly the return hose was fitted with a T pipe junction, connected to a flexible, double walled, CO_2 filled, flexible gas bag (25 L maximum volume). This gas bag provided a second CO_2 reservoir and balanced the pressure through a reduction in volume as CO_2 was consumed by the algae culture. Constructing the sparging and recapturing unit as a pipe in pipe device allowed for the apparatus to be very compact, which minimised disturbance (in terms of mixing and illumination) of the algal cultures with CO_2 addition.

2.2.1.7 First Laboratory Batch Experiment

This experiment was designed to investigate the effects of CO_2 addition on nutrient removal and algae growth in algal wastewater cultures under controlled laboratory conditions over a period of 8 days. Four glass microcosms were placed in the CT room on magnetic stirrer plates (Section: 2.2.1.1) and filled with 1.5 L anaerobically digested sewage (Section: 2.2.1.2), and 0.5 L of algae inoculum taken form the eastern Ruakura HRAP (Section: 2.2.1.3). One of the four algal cultures was fitted with the pH controller (Section: 2.2.1.5) and CO_2 addition assembly (Section: 2.2.1.6) which restricted the maximum culture pH to 8.0. The remaining three algal cultures were controls without CO_2 addition which tested for the replicability of the experimental setup (Figure: 2.2).

The cultures were sampled and analysed for pH, temperature, DRP, NH_4-N and TSS (Sections 2.1.1.1, 2.1.1.2 and 2.1.1.3) on day 0 and daily from day 4 to day 8. Samples were taken between 5.30 pm and 6.30 pm (after 10 to 11 hours of illumination) as the cultures attained the maximum daily pH at this time. Microscopic analysis of the algal cultures was

conducted on day 0, day 4 and day 6. Algae and zooplankton were identified and ranked according to their dominance in the culture.

Figure 2.2: Laboratory batch experiment with one algal culture microcosm with CO_2 addition (front right) and three control algal culture microcosms, grown under constant conditions.

2.2.1.8 Second Laboratory Batch Experiment

This experiment replicated the first experiment with minor alternations. Preliminary experiments (data not shown) showed that algal cultures are susceptible to grazer (e.g. rotifer) activity, so both, the anaerobically digested sewage and the algal inoculum, were passed through a 54 micron sieve to remove large grazers. An additional microcosm filled with 2 L screened sewage (without algae inoculum or CO_2 addition) was also tested to evaluate the processes happening in a control microcosm without algal activity (non-algal control). The cultures were sampled daily between 5.30 pm and 6.30 pm and analysed for pH, temperature, DRP, NH_4-N and TSS. Microscopic analysis of the algal cultures was conducted on days 1, 4, 6 and 8. Algae and zooplankton were identified and ranked according to their dominance in the culture.

2.2.1.9 Third Laboratory Batch Experiment

This experiment was a replicate of the first two experiments with minor alternations to confirm the previous results. The algae inoculum used in this experiment was grown up on Ruakura sewage (see 2.2.1.2) under laboratory conditions from a water sample taken from the surface of an anaerobic pond treating dairy farm wastewater. The inoculum was filtered through a 54 micron sieve to remove large grazers. The microcosms were set up by mixing 0.3 L of algal inoculum, with 1.7 L of anaerobically digested sewage, which had been frozen for several days to kill any algae and grazers.

As the previous experiments showed very good replication between the three algae control cultures only one algae control culture was compared to the culture with CO_2 addition. Two non-algae controls without CO_2 addition or algae inoculum were set up using 2 L of the previously frozen sewage. As the previous experiment showed that algae eventually grew up in the non-algal control, one of these cultures was kept under continual darkness beneath a plastic bucket to prevent algal growth.

The cultures were sampled daily between 5.30 pm and 6.30 pm and analysed for pH, temperature, DRP, NH_4-N and TSS. Microscopic analysis of the algal cultures was conducted on days 3, 7 and 10. Algae and species were identified and ranked according to their dominance in the culture.

2.2.2 Outside Mesocosm Experiments

2.2.2.1 Site

Outside mesocom experiments were carried out at NIWA's pilot-scale Advanced Pond System at Ruakura, Hamilton, New Zealand.

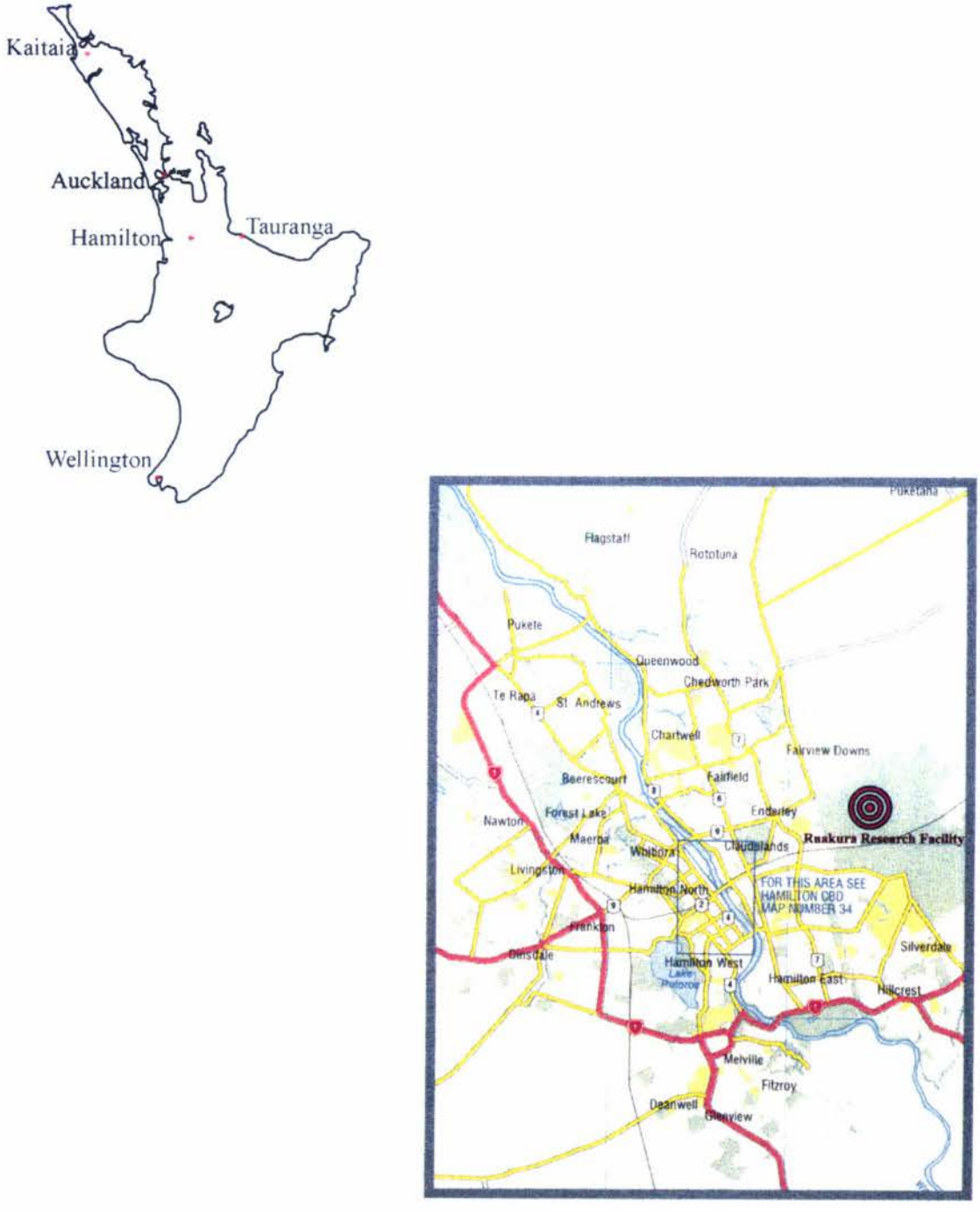

Figure 2.3: Location of the Ruakura research facility, Hamilton, New Zealand (from Mandeno 2003).

The site is located at -37.77879 latitude (dec. deg) and 175.31271 longitude (dec. deg) in the upper part of New Zealand's North Island at an altitude of 40 m above sea level (Figure: 2.3). This location experiences a semi – maritime climate with mild and wet winters and moderately warm but often humid summers.

A 2 m by 3 m platform 30 cm high, made of plywood, or a pallet was used as levelled base for the experimental set up, and provided protection from mud and moisture from the ground.

2.2.2.2. Outside Mesocosms

All outside mesocosm experiments were carried out using white 20 L High Density Polyethylene (HDPE) plastic buckets (surface area: 750 cm^2; depth: 27 cm) (Figure: 2.4). This culture depth is typical for HRAP in the field (Craggs et al. 2002a; Garcia et al. 2006). The surface lit conditions provided by the bucket mesocosms were a more realistic simulation of HRAP conditions than the glass microcosms used in the laboratory experiments. The mesocosms were placed on magnetic stirrer plates (Model: N1471, Industrial Equipment & Control Pty. Ltd. Australia) and equipped with 30 mm long round magnetic spin bars, which at ~100 rpm provided for constant mixing of the cultures.

2.2.2.3 pH Controllers

During the winter outside mesocosm experiment the pH controller described under Section 2.2.1.5 was used. During later experiments two purpose-built (Gorge Payne at NIWA Hamilton) pH controller/logger units were employed. The units consisted of a gel type pH electrode, a relay switching a mains power circuit, and a programmable logger/controller (Starlogger Model 6004C/128K, Unidata, Australia). The unit was programmed to restrict the pH level to 8.0, and log the pH in 5 minute intervals.

2.2.2.4 Local Weather Data

Local weather data including solar insolation and air temperature was collected during the outside mesocosm experiments from the NIWA weather station located at Ruakura (Figure: 2.3) (agent number 12616; network number C75733), approximately 300 m away from our

experimental site. The data was accessed through the NIWA "cliflo" climate date base online service. Due to maintenance work no insolation data was available form the Ruakura station during January and February 2006. Therefore insolation data provided by the Toenepe weather station (agent number 23908; network number B75752) located ~30km distant from the experimental site was used for the summer outside mesocosm experiment (2.2.2.6) an the high $fBOD_5$ experiment with glucose (2.2.27).

2.2.2.5 Winter Outside Mesocosm Experiment

This experiment investigated the effects of CO_2 addition on algae growth under outside conditions and was carried out at the end of the southern hemisphere winter, from the 29th August to the 28th September 2005. Four mesocosms (Section: 2.2.2.2) were filled with 16 L of anaerobically digested sewage (Section: 2.2.1.2), and 4.5 L of algae inoculum. During the first week of the experiment the cultures were grown in batch mode and were sampled and analysed daily for temperature, pH and TSS in the early afternoon (between 2 pm and 3 pm) when algal activity was highest. From the start of the second week of the experiment (day 8) the cultures were converted to semi-continuous mode by daily replacement of 2.5 L of algal culture with 2.5 L of Ruakura anaerobically digested sewage. This was done in the early afternoon (between 2pm and 3pm) when algae culture pH was at the daily maximum. On day 5 two of the cultures (C1 and C2) were equipped with a pH controller and CO_2 addition assembly (Sections 2.2.1.5 and 2.2.1.6) and the other two cultures were left as algal controls (A1 and A2) (Figure: 2.4). The pH controllers were adjusted to maintain a maximum culture pH of 8.0 through the addition of pure CO_2. Technical problems with one of the controllers prevented adequate CO_2 addition and pH control until day 10 in one of the cultures and these problems were not completely resolved throughout the experiment.

The drawn off algal culture was sampled and analysed daily for temperature, pH and TSS. Microscopic analysis of the algal cultures was conducted on days 0, 4, 10, 17, 22 and 29. Algae and zooplankton were identified and ranked according to their dominance in the culture. Throughout the experiment maximum and minimum air temperature, daily insolation and rainfall data were obtained from the weather station described under 2.2.2.4.

Figure 2.4: Outside mesocosm experimental setup showing four algal culture mesocosms, two with pH controllers and CO_2 addition (front) and two control cultures without CO_2 addition (back).

2.2.2.6. Summer Outside Mesocosm Experiment

This experiment was carried out during the southern hemisphere summer between the 23rd and 31st of January 2006. Four mesocosms (Section: 2.2.2.2) were set up; two with pH control and CO_2 addition (Sections 2.2.1.6 and 2.2.2.3) to restrict the maximum culture pH to 8.0 (C1 and C2), and two controls without CO_2 addition (A1 and A2). All mesocosms were filled with 17 L of primary sewage from the Hamilton wastewater treatment plant at Pukete, which was screened through a 500 micron sieve in order to remove large particulate matter. The raw municipal sewage was used to generate high initial $fBOD_5$ concentrations in the mesocosms. High rate algal pond water (2 L) from the (western) Ruakura HRAP was used as the algae inoculum.

The mesocosm algae cultures were operated in batch mode and were sampled daily and analysed for temperature, pH, TSS, DRP, NH_4-N and *E. coli*. The $fBOD_5$ concentrations were also analysed between day 0 and day 5. Microscopic analysis of the algal cultures was conducted on days 0, 4, 6 and 8. Algae and zooplankton were identified and ranked according to their dominance in the culture.

2.2.2.7 High $fBOD_5$ Experiment with Glucose

This experiment was primarily designed to investigate how CO_2 addition and consequently reduced culture pH effects $fBOD_5$ removal in algae wastewater batch cultures with raised initial $fBOD_5$ levels. Previous experiments (Section: 2.3.2.6) did not evaluate $fBOD_5$ removal under high pH conditions as the $fBOD_5$ was almost completely removed within the first 2 days in algal cultures both with and without CO_2 addition, before algal growth had raised the pH of the culture without CO_2 addition.

This experiment was carried out during the southern hemisphere summer between the 16th and 20th of February 2006. All mesocosms (Section: 2.2.2.2) were filled with 13 L of HRAP water (Section: 2.2.1.3) and 4 L of 500 micron screened primary sewage from the Hamilton wastewater treatment plant at Pukete. Two of the mesocosms (C1 and A1) were spiked with ~2.0 g of glucose to raise initial $fBOD_5$ levels by approximately five times to 110 mg/L. One glucose spiked mesocosm (C1) and one unspiked mesocosm (C2) were set up with pH controllers and CO_2 addition assemblies (Section: 2.2.2.3 and 2.2.1.6) which restricted the maximum pH to 8. The two remaining spiked (A1) and unspiked mesocosms (A2) were left as control cultures without CO_2 addition. The batch operated mesocosms were sampled daily for temperature, pH, TSS, DRP, NH_4-N, *E. coli* and $fBOD_5$. Microscopic analysis of the algal cultures was conducted on days 0, 3, and 4. Algae and zooplankton were identified and ranked according to their dominance in the culture.

2.2.2.8 High $fBOD_5$ Experiment with Egg Material

This experiment was a further investigation of how CO_2 addition and consequently reduced culture pH effects $fBOD_5$ removal in algae wastewater batch cultures with raised initial $fBOD_5$ levels. For this experiment homogenised egg solution was used to raise initial $fBOD_5$ levels, as unlike glucose (Abeliovich and Weisman 1978), egg cannot be directly absorbed by algae, but was still water soluble.

This experiment was carried out at the end of the southern hemisphere summer between the 1st and the 4th of March 2006. All mesocosms were filled with 19 L of HRAP water (Section: 2.2.1.3). Initial $fBOD_5$ levels were raised by spiking with a homogenised solution of equal volumes of chicken egg and deionised water. Two of the mesocosms (high) were spiked with

8 ml of egg solution the remaining two mesocosms (low) were spiked with 4 ml of egg solution targeting $fBOD_5$ levels of 100 mg/L and 50 mg/L respectively. One 8 ml egg spiked mesocosm and one 4 ml egg spiked mesocosm were set up with pH controllers and CO_2 addition assemblies (Section: 2.2.2.3 and 2.2.1.6) which restricted the maximum pH to 8. The two remaining mesocosms were left as controls without CO_2 addition. The batch operated mesocosms were sampled daily for temperature, pH, TSS, DRP, NH_4-N, *E. coli* and $fBOD_5$. Microscopic analysis of the algal cultures was conducted on days 0, 1, and 3. Algae and zooplankton were identified and ranked according to their dominance in the culture.

2.2.3 Carbon Dioxide Addition to Outside Pilot-Scale High Rate Algal Ponds

2.2.3.1 Ruakura Pilot-Scale High Rate Algal Ponds

NIWA operates a pilot-scale advanced pond system (APS) (Figure: 2.5) at the Ruakura research campus (Figure: 2.3) (Section: 2.2.2.1). The system receives raw municipal sewage from the main Ruakura sewer. The sewage is pumped to a reservoir tank (2 m^3) from which it is pumped into the first stage of the system, an anaerobic digester. The digester is filled in 3-hour intervals. This unheated and unmixed anaerobic digester (8.5 m^3) with a hydraulic retention time of ~4 days mimics an anaerobic wastewater stabilization pond. The effluent flow from the anaerobic digester is measured using a tipping bucket, which also splits the flow evenly between two high rate algal ponds (eastern HRAP and western HRAP). These identical ponds are single loop raceways with a depth of 30 cm, surface area of 32 m^2, volume of 8 m^3 and a hydraulic retention time of ~8 days. Mixing (horizontal water velocity ~15cm/s) in both ponds is provided by 8-blade galvanised steel paddlewheel driven by a 75 W single phase electric motor. The effluent from each HRAP flows into two separate pond treatment trains consisting of two algae settling ponds, and four maturation ponds in series.

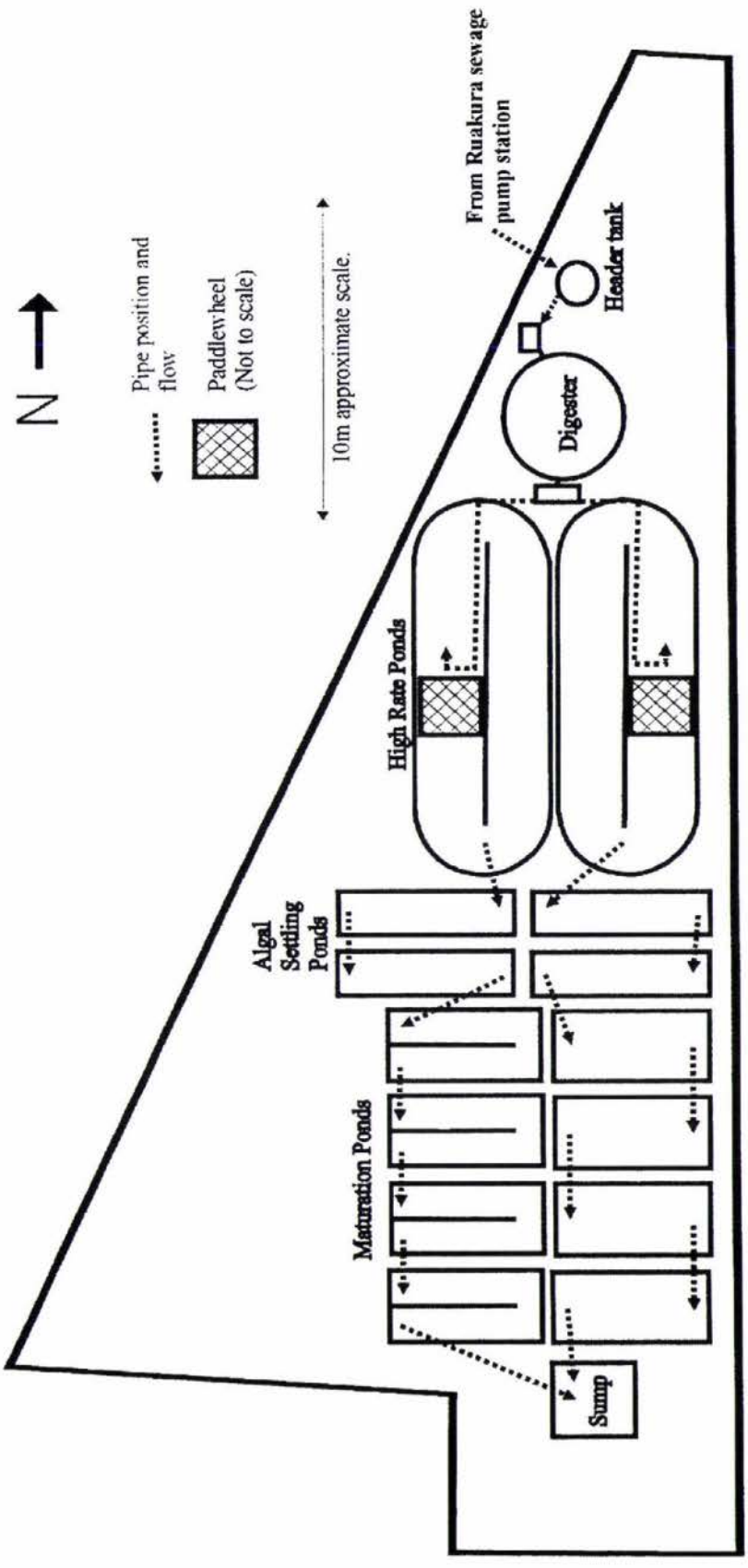

Figure 2.5: Schematic diagram of the Ruakura pilot-scale Advanced Pond System (from Mandeno 2003).

2.2.3.2 Carbon Dioxide Addition

The eastern Ruakura HRAP was equipped with a pH controller (Model Siesta pHSP, DEMA, Australia) and CO_2 addition assembly (Figure: 2.6) which sparged food grade bottled CO_2 (6.8 kg Size D bottle, BOC Gas, Hamilton, New Zealand) into the pond water, without gas recapture (Figure: 2.8).

Figure 2.6: High rate algal pond pH controller and CO_2 gas bottle.

The pH controller measured the pond water pH at 30 second intervals using a gel type pH probe. When the pond water pH exceeded the pH threshold set on the controller, a solenoid valve opened, delivering CO_2 from the gas cylinder through the gas line to the pond until the pond water pH was reduced to 0.2 pH units below the pH threshold. The CO_2 was sparged

into the bottom of the pond using a sparging board (Figure: 2.7). This consisted of a wooden board weighted down with two 5 kg diving weights, with eight aquarium air stones fixed along the top of the board and connected by 4 mm aquarium hose to a manifold, which was connected to the solenoid valve and gas cylinder by 13 mm aquarium tubing.

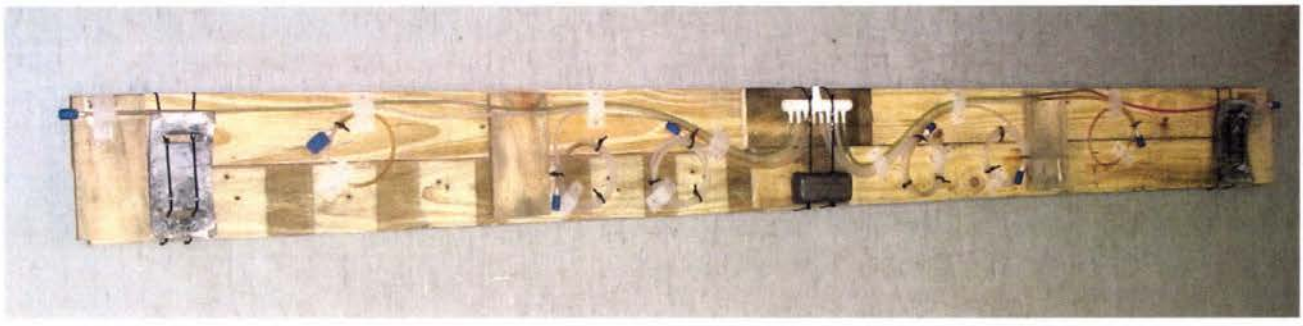

Figure 2.7: Carbon dioxide sparging board with eight aquarium air stones.

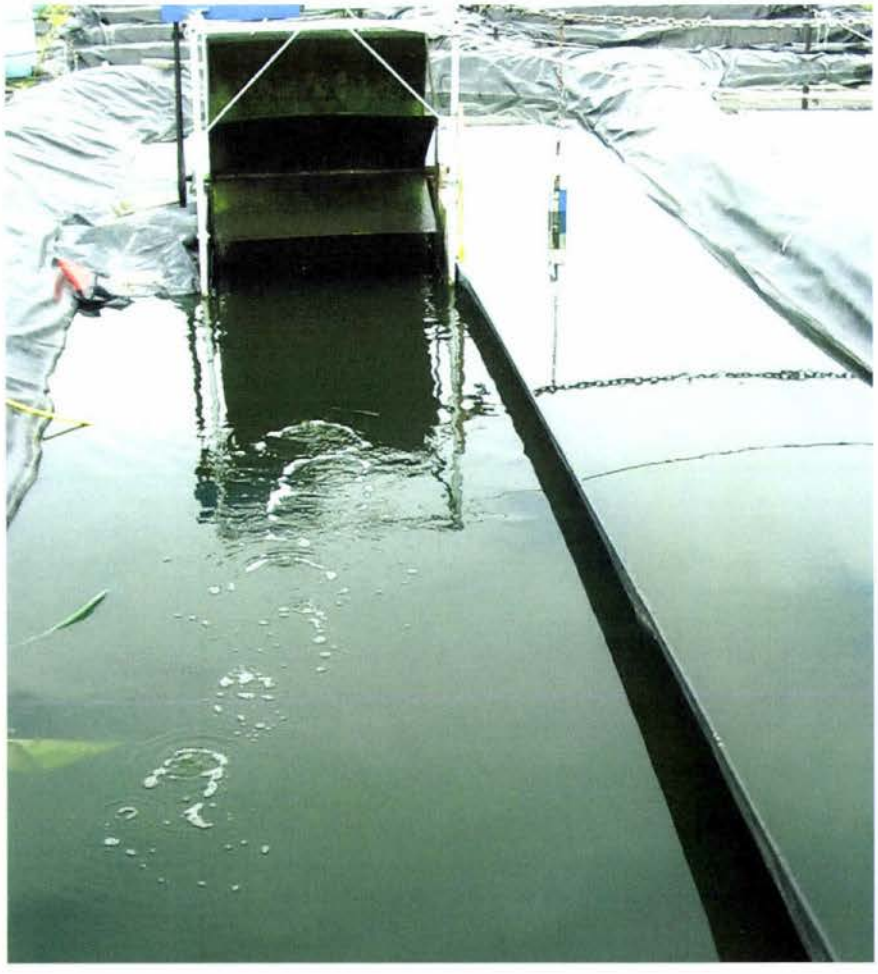

Figure 2.8: Carbon dioxide addition to the eastern HRAP close to the paddlewheel, where water column turbulence is greatest.

The CO_2 addition assembly was installed in the eastern HRAP on the 5th September. Algal growth and wastewater treatment in the eastern HRAP with CO_2 addition was compared to the western HRAP (control pond) over four months (August to December 2005). During the experimental period, pond water temperature, pH, TSS, DRP and NH_4-N, and algae and zooplankton abundance were monitored at, at least, weekly intervals in both ponds. Samples were taken in the early afternoon (1pm to 3 pm) when algae activity was highest.

Three intensive studies of the pond water physical characteristics (temperature, pH and dissolved oxygen) were made using datasondes (Datasonde 4a, Hydrolab Corporation, Austin, Texas, USA) (Figure: 2.9) which were deployed in both outside pilot-scale HRAP, and provided detailed (15 minute interval) data (Figure: 2.10). The first study was conducted at the end of winter (end of August / early September) before the installation of CO_2 addition to the eastern HRAP. The other studies were conducted after the installation of CO_2 addition to the eastern HRAP in the middle of spring (late September to early October) and at the beginning of summer (late November to early December).

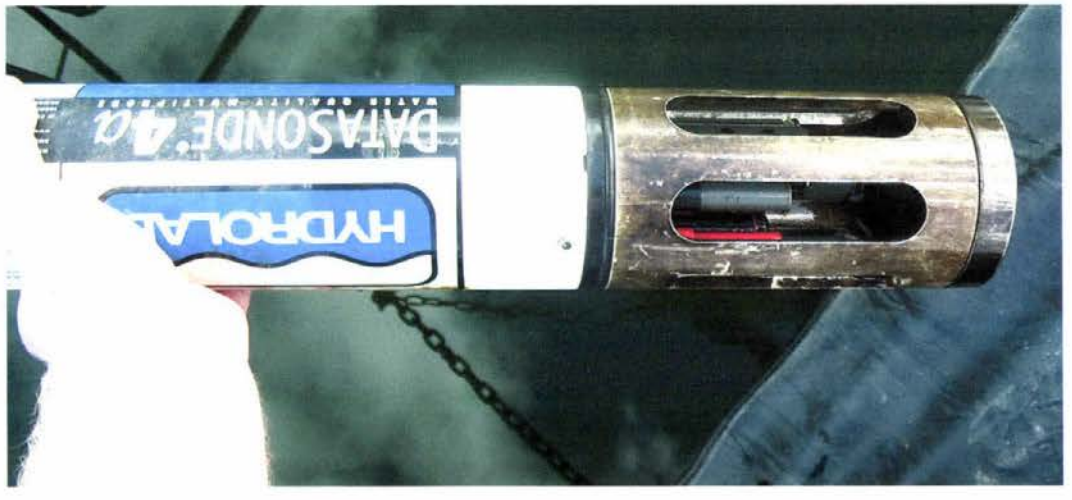

Figure 2.9: Close-up of a Hydrolab datasonde 4a, deployed in one of the pilot-scale HRAP at Ruakura. The individual probes are protected by a solid guard.

The pH and DO probes of the datasondes were calibrated on a weekly basis following the procedures described in the user manual, (pH calibration with buffer solutions at pH 4, 7 and 10; Bonnet Equipment Pty. Ltd. Auckland, New Zealand). The logged data was downloaded using the "Procomm" software package provided by Hydrolab Corporation, Austin, Texas, USA.

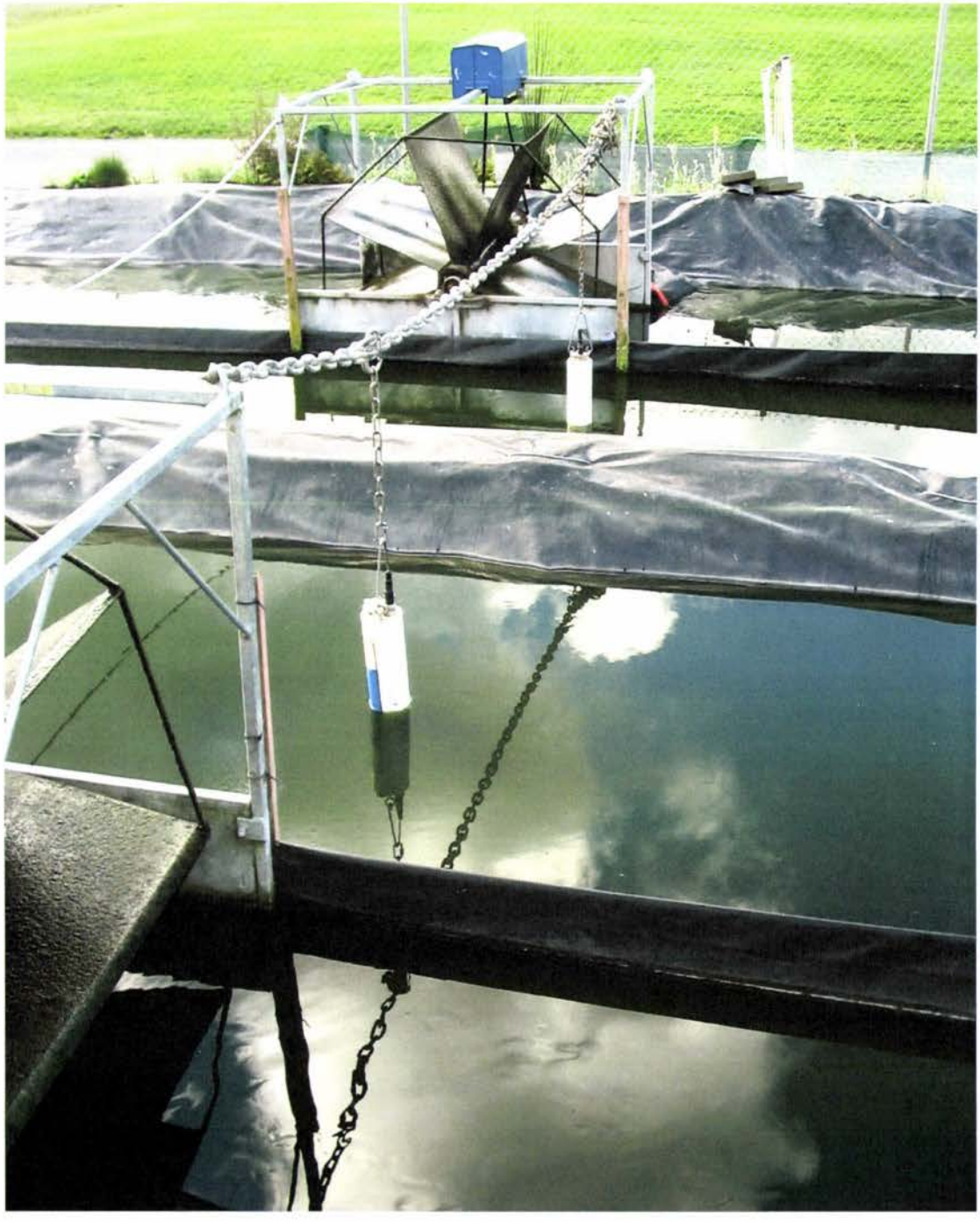

Figure 2.10: Datasondes in the eastern and western Ruakura pilot-scale HRAP.

3. Results

3.1. Laboratory Batch Experiments

3.1.1. First Laboratory Batch Experiment

This batch experiment under controlled laboratory conditions (20°C) investigated the effects of CO_2 addition on nutrient removal and algal growth in microcosm cultures grown on anaerobically digested sewage over eight days.

Maximum daily temperatures in the culture with CO_2 addition gradually increased from 20.3°C to 20.9°C over the 8 day period while those of the 3 control cultures fluctuated between 19.9°C and 20.4°C.

Microscopic analysis showed that the algal inoculum, taken from the eastern CO_2 added Ruakura HRAP, was dominated by the algae *Dictyosphaerium sp.* (mainly as large colonies of 24 or more cells) and some smaller, single celled algae, mainly *Monorapidium sp.* and *Nephroclamis sp.*. While the 3 control cultures without CO_2 addition remained dominated by large *Dictyosphaerium sp.* colonies (Figure: 3.1) until day 8, the *Dictyosphaerium sp.* algae population in the culture with CO_2 addition had changed to a mixture of smaller colonies (2 to 4 cells) and single cells (Figure: 3.2) by day 4 of the experiment.

On day 8 the large *Dictyosphaerium sp.* colonies in all 3 control cultures (without CO_2 addition) died and the colour of the culture changed from green to brown. Very low numbers of protozoa (*Paramecium sp.*) were detected in all 4 experimental cultures throughout the experimental period, with slightly higher numbers in the culture with CO_2 addition.

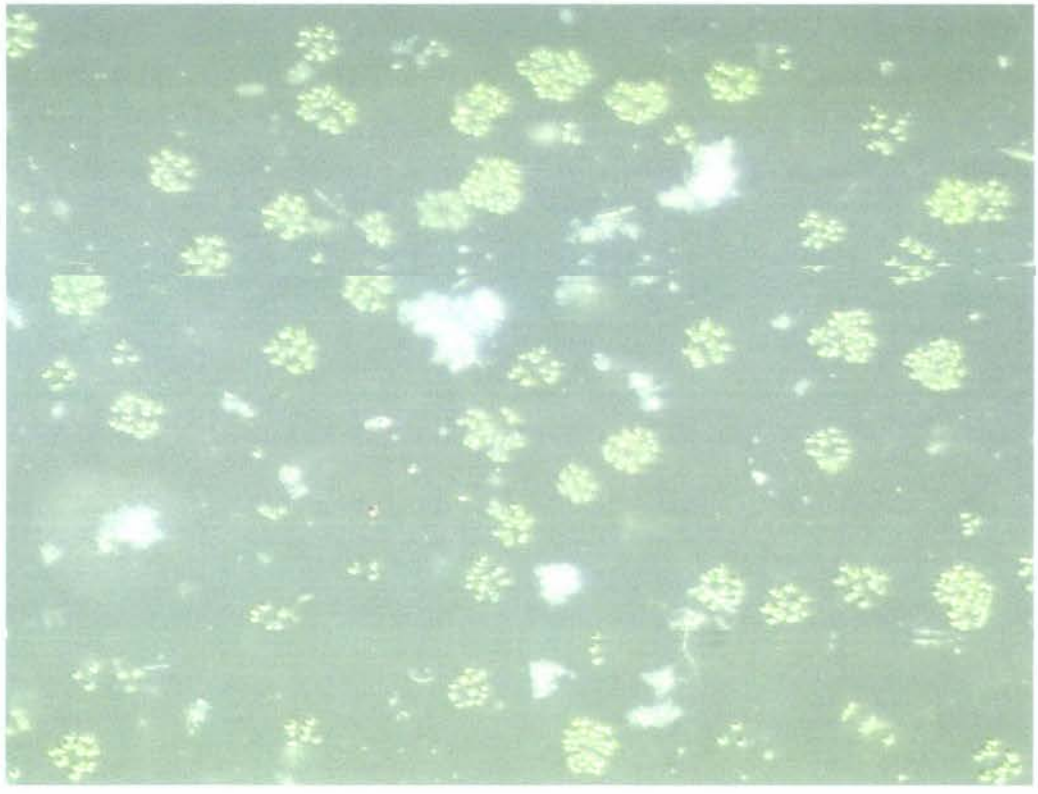

Figure 3.1: Large colonies of *Dictyosphaerium sp.* algae, grown under constant conditions over 4 days without CO_2 addition

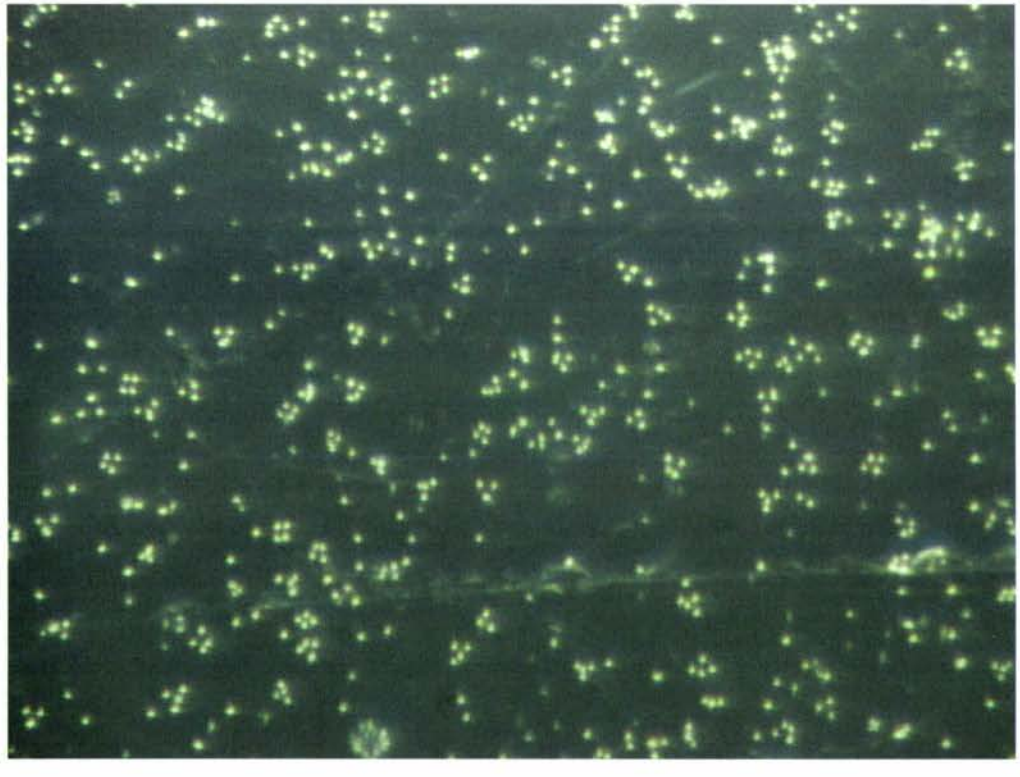

Figure 3.2: Small colonies and single cells of *Dictyosphaerium sp.* algae, grown under constant conditions over 4 days with CO_2 addition.

Algal biomass (indicated by TSS), is shown in Figure 3.3. By the end of the experiment the TSS concentration in the culture with CO_2 addition (595 mg/L) was over twice those of the control cultures without CO_2 addition (mean 245 mg/L). All three control cultures had very similar values for TSS (and other parameters measured) throughout the experiment (Appendix B), indicating that the experimental setup provided good replication of results. Therefore control culture data for each parameter is presented as a mean with error bars indicating the maximum and minimum values of the 3 control cultures (Figures: 3.3, 3.4, 3.5 and 3.6).

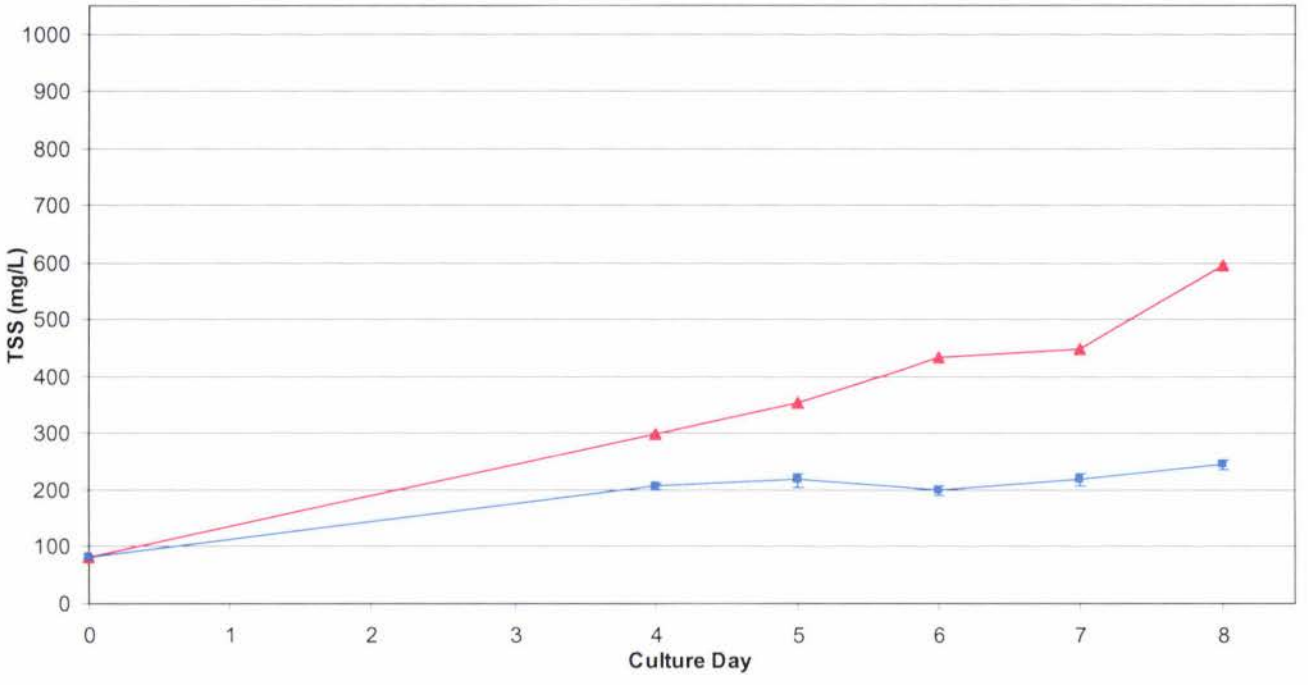

Figure 3.3: TSS concentrations in the culture with CO_2 addition (▲) and 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) measured over 8 days.

The pH controller effectively maintained the maximum culture pH below 8 in the culture with CO_2 addition (Figure: 3.4). In contrast, the daily maximum pH of control cultures (without CO_2 addition) increased from 7.01 at the beginning of the experiment to 10.79 on day 4, and was maintained at values between pH 10 and pH 11 for the remainder of the experimental period. This resulted in a maximum daily difference of 2 to 3 pH units between the control cultures and the culture with CO_2 addition from day 4 onwards.

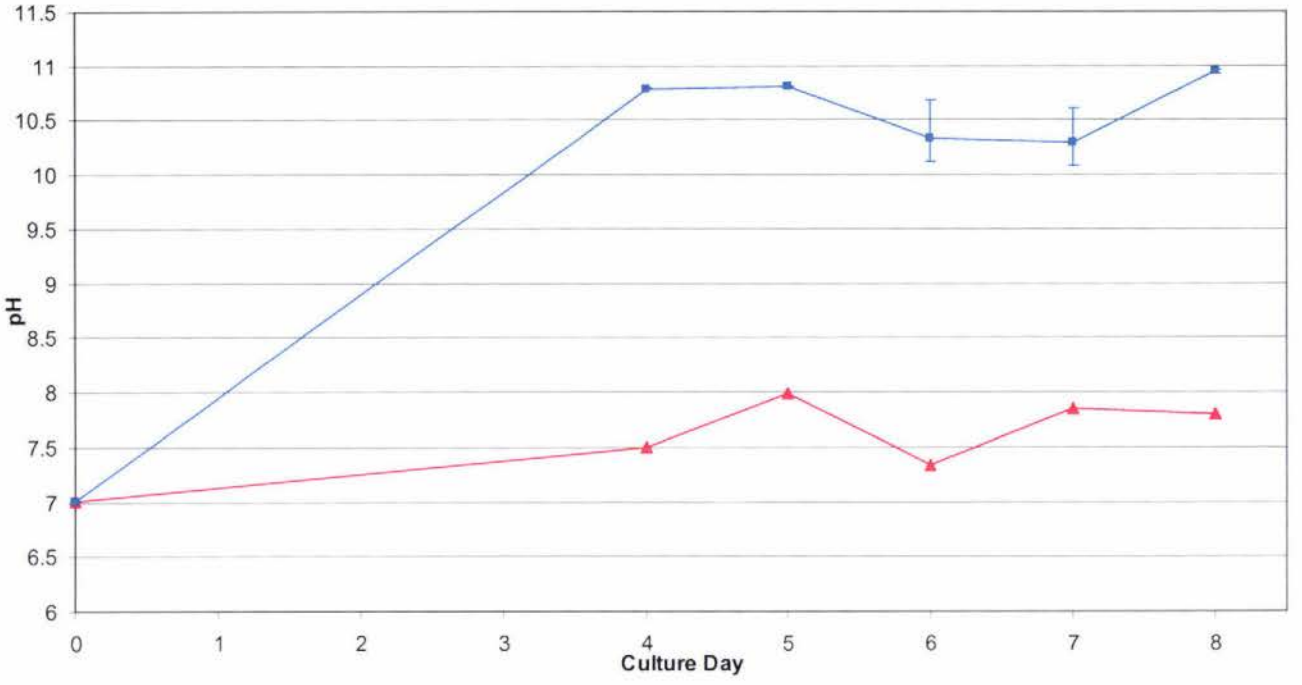

Figure 3.4: Maximum day-time pH in the culture with CO_2 addition (▲) and 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) measured over 8 days.

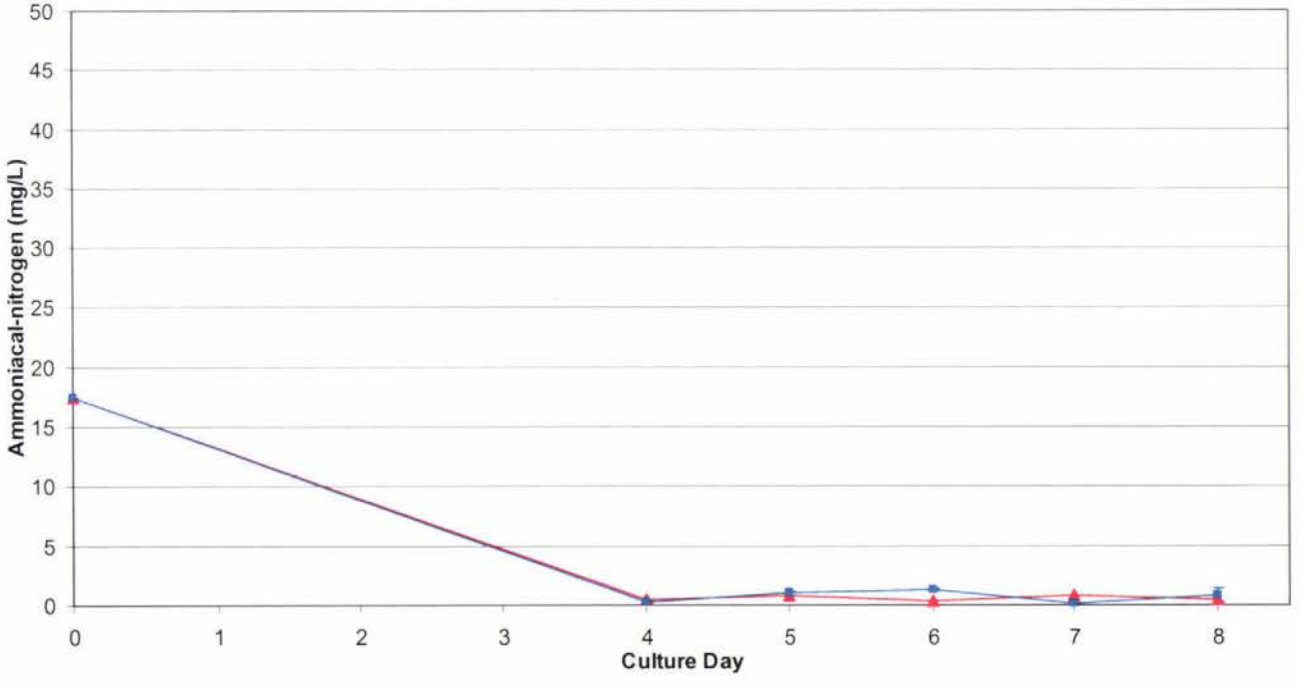

Figure 3.5: Ammoniacal-nitrogen concentrations in the culture with CO_2 addition (▲) and 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) measured over 8 days.

Ammoniacal-nitrogen concentrations in cultures with and without CO_2 addition were reduced from 17.5 mg/L to below 2 mg/L in the first 4 days of the experiment and remained below this level for the remainder of the experiment (Figure: 3.5). The low NH_4-N concentration after day 4 of the experiment did not appear to limit algal growth in the culture with CO_2 addition (Figure: 3.3).

Dissolved Reactive Phosphorus (DRP) concentrations in the culture with CO_2 addition were reduced from 5.6 mg/L to 3.0 mg/L in the first 4 days of the experiment, and then to 0.5 mg/L by day 6 and remained below this level for the remainder of the experiment (Figure: 3.6). During the first 4 days of the experiment DRP removal in the control cultures, which reduced the DRP concentrations from 5.6 mg/L to 1.8 mg/L, was higher than in the culture with CO_2 addition. Following day 4, control culture DRP concentrations gradually increased again to 3.3 mg/L by day 8 (Figure: 3.6). The low DRP concentrations in the culture with CO_2 addition on days 6 to 8 did not appear to restrict algal growth (Figure: 3.3).

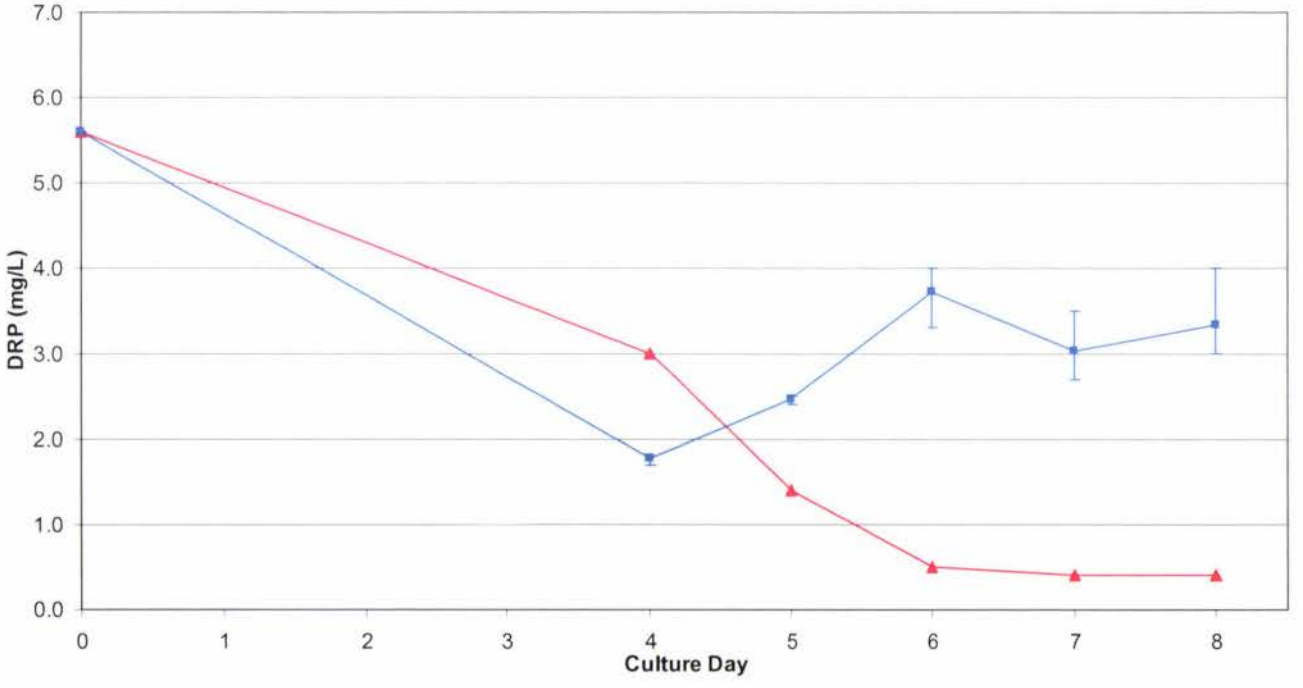

Figure 3.6: Dissolved reactive phosphorus (DRP) concentrations in the culture with CO_2 addition (▲) and 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) measured over 8 days.

3.1.2. Second Laboratory Batch Experiment

The second laboratory batch experiment replicated the first experiment, investigating the effects of CO_2 addition on nutrient removal and algal growth in microcosm cultures grown on anaerobically digested sewage over a period of 13 days. However large grazers were removed from both the digested sewage and algal inoculum using a sieve and an additional mesocosm without an algal inoculum was tested.

Maximum daily temperatures in all of the experimental cultures were similar throughout the experimental period. Maximum daily temperatures in the culture with CO_2 addition were about 0.5°C warmer than those of the control cultures and the non-algal control which all fluctuated between 19.6°C and 20.5°C throughout the 13-day experiment,

Microscopic analysis of the 4 algae cultures and the non-algal control on days 1, 4, 6 and 8 showed that all algae cultures were dominated by the single celled algae *Monorapidium sp.* and *Nephroclamis sp.* throughout the experimental period. These species also established in the non-algal control (indicated by a visible green colour) by day 4, effectively creating another control culture. The 3 control cultures, the algae culture with CO_2 addition and the non-algal control all supported a small population of zooplankton. *Peritriches sp.* were present in all 5 cultures in low and constant numbers throughout the experiment. *Paramecium sp.* were also present in all 5 cultures and abundance increased slightly throughout the experimental period, but particularly in the algae culture with CO_2 addition and the non-algal control.

TSS concentrations in all cultures initially increased on day 1 (Figure: 3.7). From day 2 the algae culture with CO_2 addition had higher algal biomass concentrations (indicated by TSS) than the 3 control cultures (which all had similar TSS levels) throughout the experiment. The growth of algae that established in the non-algal control from day 4 was at a similar rate to those in the 3 control cultures, but with a lag of ~4 days.

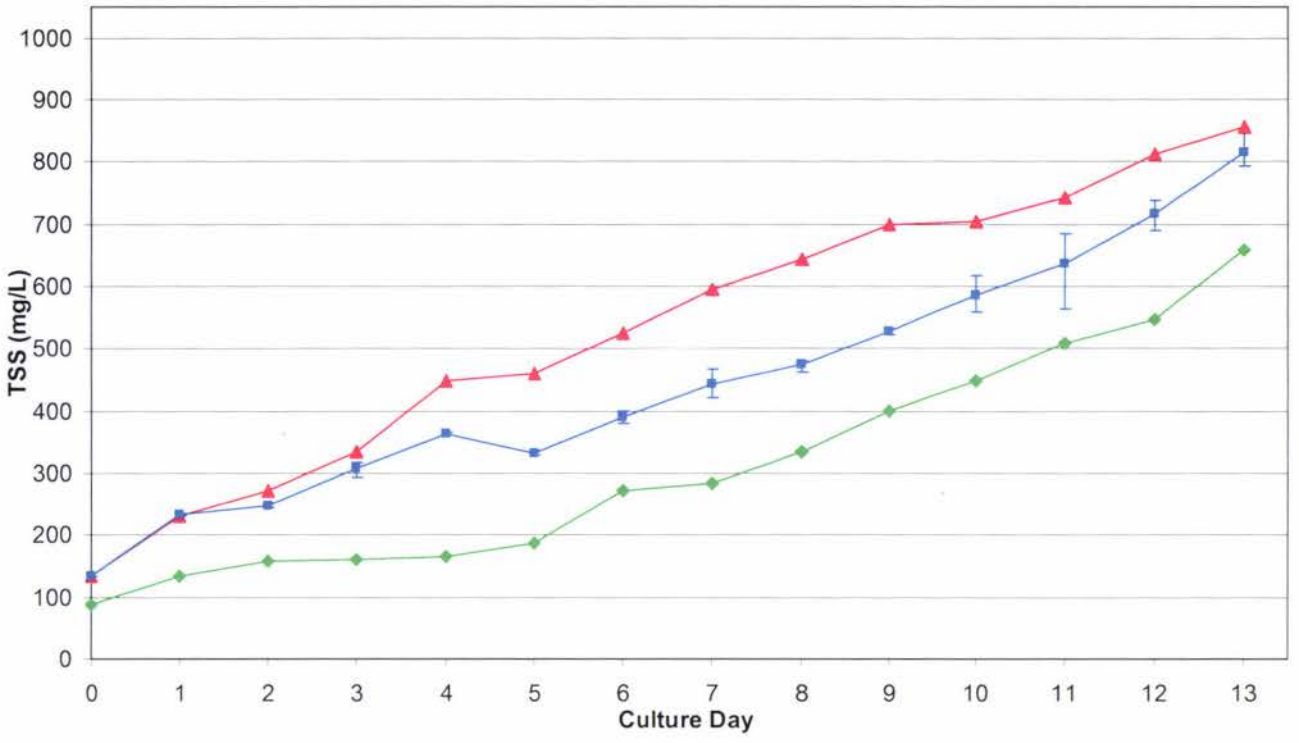

Figure 3.7: TSS concentrations in the culture with CO_2 addition (▲), 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) and the non-algal control (without CO_2 addition) (♦) measured over the 13 day experimental period.

The maximum day-time pH in the 3 control cultures were very similar and increased from 7.01 to 10.74 over the first 4 days of the experiment after which the pH was maintained between 10.40 and 10.77 for the rest of the experiment (Figure: 3.8). The pH change in the non-algal control (maximum between 10.77 and 10.90) was very similar to the control cultures, once algae had established (Figure: 3.8). In the algae culture with CO_2 addition, the pH controller set at pH 8.0, effectively maintained the maximum day-time pH within a narrow range (pH 7.82 to 8.15) from day 2 onwards (Figure: 3.8). This resulted in a maximum day-time pH difference of more than 2.5 units between the CO_2 added culture and the control cultures from day 4 until the end of the experiment.

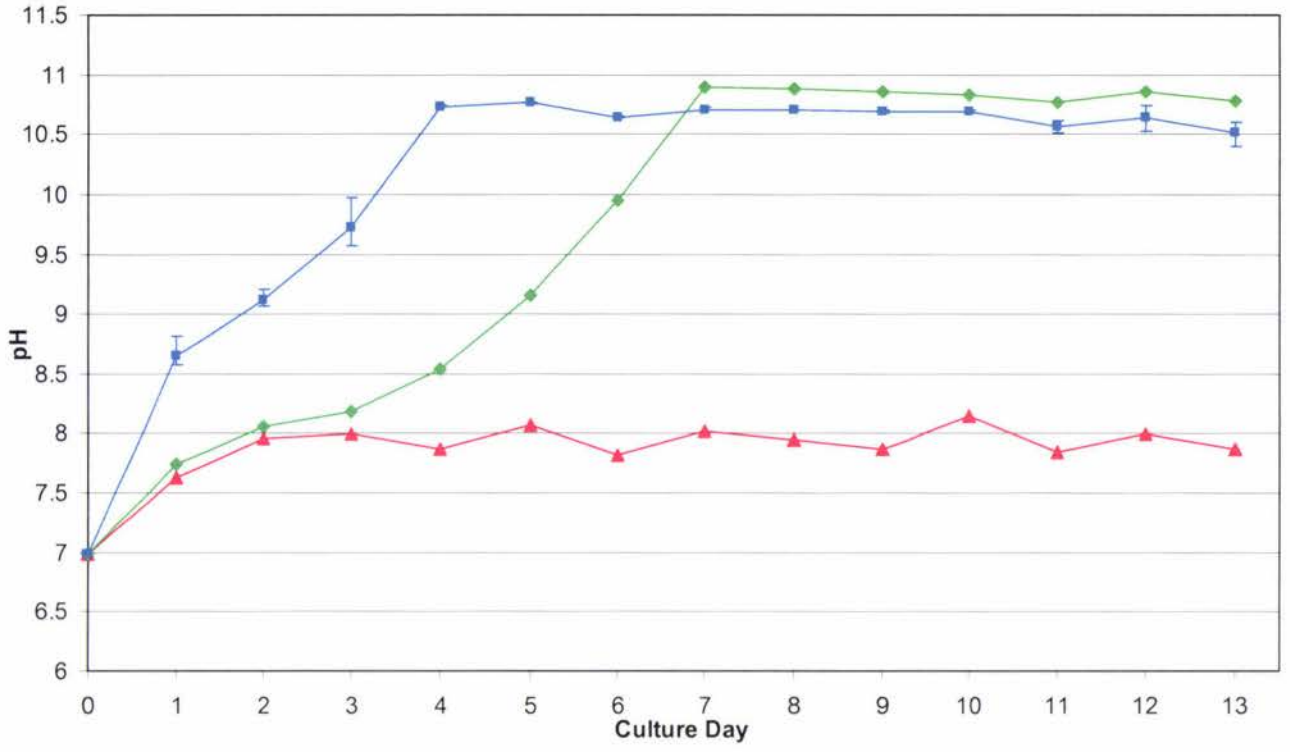

Figure 3.8: Maximum day-time pH in the culture with CO_2 addition (▲), 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) and the non-algal control (without CO_2 addition) (♦) measured over the 13 day experimental period.

The NH_4-N concentrations declined rapidly in all cultures on day 1, although greater removal was observed in the algal cultures compared to the non-algal control, and by day 2 all algal cultures had removed more than 50% of the NH_4-N (Figure: 3.9). Ammoniacal-nitrogen was completely removed in the control cultures by day 4 and in the culture with CO_2 addition by day 6. Ammoniacal-nitrogen was also completely removed in the non-algal control by day 7 following the establishment of algae (Figures: 3.7 and 3.9). The complete removal of NH_4-N in all cultures by day 7 did not appear to limit algal growth, as all cultures kept on growing for the remainder of the experimental period (Figure: 3.7).

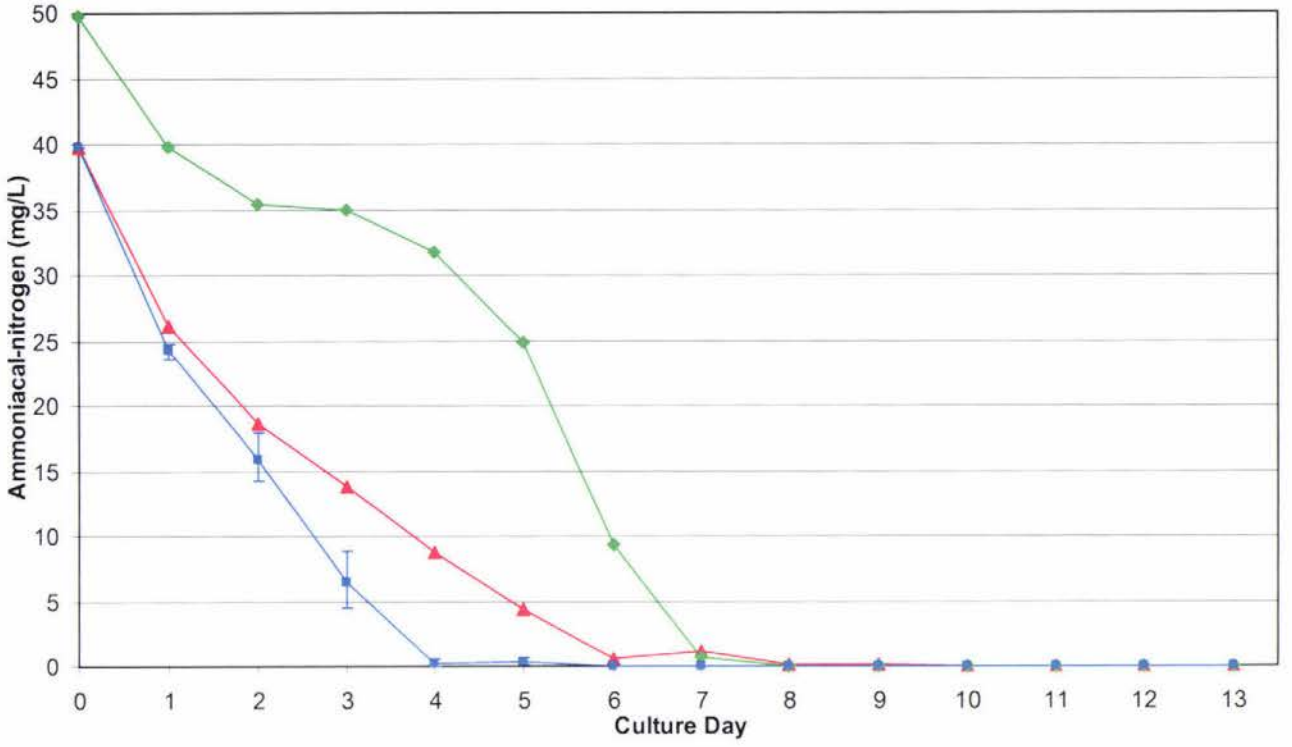

Figure 3.9: Ammoniacal-nitrogen concentrations in the culture with CO_2 addition (▲), 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) and the non-algal control (without CO_2 addition) (♦) measured over the 13 day experimental period.

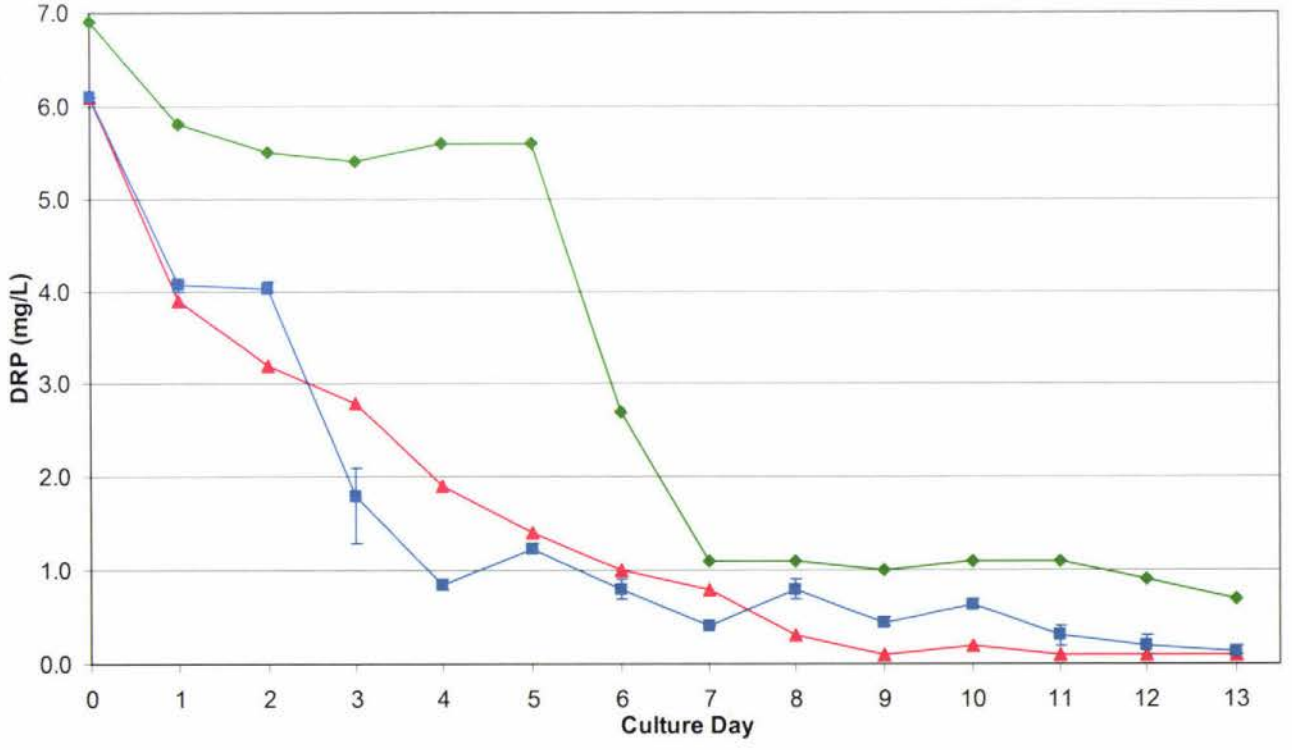

Figure 3.10: Dissolved reactive phosphorus (DRP) concentrations in the culture with CO_2 addition (▲), 3 controls without CO_2 addition (■ mean values with error bars indicating the maximum and minimum values) and the non-algal control (without CO_2 addition) (♦) measured over the 13 day experimental period.

The DRP concentrations declined rapidly in all cultures on day 1, in a similar way to the NH_4-N concentrations (Figures: 3.9 and 3.10). From then on DRP levels in the algae culture with CO_2 addition declined at a constant rate with almost complete removal (0.1 mg/L) by day 9 (Figure: 3.10). DRP removal in the control cultures and the non-algal control (once algae had established) occurred rapidly (declining to ~1 mg/L within 2 days) from day 2 and day 5 respectively and then declined at much slower rate over the rest of the experiment (Figure: 3.10).

3.1.3 Third Laboratory Batch Experiment

The third laboratory batch experiment replicated the first and second laboratory batch experiments, investigating the effects of CO_2 addition on nutrient removal and algal growth in microcosm cultures grown on anaerobically digested sewage over a period of 10 days. However, large grazers were removed from the algal inoculum using a sieve and the digested sewage was frozen to kill any algae and grazers present. Nutrient removal and algal growth in the culture with CO_2 addition was compared to those in an algae control culture without CO_2 addition and two non-algae controls, of which, one was kept in continual darkness.

Maximum daily temperatures were constant in all of the experimental cultures throughout the 10-day experimental period. Maximum daily temperatures in the culture with CO_2 addition (~21°C) were about 0.5°C warmer than those of the algal control culture which were about 0.25°C warmer than those of the light non-algal control (~20.25°C). Covering the dark non-algal control resulted in a temperature that was more than 4°C higher than the light non-algal control.

Microscopic analysis of the laboratory cultured algae inoculum used in this experiment showed that it was dominated by the colonial algae *Scenedesmus sp.* (clusters of 3 to 4 cells), and the single celled algae, *Monorapidium sp.* and *Nephroclamis sp.* The inoculum was zooplankton free; apart from small numbers of *Paramecium sp.* Microscopic analysis of the algae cultures on days 3, 7 and 10 showed that the dominance of *Scenedesmus sp.* increased slightly throughout the experimental period, particularly in the algae culture with CO_2 addition. No algae were found in the dark non-algal control, but by day 8 algae established in the light non-algal control which was visibly green. Microscopic analysis of this culture on day 10 showed that it was dominated by the single celled algae *Monorapidium sp.* and *Nephroclamis sp.* with some *Scenedesmus sp.* also present. Throughout the experiment the algae culture with CO_2 addition and the algae control culture both had low numbers of *Paramecium sp.*, and low numbers of *Peritriches sp.* were also observed towards the end of the experiment.

TSS levels in all cultures initially increased on day 1 (Figure: 3.11). From day 3, the algae culture with CO_2 addition had higher algal biomass (indicated by TSS) than the control culture throughout the experiment and had nearly double (1.8 times) the growth rate between days 4 and 10 (Figure: 3.11). TSS levels in both non-algal controls declined from day 1 onwards, except after day 8 in the light non-algal control, when levels began to rise due to the establishment of algae in the culture.

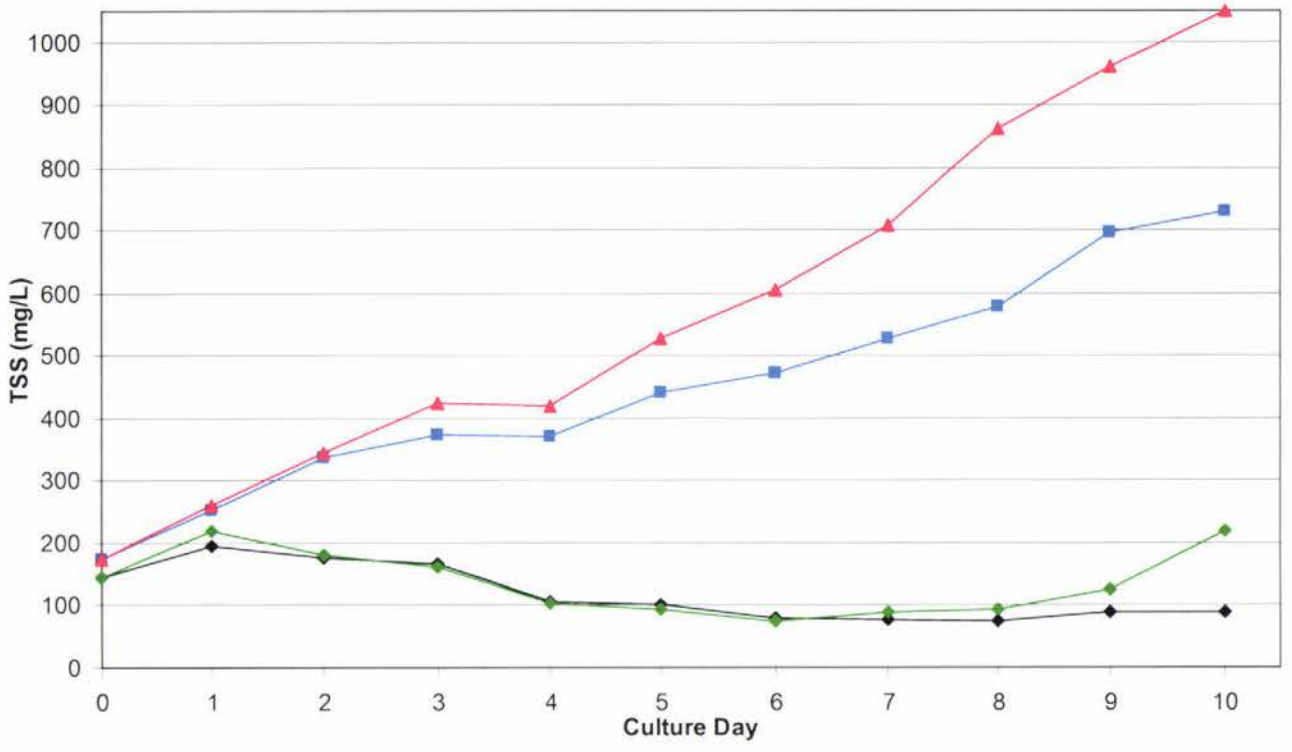

Figure 3.11: TSS concentration in the algae culture with CO_2 addition (▲), the control culture without CO_2 addition (■), the light non-algal control (without CO_2 addition) (♦) and the dark non-algal control (without CO_2 addition) (♦) over the 10 day experimental period.

The maximum day-time pH in the control culture increased from 7.4 to 10.8 over the first 3 days of the experiment after which the pH was maintained between 10.7 and 11.0 for the rest of the experiment (Figure: 3.12). The pH changes in the dark non-algal control (maximum between 7.5 and 8.2) were very similar to those in the light non-algal control, until algae began to establish in the light non-algal control from day 8, increasing the pH to 9.5 by day 10 (Figure: 3.12). The pH controller, in the algae culture with CO_2 addition, effectively maintained the maximum day-time pH within a narrow range (pH 7.5 to 8.1) from day 2 onwards (Figure: 3.12). This resulted in a maximum day-time pH difference of more than 2.75 units between the CO_2 added culture and the control cultures from day 3 until the end of the experiment.

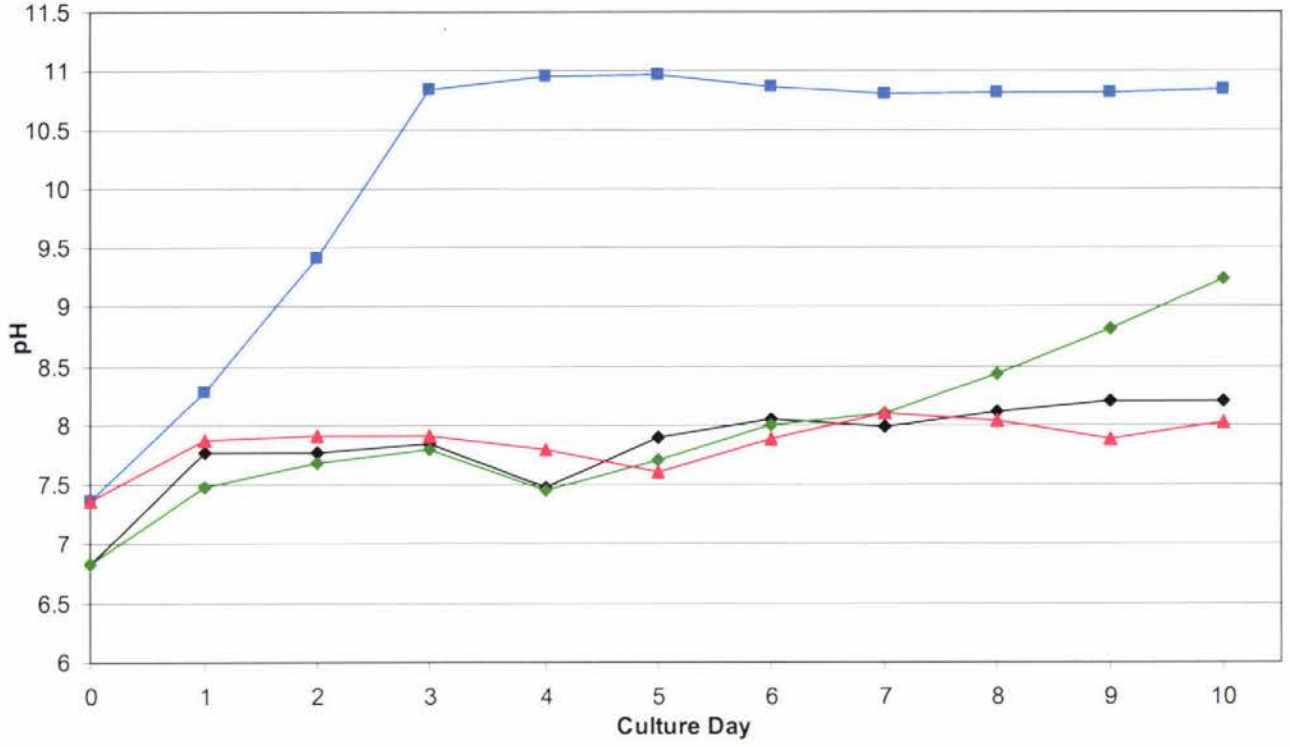

Figure 3.12: Maximum day-time pH in the algae culture with CO_2 addition (▲), the control culture without CO_2 addition (■), the light non-algal control (without CO_2 addition) (♦) and the dark non-algal control (without CO_2 addition) (♦) over the 10 day experimental period.

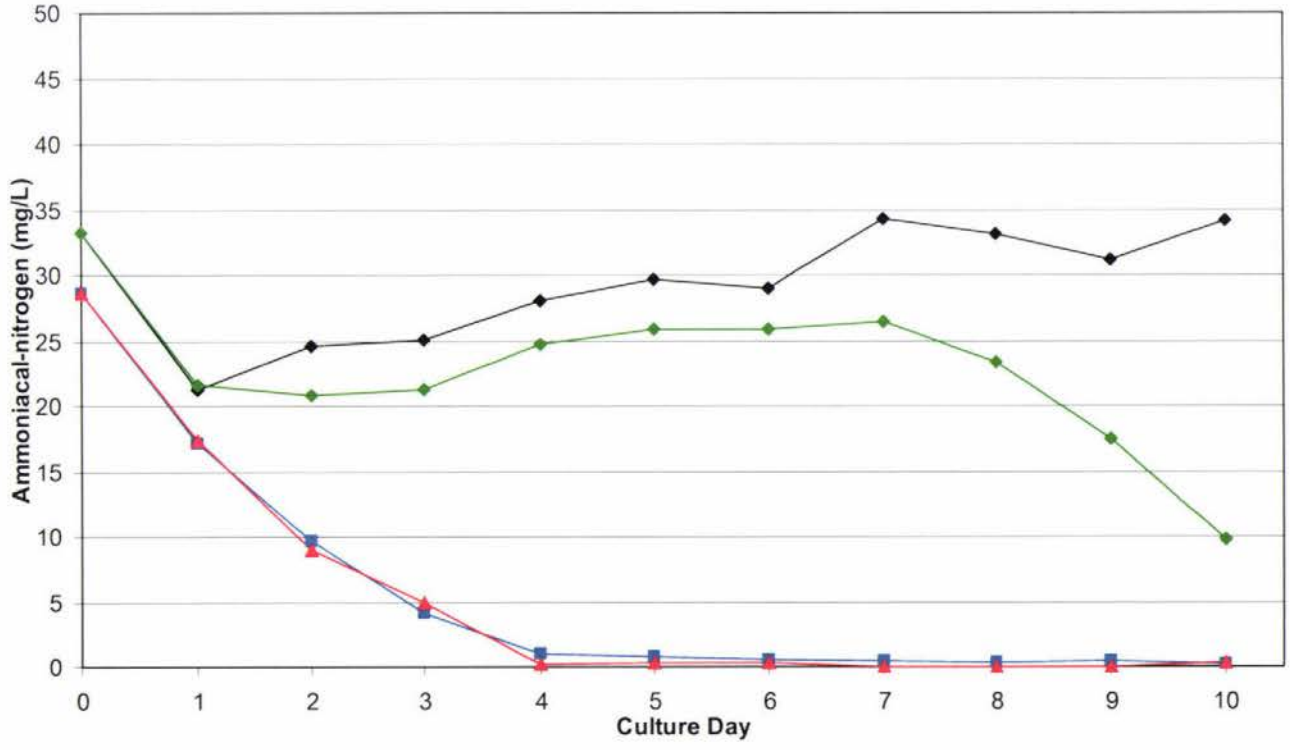

Figure 3.13: Ammoniacal-nitrogen concentration in the algae culture with CO_2 addition (▲), the control culture without CO_2 addition (■), the light non-algal control (without CO_2 addition) (♦) and the dark non-algal control (without CO_2 addition) (♦) over the 10 day experimental period.

The NH_4-N concentrations declined sharply in all cultures on day 1 (Figure: 3.13). Ammoniacal-nitrogen was almost completely removed in both, the algal culture with CO_2 addition and the algal control culture by day 4. Ammoniacal-nitrogen levels in both non-algal controls increased from day 1 onwards, except from day 8 in the light non-algal control, when levels began to decline corresponding to the establishment of algae in the culture. The complete removal of NH_4-N in both algal cultures by day 4 did not appear to limit algal growth, as the cultures kept on growing for the remainder of the experimental period (Figure: 3.11).

The DRP concentrations declined sharply (~70% removal) in all cultures on day 1 in a similar way to the NH_4-N concentrations (Figures: 3.13 and 3.14). By day 2, DRP was almost completely removed (<0.2 mg/L) in the algae culture with CO_2 addition and levels remained below this value for the remainder of the experiment (Figure: 3.14). DRP removal in the algal control was slower with levels only declining to 0.2 mg/L by day 7. DRP levels in both non-algal controls increased from day 1 onwards, except from day 8 in the light non-algal control, when levels began to decline corresponding to the establishment of algae in the culture.

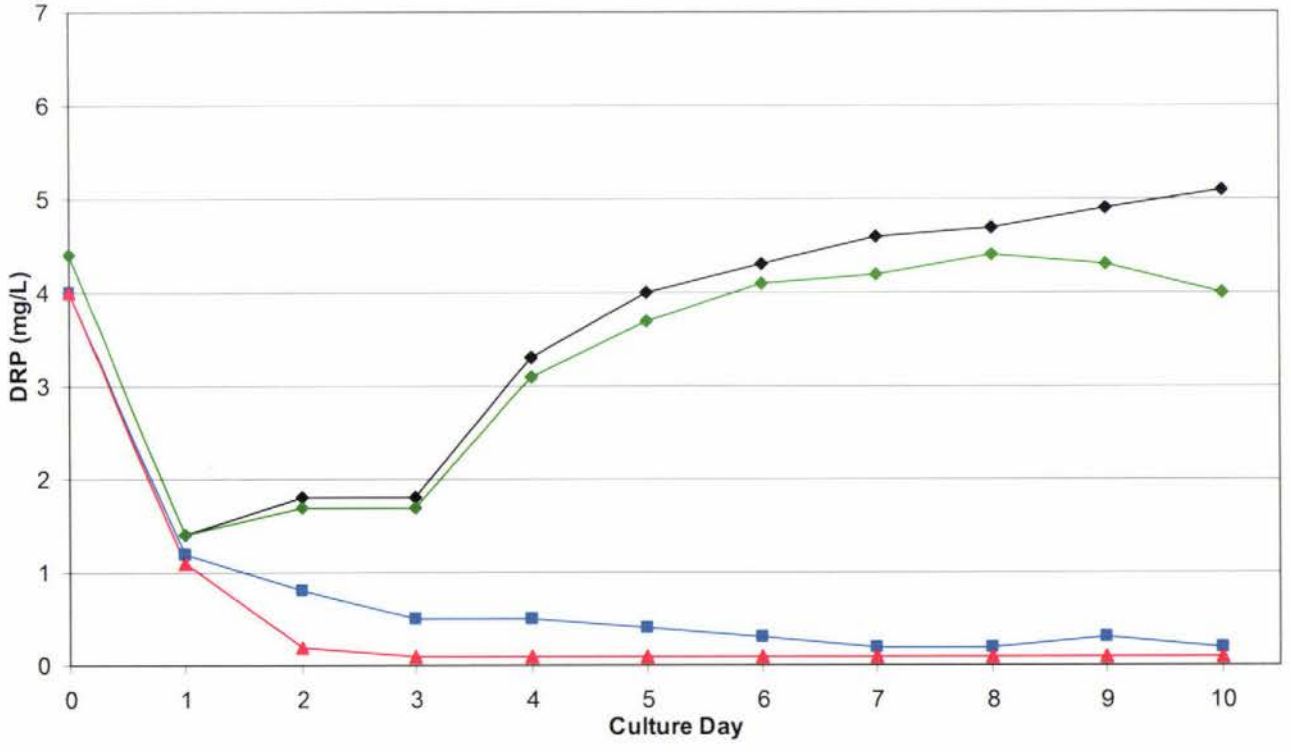

Figure 3.14: Dissolved reactive phosphorus (DRP) concentration in the algae culture with CO_2 addition (▲), the control culture without CO_2 addition (■), the light non-algal control (without CO_2 addition) (♦) and the dark non-algal control (without CO_2 addition) (♦) over the 10 day experimental period.

3.2 Outside Mesocosms Experiments

3.2.1 Winter Outside Mesocosm Experiment

This experiment studied the effects of CO_2 addition on algal growth and species composition in 4 mesocosm cultures (20 L) grown on anaerobically digested sewage under outside conditions at the end of the southern hemisphere winter (August/September). The mesocosms were initially operated as batch cultures for 1 week. From the second week onwards the mesocosms were operated in semi-continuous cultures by daily replacement of 2.5 L of the culture with 2.5 L of anaerobically digested sewage. Two of the mesocosms were equipped with CO_2 addition and pH control.

Ambient conditions (maximum and minimum air temperature, solar insolation and rainfall) were quite variable throughout the experimental period (Figures: 3.15, 3.16) Daily air temperatures varied between 12 and 20°C (maximum) and 0 and 13°C (minimum) (Figure: 3.15). Daily insolation values varied between ~6 and ~19 MJ/m^2 over the course of the experiment. Several high rainfall events (>10 mm/d) occurred between day 17 and 25, corresponding to the periods of lowest insolation and temperature (Figures: 3.15, 3.16).

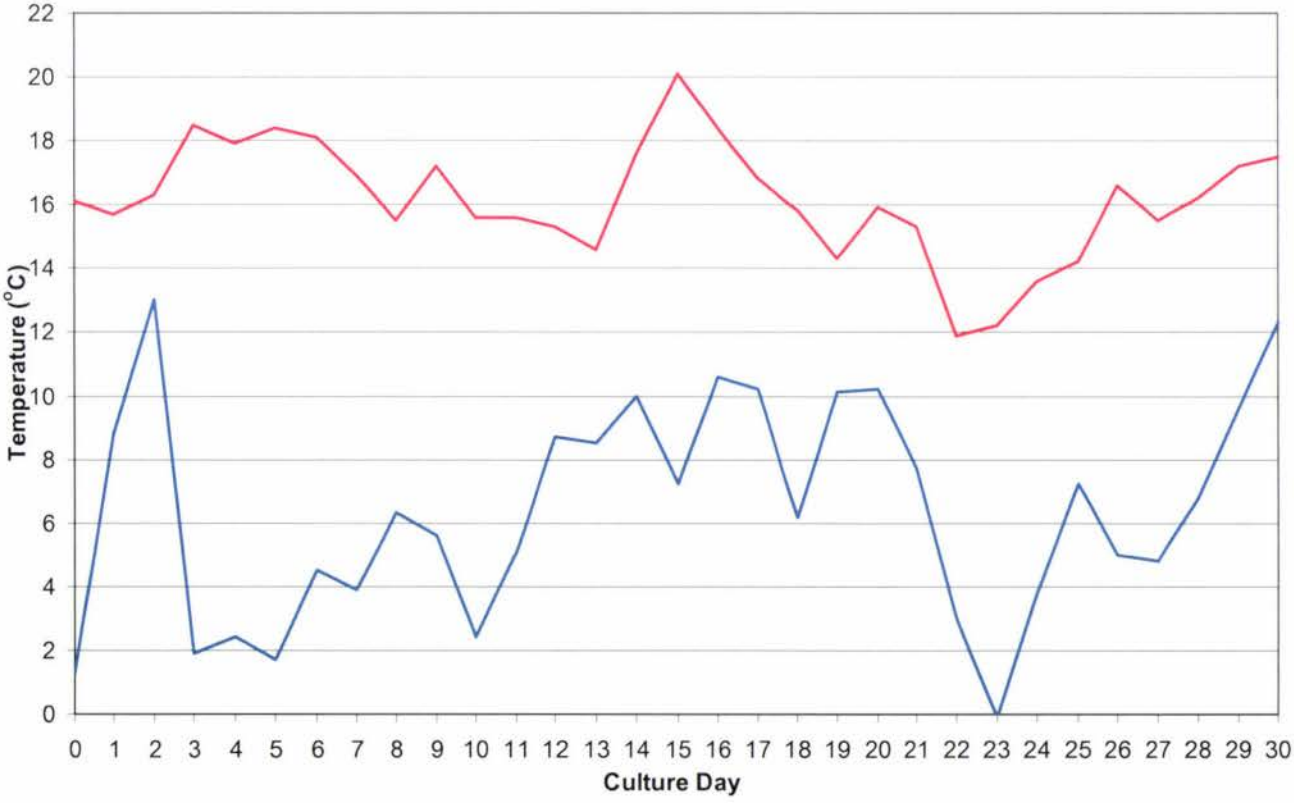

Figure 3.15: Daily maximum (—) and minimum (—) air temperature during the winter outside mesocosm experiment.

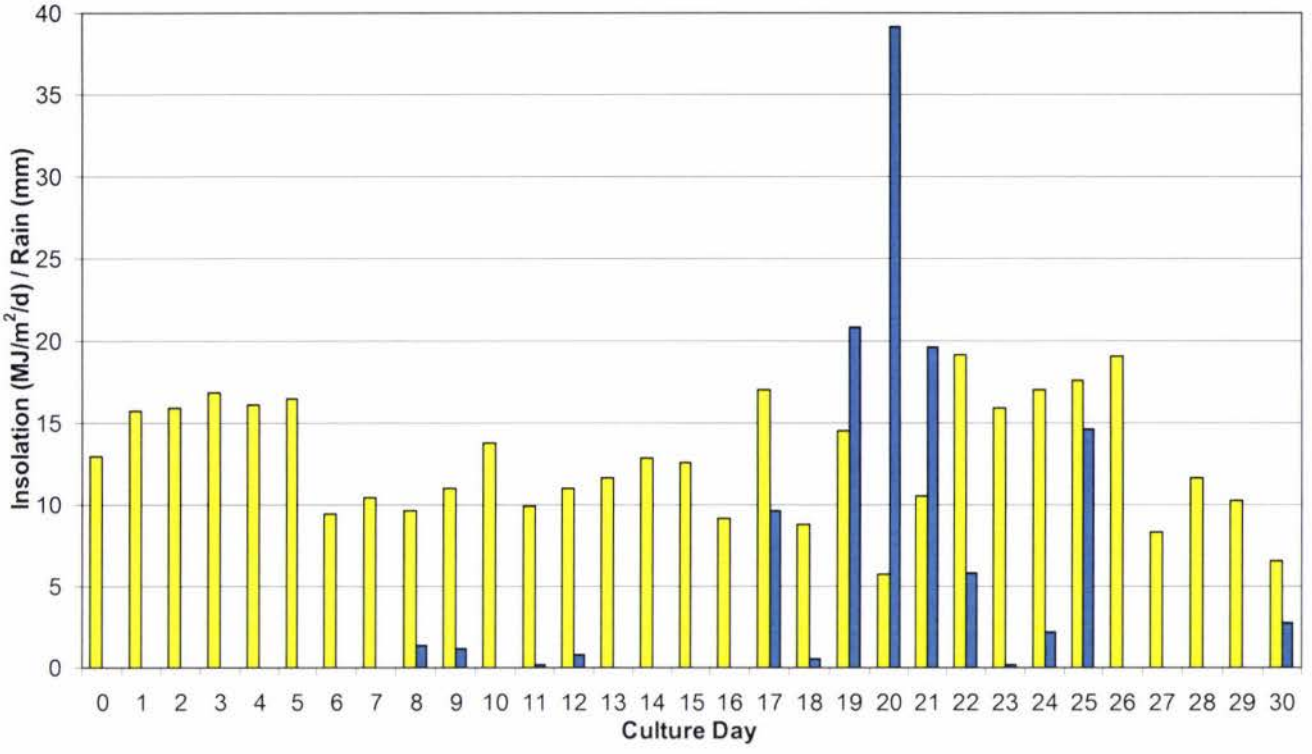

Figure 3.16: Daily insolation (■) and rainfall (■) during the winter outside mesocosm experiment.

Microscopic analysis of the algae inoculum showed that is was dominated by several algae species including: *Dictyosphaerium sp., Actinastrum sp., Scenedesmus sp., Closterium sp.*, and *Pediastrum sp.* During the experiment the colonial algae *Dictyosphaerium sp.* became dominant in all 4 mesocosms, with large colonies (>24 cells) remaining in the algal controls but with small colonies (<4 cells) in the cultures with CO_2 addition. Numbers of the other algae all declined, except those of *Closterium sp.* which remained unchanged.

During the first 3 weeks of the experiment all 4 mesocosms had low numbers of zooplankton (including *Paramecium sp., Brachionus sp., Peritriches sp.*), with slightly higher numbers in the cultures with CO_2 addition. From day 22 onwards, zooplankton numbers (especially *Brachionus sp.*) increased in all 4 mesocosms, which led to the complete grazing of one of the cultures with CO_2 addition (C2) and one of the controls (A2) during the last week of the experiment.

TSS levels increased to more than 300 mg/L in all cultures during the first week under batch mode (Figure: 3.17). On day 8, when operation was changed to semi-continuous mode, dilution of the cultures with digested sewage initially reduced TSS levels. From day 8 and throughout the remainder of the experiment, the algae cultures with CO_2 addition had higher (~20%) TSS levels (up to 100 mg/L higher) than the control cultures (Figure: 3.17). However, duplication of the TSS levels between the two algal controls was much better than that between the two cultures with CO_2 addition with TSS levels in C2 generally greater than those in C1. From day 22 onwards, TSS levels in all of the cultures declined as a result of zooplankton grazing, which was exacerbated by high rainfall between days 19 to 21. (Figures: 3.16 and 3.17).

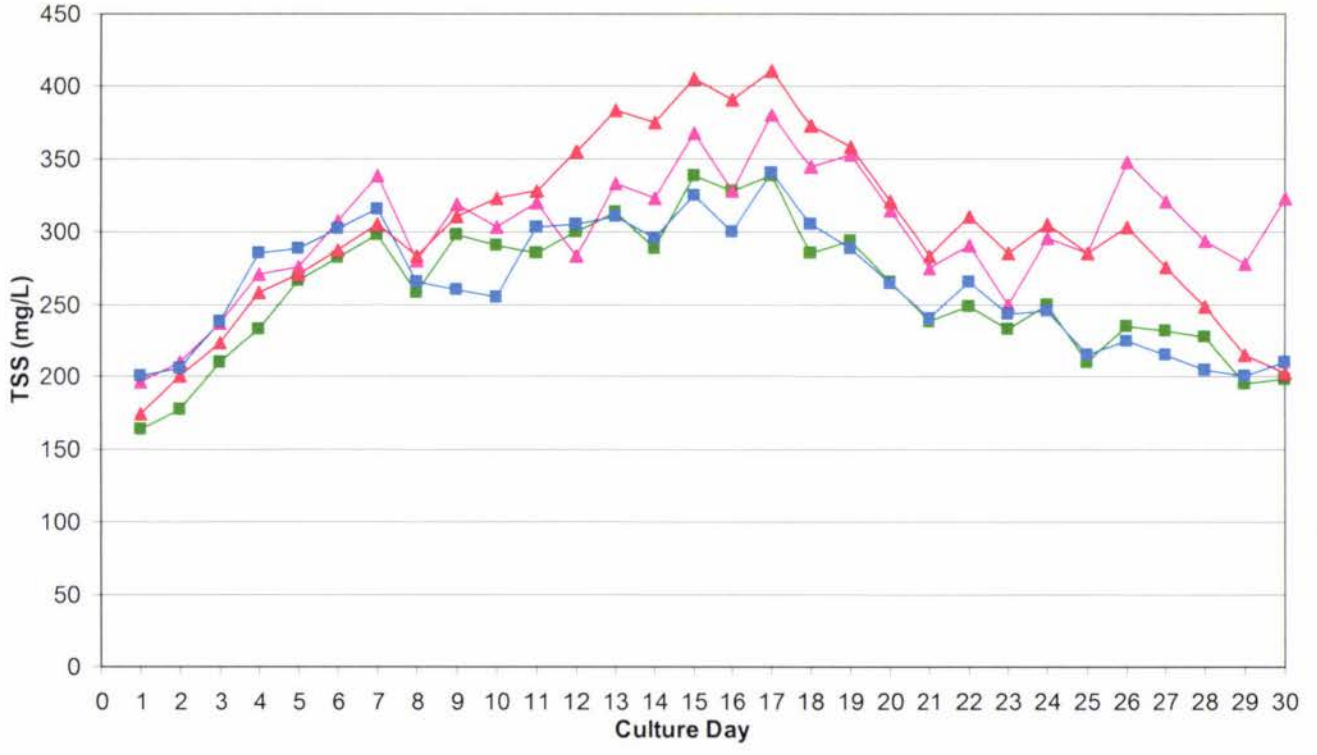

Figure 3.17: TSS concentrations in the cultures with CO_2 addition (C1 ▲, C2 ▲) and algal control cultures without CO_2 addition (A1 ■, A2 ■) over the 4 week experimental period.

The maximum day-time pH in all of the cultures increased (to >9.0) during the week under batch operation (Figure: 3.18) and following the change to semi-continuous operation the pH of both control cultures remained at this level throughout the experiment. Carbon dioxide addition from day 5 restricted the maximum day-time pH level in mesocosm C2 to below pH 8.0 for the remaining experimental period, except when the pH controller malfunctioned between days 19 to 22 as a result of a rain event (Figure: 3.16). Technical problems with the

pH controller in mesocosm C1 led to inconsistent CO_2 addition and widely varying maximum day-time pH values throughout the experiment. A maximum day-time pH difference of more than 1.75 units was achieved between both of the CO_2 added cultures and the control cultures from day 10 until the end of the experiment.

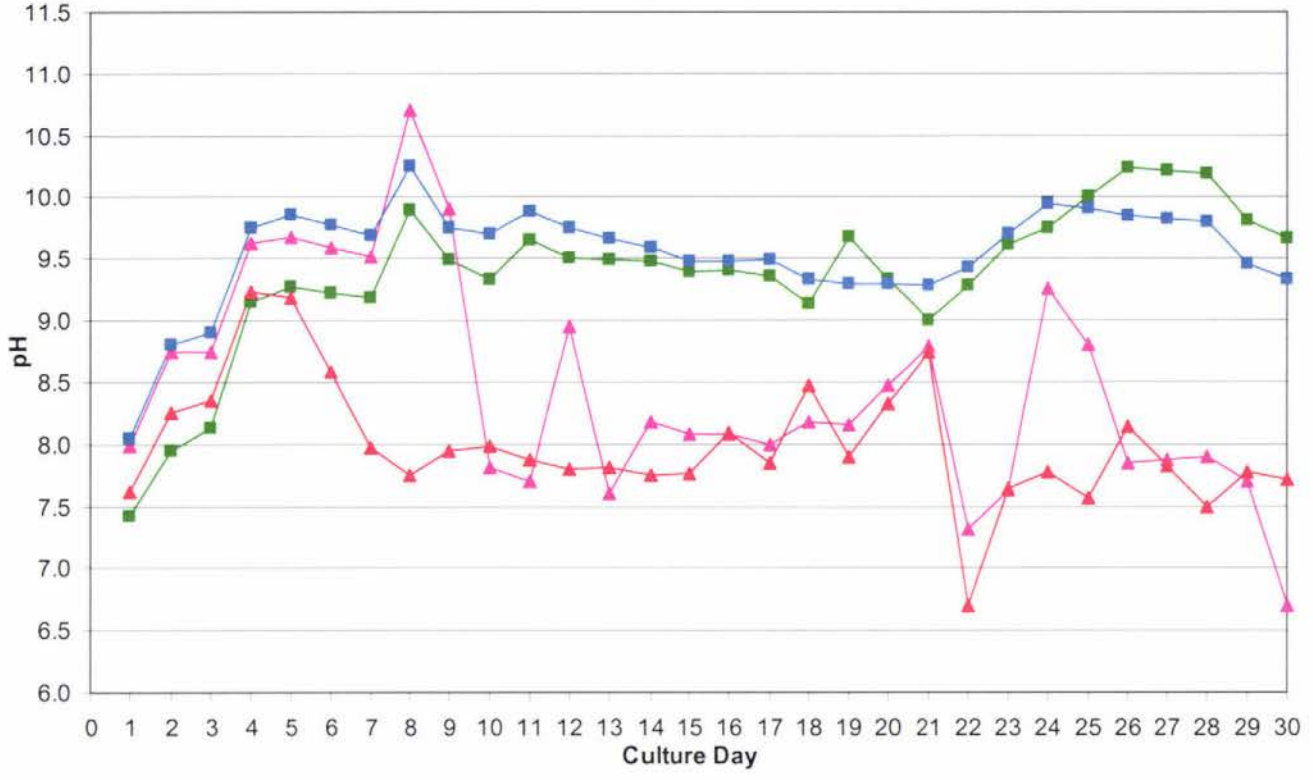

Figure 3.18: Maximum day-time pH in the cultures with CO_2 addition (C1▲, C2▲) and algal control cultures without CO2 addition (A1■, A2■) over the 4 week experimental period.

3.1.2 Summer Outside Mesocosm Experiment

This experiment repeated the batch culture part of the previous experiment over 8 days during the southern hemisphere summer (January). The four mesocosms (two with pH control and CO_2 addition, and two controls) were set up using primary sewage effluent to increase initial $fBOD_5$ concentrations and HRAP water as the algae inoculum. The mesocosms were sampled daily and analysed for temperature, pH, and concentrations of TSS, DRP, NH_4-N, *E. coli* and $fBOD_5$.

Ambient conditions (maximum and minimum air temperature, solar insolation and rainfall) were quite variable throughout the experimental period (Figures: 3.19 and 3.20) Daily air temperatures varied between 23 and 28°C (maximum) and 11 and 20°C (minimum), gradually increasing over the experimental period and were about 10°C higher than those measured during the winter experiment (Figures: 3.15 and 3.19). Daily insolation values varied between 2.7 and 24.7 $MJ/m^2/d$ over the course of the experiment (Figure: 3.20). Several high rainfall events (up to 38 mm/d) occurred on days 0, 1 and 2, corresponding to the periods of lowest insolation and temperature.

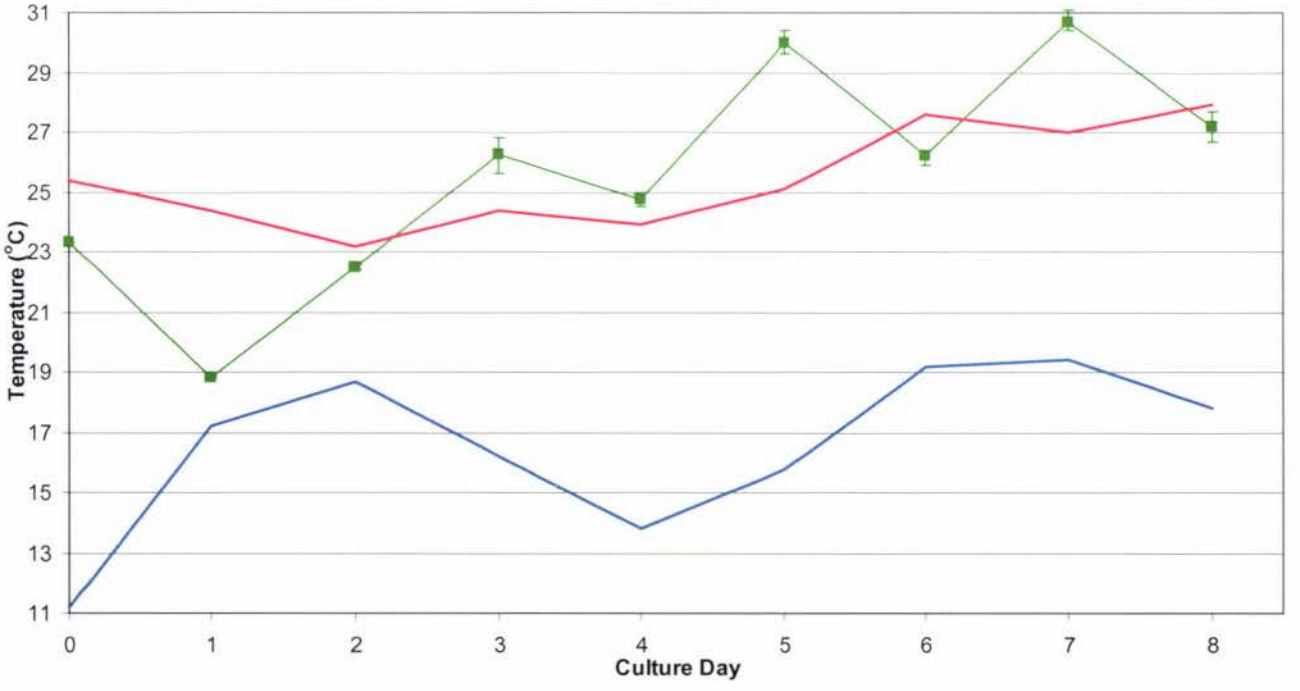

Figure 3.19: Daily maximum (—) and minimum (—) air temperature and maximum culture temperature of 4 mesocosms (■ mean values with error bars indicating the maximum and minimum values) during the summer outside mesocosm experiment.

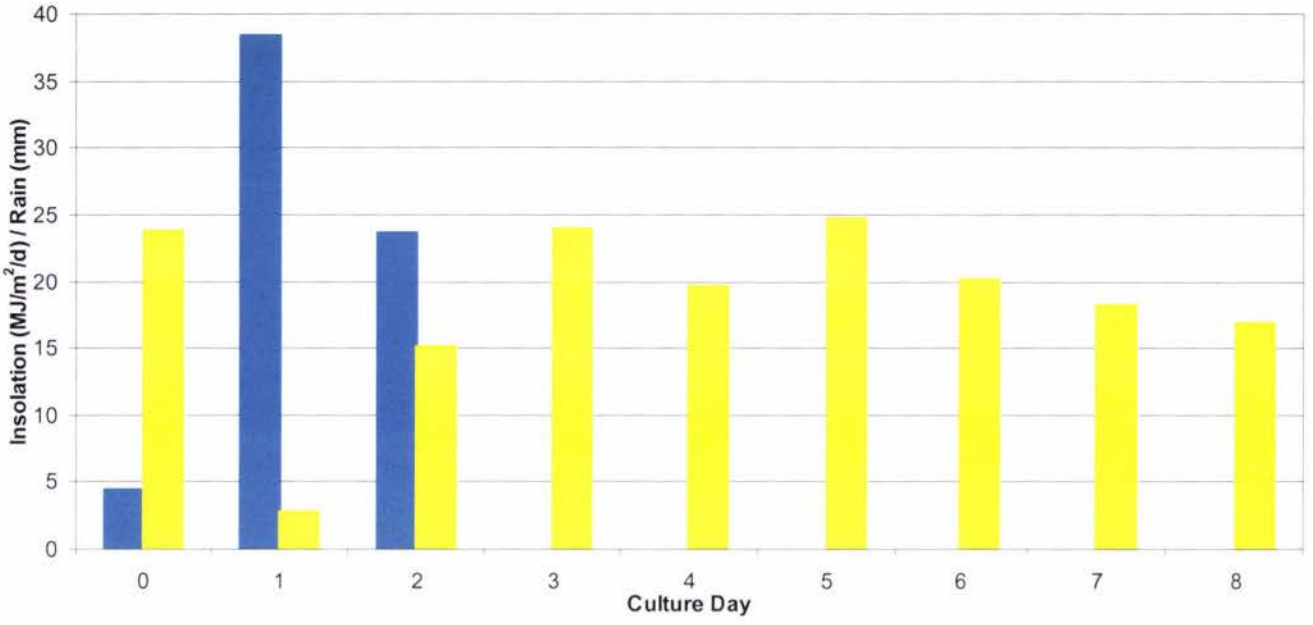

Figure 3.20: Daily insolation (■) and rainfall (■) during the summer outside mesocosm experiment.

The average maximum day-time temperature of all 4 mesocosm cultures varied from day to day but gradually increased over the experimental period with a maximum of 30.5°C on day 7 (Figure: 3.19). The lowest culture temperatures coincided with the rainfall during the first days of the experiment.

Microscopic analysis of the algae inoculum showed that it was dominated by the single celled algae *Nephrochlamis sp.* and *Monorapidium sp.* which continued to dominate all cultures throughout the experiment. *Paramecium sp.* and *Peritriches sp.* numbers were low in all 4 mesocosms throughout the experiment, with slightly higher numbers in the cultures with CO_2 addition. However, *Brachionus sp.* numbers increased in all 4 mesocosms from day 4, and grazing led to a change in culture colour from green to yellow-brown on day 8, although this occurred to a lesser extent in one of the cultures with CO_2 addition (C2).

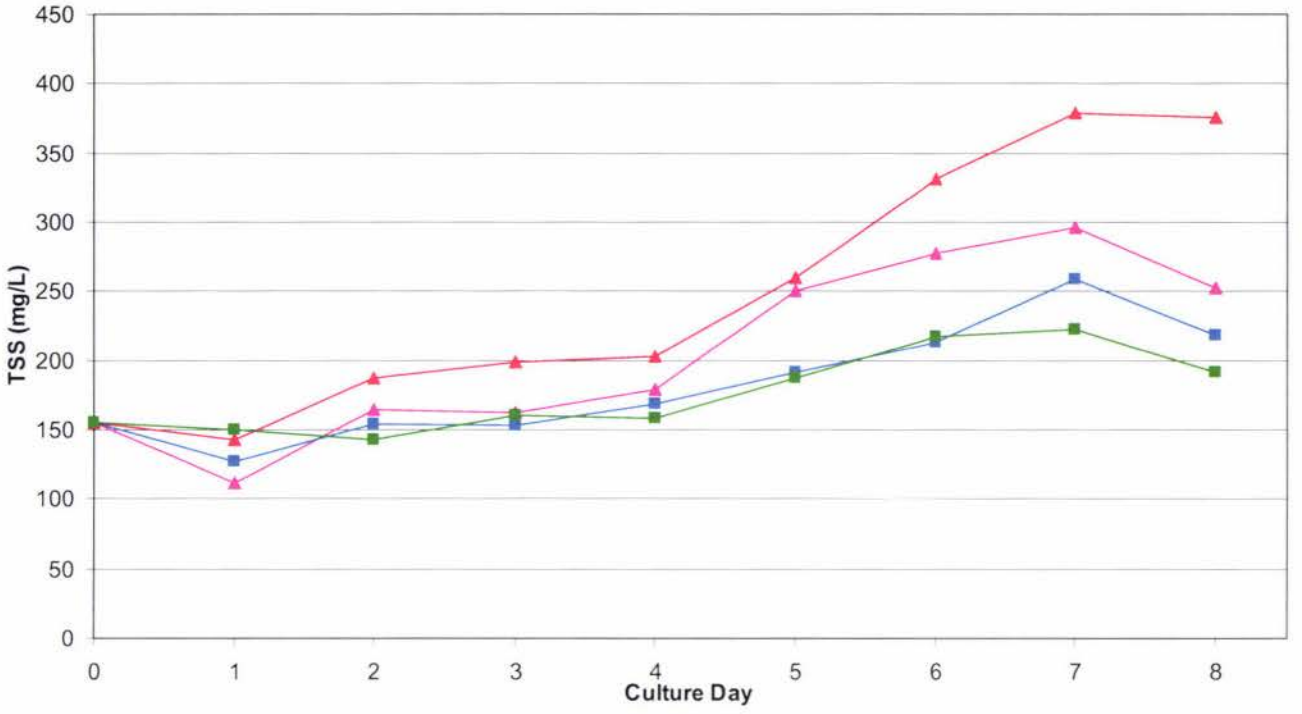

Figure 3.21: TSS concentrations in the cultures with CO_2 addition (C1 ▲, C2 ▲) and algal control cultures without CO_2 addition (A1 ■, A2 ■) over the 8 day experimental period.

TSS levels in all cultures initially decreased on day 1 (coinciding with a rainfall event) and then increased until day 8 when grazing by *Brachionus sp.* caused a decline particularly in cultures C1, C2 and A1, (Figure: 3.21). From day 2 and throughout the experiment, the algae cultures with CO_2 addition both had higher TSS levels (up to 125 mg/L higher) than the control cultures (Figure: 3.21). Duplication of the TSS levels between the two algal controls was again much better than that between the two cultures with CO_2 addition with greater TSS levels in C2 than in C1 (Figure: 3.21).

The maximum day-time pH of both control cultures increased almost linearly from day 2 to day 7 (pH ~10.5) after which levels declined on day 8 as a result of grazing (Figure: 3.22). Carbon dioxide addition restricted the maximum day-time pH level in mesocosms C1 and C2 to below pH 8.2 for the whole experiment, although the controllers, particularly in mesocosm C1 tended to overshoot, reducing culture pH to as low as 6.5 on several occasions. A maximum day-time pH difference of more than 2.5 units was achieved between both of the CO_2 added cultures and the control cultures during the experiment.

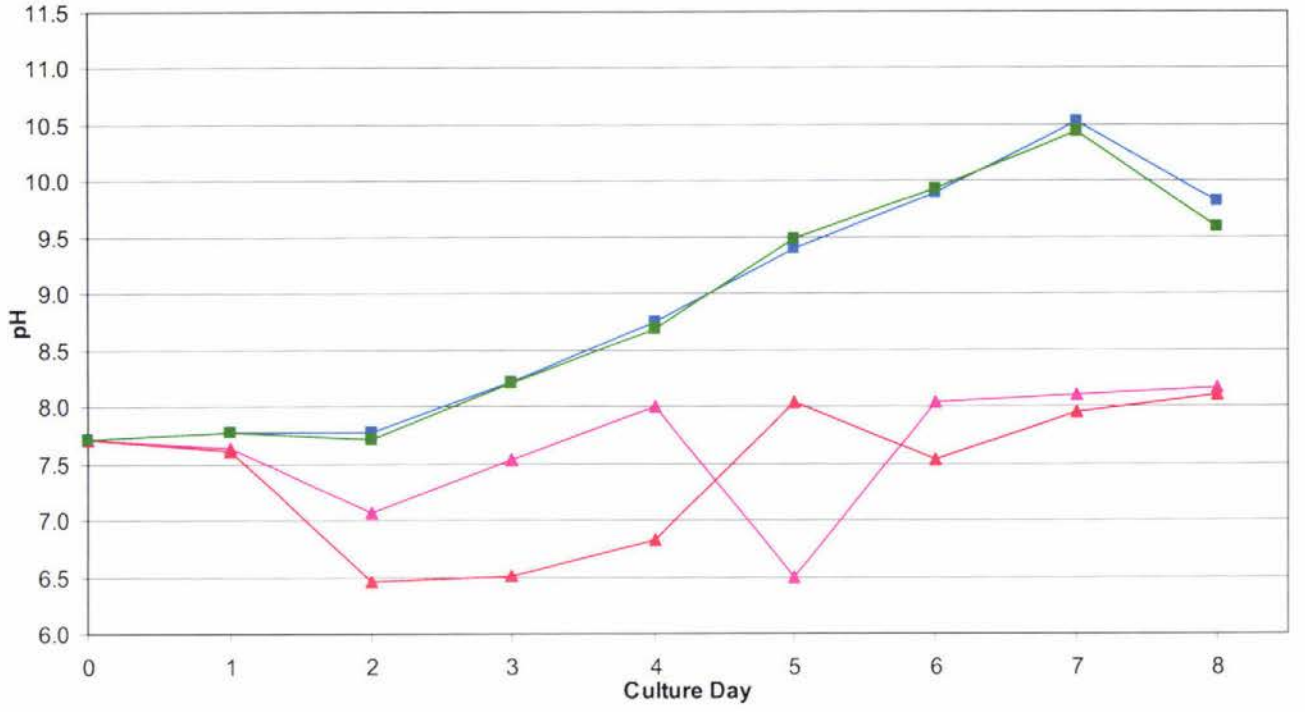

Figure 3.22: Maximum day-time pH in the cultures with CO_2 addition (C1▲, C2▲) and algal control cultures without CO_2 addition (A1■, A2■) over the 8 day experimental period.

The NH_4-N concentrations declined uniformly in all cultures until day 3 (Figure: 3.23). From day 4 onwards, NH_4-N was removed faster by the algal control cultures than the algal cultures with CO_2 addition. By day 6 NH_4-N concentrations remaining within the algal cultures with CO_2 addition were at least 5 mg/L higher than those in control cultures. Overall, NH_4-N removal by the cultures with CO_2 addition was higher in C2 than in C1. On day 8 NH_4-N levels began to rise in most of the cultures due to grazer activity.

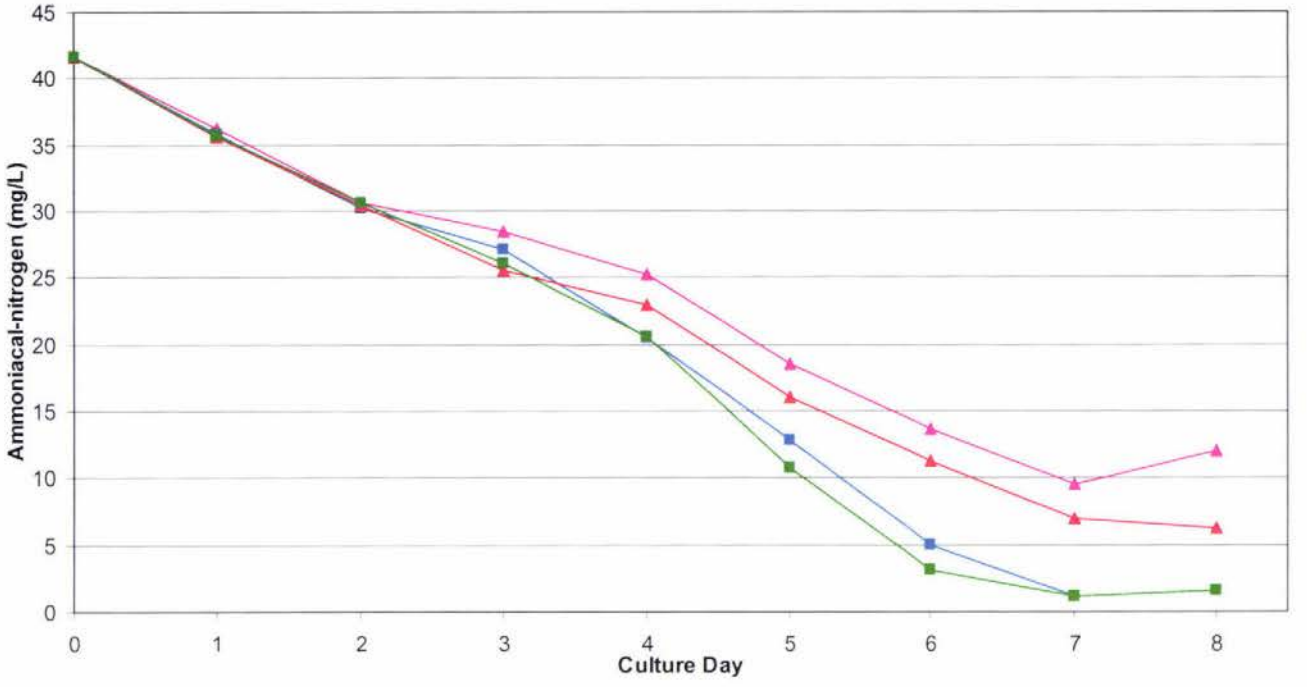

Figure 3.23: Ammoniacal-nitrogen concentrations in the cultures with CO_2 addition (C1▲, C2▲) and algal control cultures without CO_2 addition (A1■, A2■) over the 8 day experimental period.

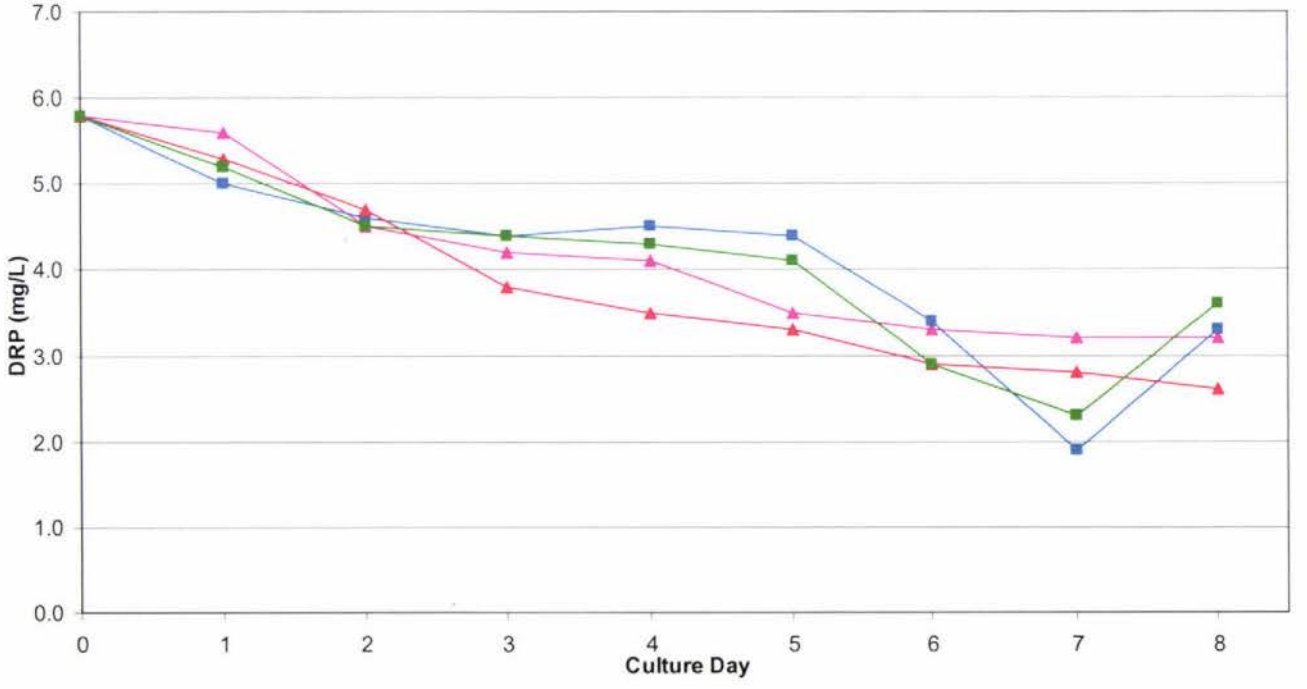

Figure 3.24: Dissolved reactive phosphorus (DRP) concentrations in the cultures with CO_2 addition (C1▲, C2▲) and algal control cultures without CO_2 addition (A1■, A2■) over the 8 day experimental period.

Similar to the NH_4-N concentrations, the DRP concentrations declined in all cultures until day 3 (Figures: 3.23 and 3.24). Between day 3 and day 5, DRP was removed faster by the algal cultures with CO_2 addition, but between day 5 and day 7 DRP was removed faster in the algal control cultures. On day 8 the control cultures show a rise in DRP levels corresponding to grazer activity and reduced culture pH.

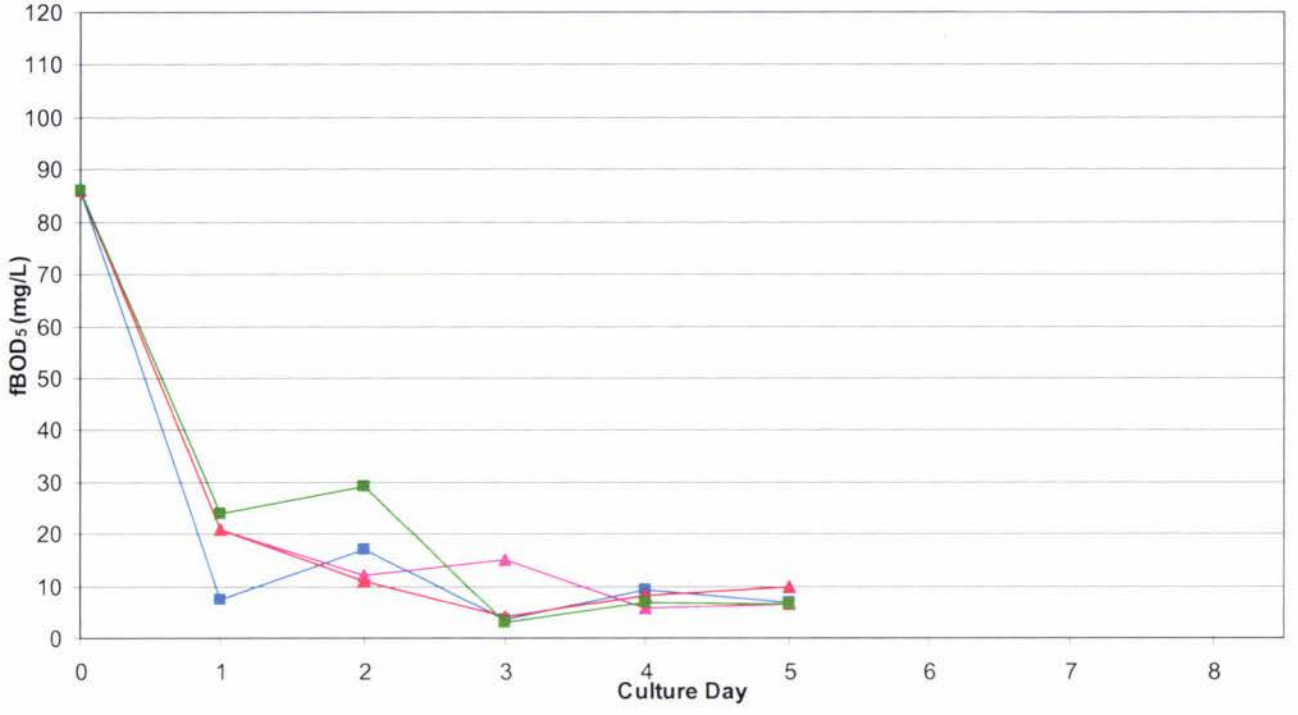

Figure 3.25: $fBOD_5$ concentrations in the cultures with CO_2 addition (C1▲, C2▲) and algal control cultures without CO_2 addition (A1■, A2■) over the first 5 days of the 8 day experimental period.

The $fBOD_5$ concentrations were reduced by at least 75% (down to <25 mg/L) in all cultures on day 1 and declined to <10 mg/L by day 4 (Figure: 3.25). No difference in $fBOD_5$ removal performance was observed between the mesocosms with CO_2 addition and the algal control mesocosms.

E. coli numbers in all four cultures remained unchanged over the first 3 days of the experiment (Figure: 3.26). From day 4 onwards, *E. coli* numbers in the cultures with CO_2 addition gradually declined at a rate of ~0.5 log unit per day, while *E. coli* numbers in the control cultures declined more rapidly at a rate of ~1 log unit per day. *E. coli* levels in the algal control cultures probably dropped further on days 7 and 8 but were below the detection limit for the dilution used.

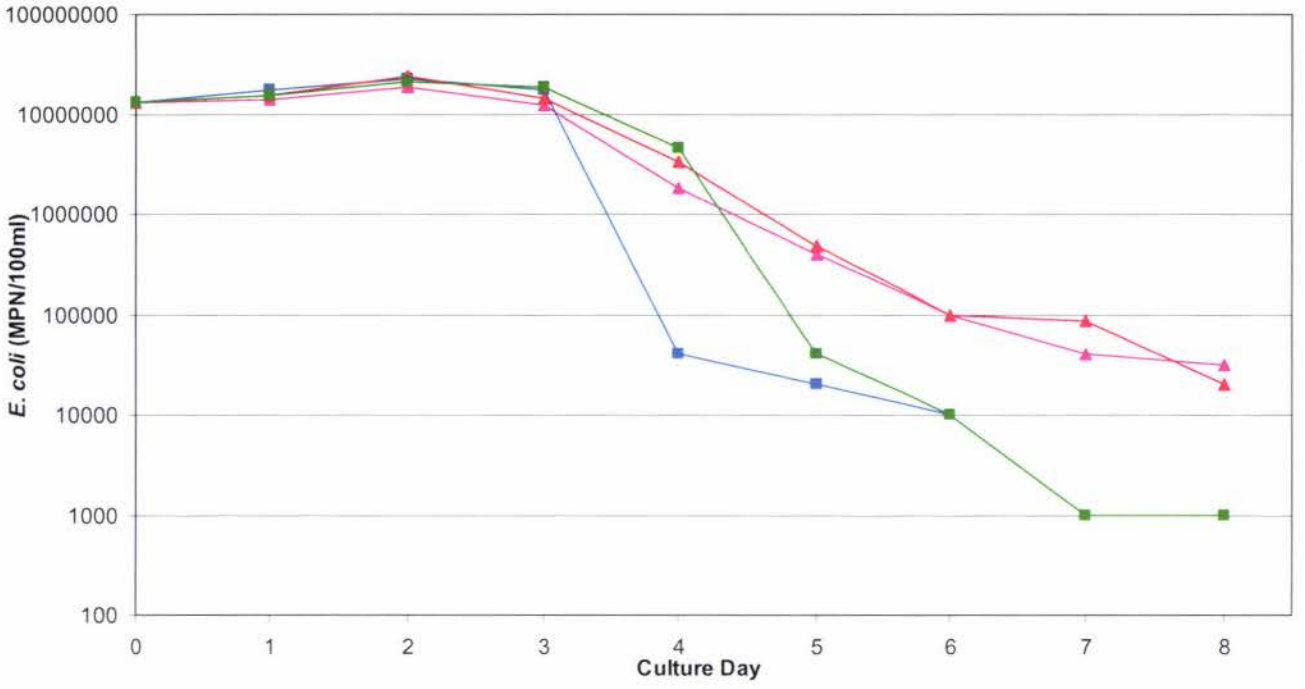

Figure 3.26: *E. coli* numbers in the cultures with CO_2 addition (C1 ▲, C2 ▲) and algal control cultures without CO_2 addition (A1 ■, A2 ■) over the 8 day experimental period.

3.2.3 High $fBOD_5$ Experiment with Glucose

This experiment repeated the previous batch culture experiment but with higher initial concentrations of both algae and $fBOD_5$ to study the influence of CO_2 addition and consequently reduced culture pH on $fBOD_5$ removal. Four mesocosms (two with pH control and CO_2 addition, C1 and C2, and two controls A1 and A2) were set up using primary sewage effluent and HRAP water as the algae inoculum. The initial $fBOD_5$ concentration in two of the mesocosms (one with (C1) and one without CO_2 addition (A1)) was raised by glucose addition. The experiment was conducted over 4 days during the southern hemisphere summer (February) and the mesocosms were sampled daily and analysed for temperature, pH, and concentrations of TSS, DRP, NH_4-N, *E. coli* and $fBOD_5$.

Ambient conditions (maximum and minimum air temperature, solar insolation and rainfall) were similar to the previous summer experiment (Figures: 3.19 and 3.20). Daily air temperatures varied between 23.2 and 26.1°C (maximum) and 10.2 and 16°C (minimum). The average maximum day-time temperature of all 4 mesocosm cultures varied between 23.0°C and 30.5°C over the 4 day experimental period. Daily insolation values (Figure: 3.27) varied between 16.5 and 23.8 $MJ/m^2/d$ over the course of the experiment. No rainfall was recorded during the experimental period.

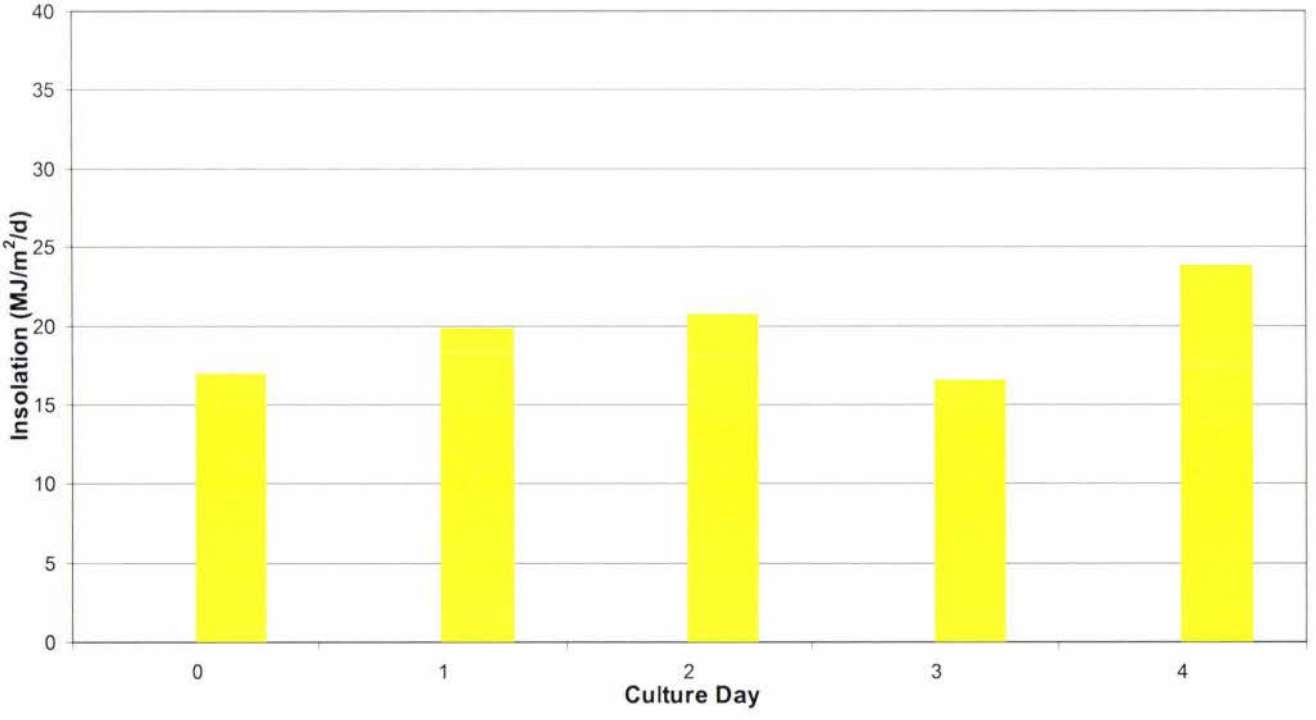

Figure 3.27: Daily insolation (■) during the summer outside mesocosm experiment.

Microscopic analysis of the algae inoculum showed that it was dominated by the single celled algae *Nephrochlamis sp.* and *Monorapidium sp.* which continued to dominate all cultures throughout the experiment. Zooplankton numbers (including *Paramecium sp.* and *Peritriches sp.*) were initially low in all 4 mesocosms and remained low in both mesocosms without glucose addition. In the cultures with glucose addition, zooplankton (particularly *Peritriches sp.*) numbers increased markedly, but unlike previous experiments, grazing did not decrease the TSS concentrations in these mesocosms (Figure: 3.28).

Initial TSS levels (~305 mg/L) were high in all mesocosms due to the large volume of the HRAP water inoculum (Figure: 3.28). TSS levels in all cultures initially increased on day 1 with the largest increase (~25% to ~390 mg/L) in the glucose containing cultures (C1 and A1) compared with (~5% to ~325 mg/L) in the cultures without glucose (C2 and A2) (Figure: 3.28). Throughout the remainder of the 4 day experiment TSS levels in all cultures remained relatively unchanged, except those in the cultures without glucose increased slightly on day 4.

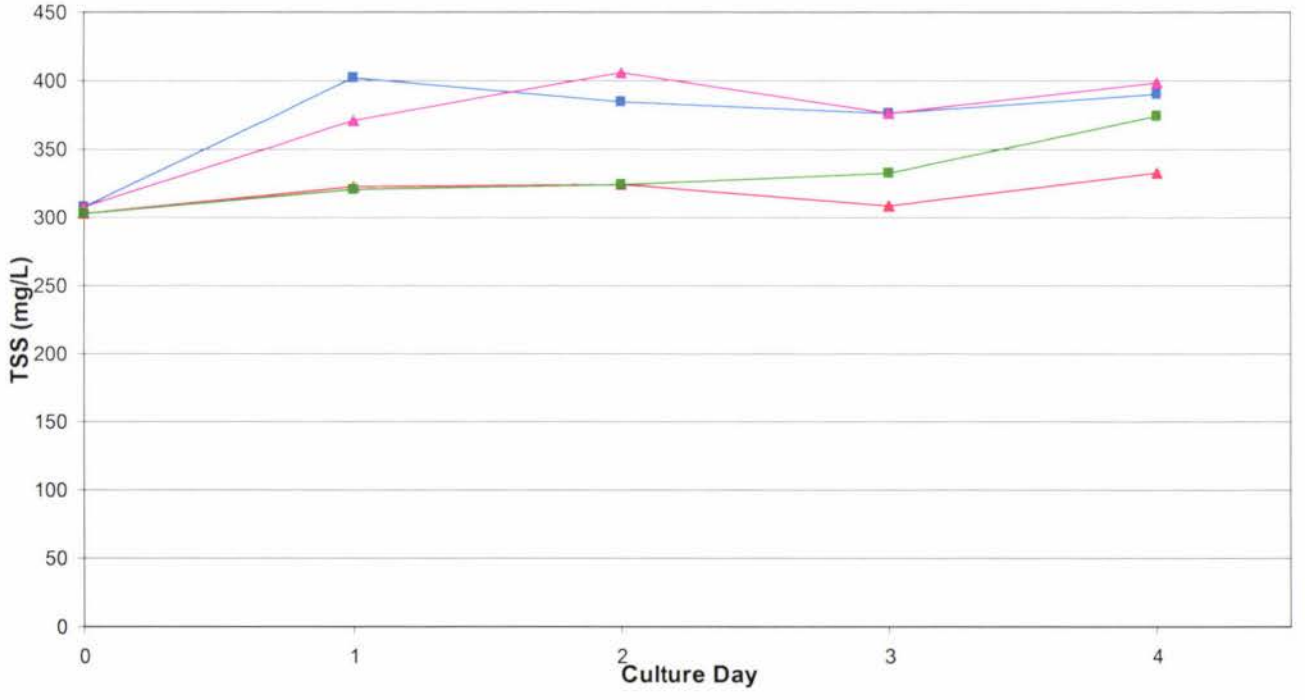

Figure 3.28: TSS concentrations in cultures with CO_2 addition (C1▲ with glucose, C2▲) and algal control cultures without CO_2 addition (A1■ with glucose, A2■) over the 4 day experimental period.

The maximum day-time pH of the control culture without glucose (A2) increased almost linearly reaching a maximum pH (~10.53) on day 4, while that of the control culture with glucose (A1) increased slightly until day 3 when it dropped to below pH 7.5 until the end of the experiment (Figure: 3.29). Carbon dioxide addition restricted the maximum day-time pH level in mesocosms C1 and C2 to below pH 7.75 for the whole experiment, although the controllers, particularly in mesocosm C2 tended to overshoot, reducing culture pH to as low as 6.5 on several occasions. A maximum day-time pH difference of more than 2.5 units was achieved between both of the CO_2 added cultures and the control culture without glucose (A2) during the experiment.

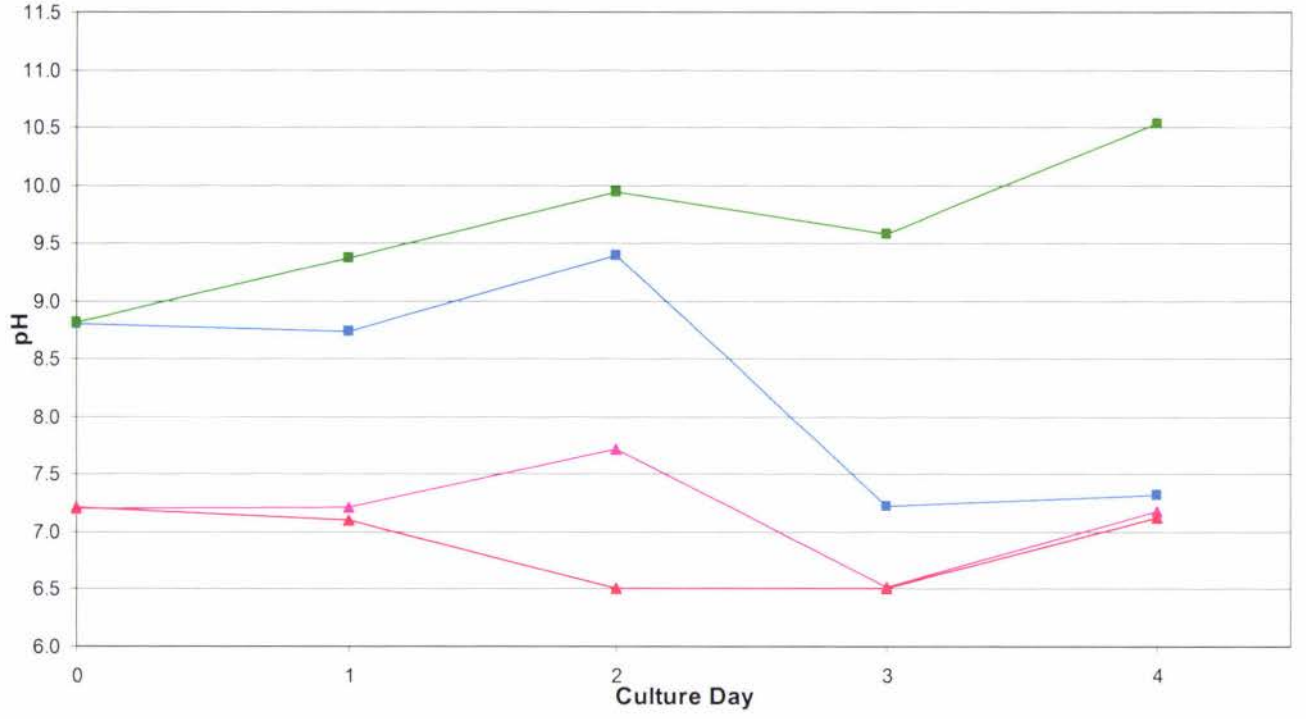

Figure 3.29: Maximum day-time pH in cultures with CO_2 addition (C1▲ with glucose, C2▲) and algal control cultures without CO_2 addition (A1■ with glucose, A2■) over the 4 day experimental period.

Initial NH_4-N concentrations were low (~7.2 mg/L) in all mesocosms due to the large volume of HRAP water used as inoculum (Figure: 3.30). For the mesocosms without glucose, NH_4-N removal was slower in the algal culture with CO_2 addition (3.3 mg/L by day 4) than in the algal control culture which reduced levels to 0 mg/L by day 4. Ammoniacal-nitrogen concentrations in the two cultures with glucose were reduced to below 0.5 mg/L by day 1 and declined further to 0 mg/L on day 4 (Figure: 3.30).

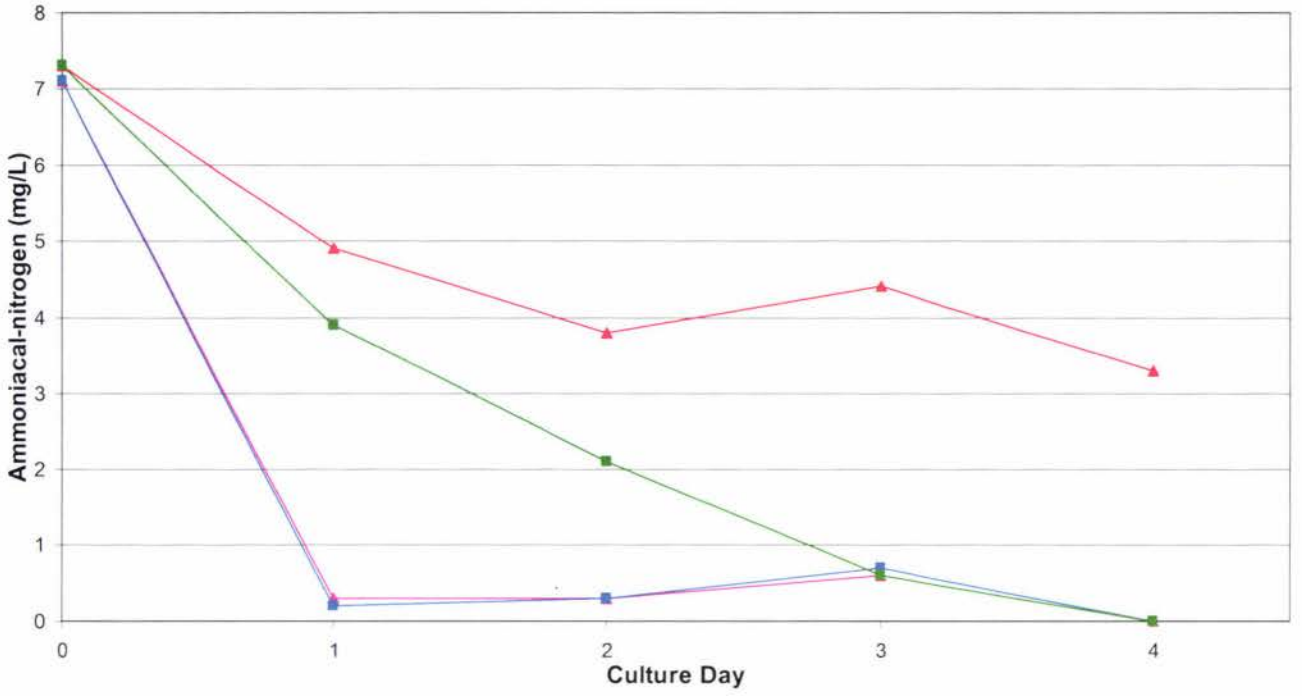

Figure 3.30: Ammoniacal-nitrogen concentrations in cultures with CO_2 addition (C1▲ with glucose, C2▲) and algal control cultures without CO_2 addition (A1■ with glucose, A2■) over the 4 day experimental period.

The low initial DRP concentrations (~2.0 mg/L) in all mesocosms (due to the large volume of HRAP water used as inoculum) were too low to compare the differences in DRP removal between the mesocosms (Appendix B).

Initial $fBOD_5$ concentrations in both of the cultures without glucose were low (20 mg/L) and were slowly reduced over the experiment (Figure: 3.29). Glucose addition increased initial $fBOD_5$ concentrations to 110 mg/L but this was almost completely removed by both cultures (~88%, reduced to ~15 mg/L) by day 1 (Figure: 3.31). No difference in $fBOD_5$ removal performance could be determined between the mesocosms with CO_2 addition and the algal control mesocosms.

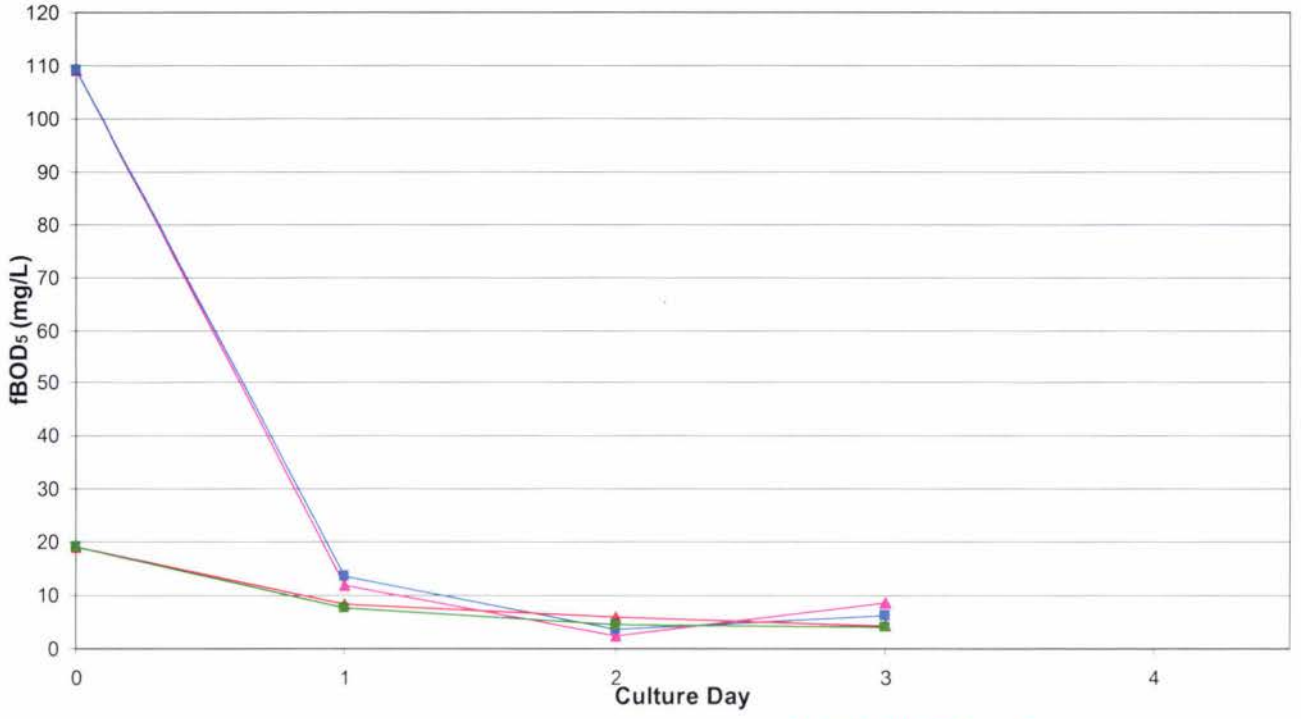

Figure 3.31: $fBOD_5$ concentrations in cultures with CO_2 addition (C1▲ with glucose, C2▲) and algal control cultures without CO_2 addition (A1■ with glucose, A2■) over the 4 day experimental period.

Escherichia coli numbers in all but the algal control culture without glucose (A2) declined at a low and constant rate (~1 log removal over the 4-day experiment) (Figure: 3.32). Between day 1 and 3, *E. coli* numbers in the control culture without glucose declined by ~5 log units. *Escherichia coli* levels in this culture probably dropped further on day 4 but were below the detection limit for the dilution used (Figure: 3.32).

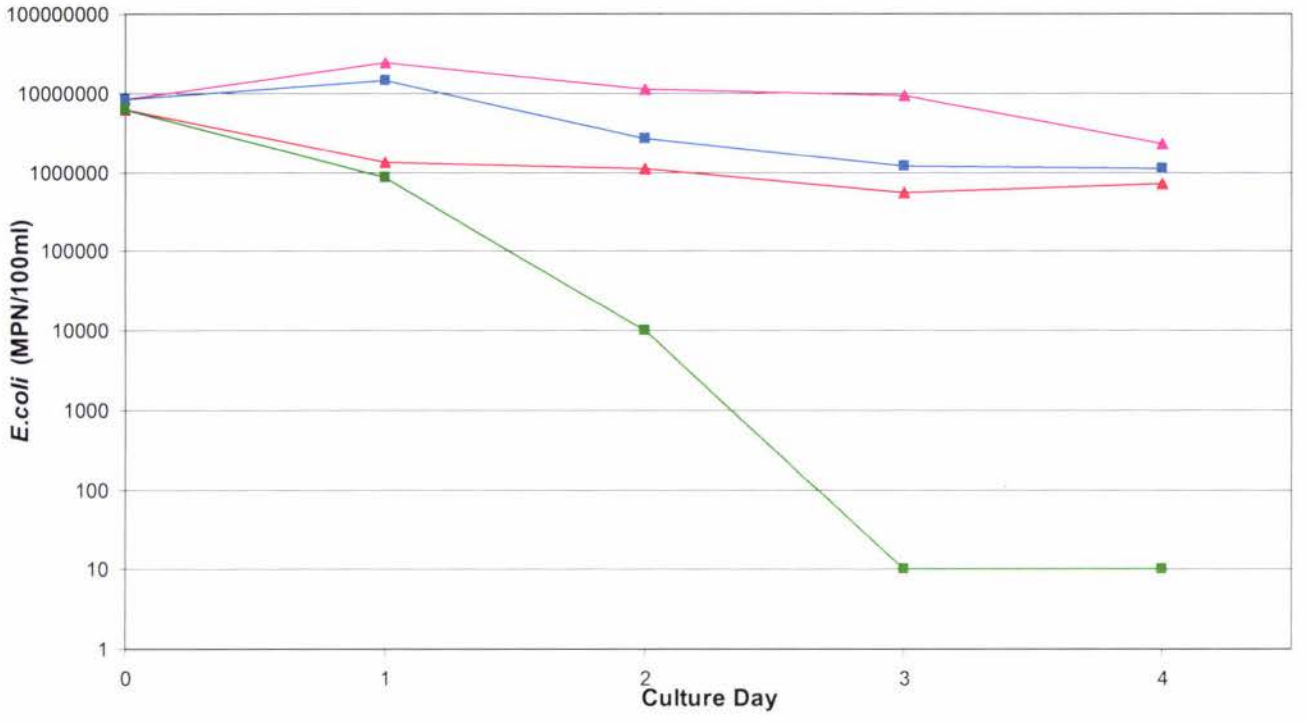

Figure 3.32: *E. coli* numbers in cultures with CO_2 addition (C1▲ with glucose, C2▲) and algal control cultures without CO_2 addition (A1■ with glucose, A2■) over the 4 day experimental period.

3.2.4 High $fBOD_5$ Experiment with Egg Material

This experiment repeated the previous batch culture experiment but used homogenised egg solution at two concentrations (high and low) rather than glucose to raise the initial $fBOD_5$ levels in the cultures. The experiment was conducted over 4 days during late summer (March) and the mesocosms were sampled daily and analysed for temperature, pH, and concentrations of TSS, DRP, NH_4-N, *E. coli* and $fBOD_5$.

Maximum and minimum air temperatures recorded during the experimental period were lower than in the previous summer experiment (Figure: 3.33). Daily air temperatures varied between 19.3 and 22.6°C (maximum) and 6.4 and 13.4°C (minimum). The average maximum day-time temperature of all 4 mesocosm cultures were also lower compared to previous experiments and varied between 20.9°C and 22.2°C over the 3 day experimental period (Figure: 3.33). Daily insolation values varied between 16.9 and 24.9 $MJ/m^2/d$ (Figure: 3.34) over the course of the experiment and were similar to those measured during the previous experiment. Only light rainfall occurred over the 3 day experimental period (Figure: 3.34).

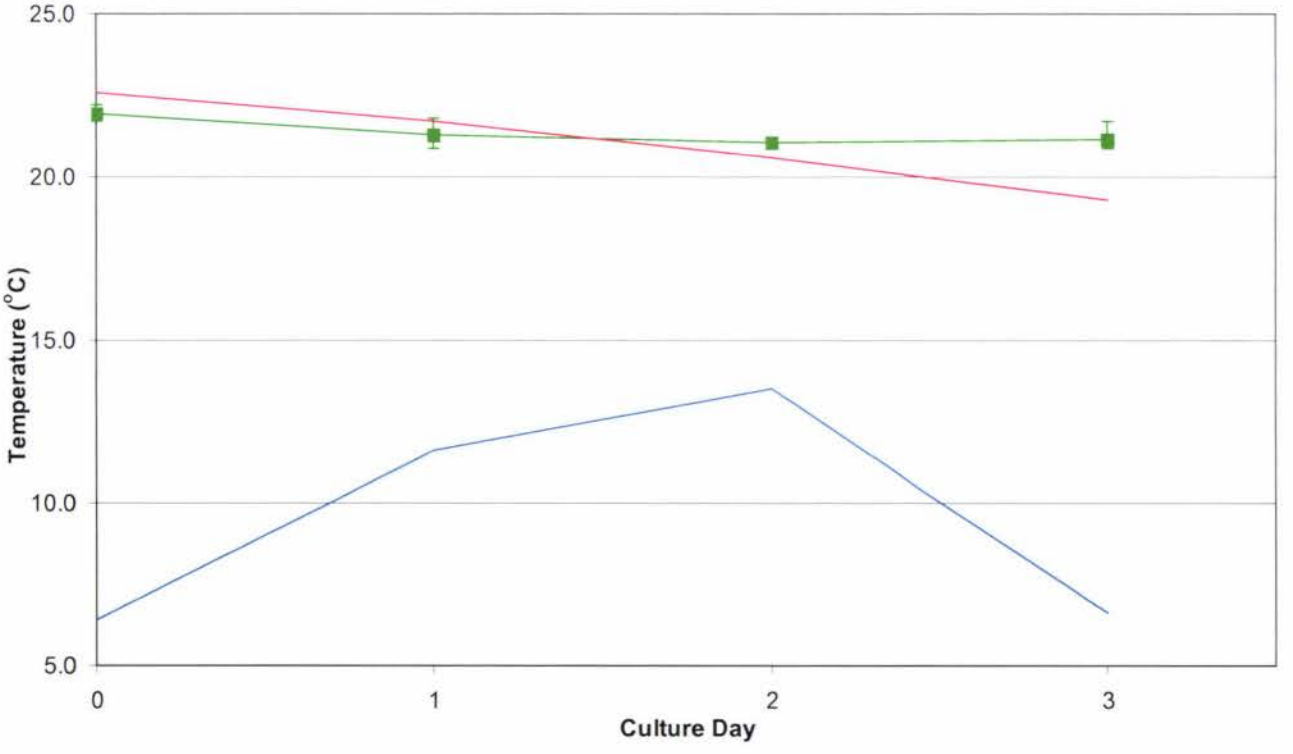

Figure 3.33: Daily maximum (—) and minimum (—) air temperature and maximum culture temperature of 4 mesocosms (■ mean values with error bars indicating the maximum and minimum values) during the 3-day experiment.

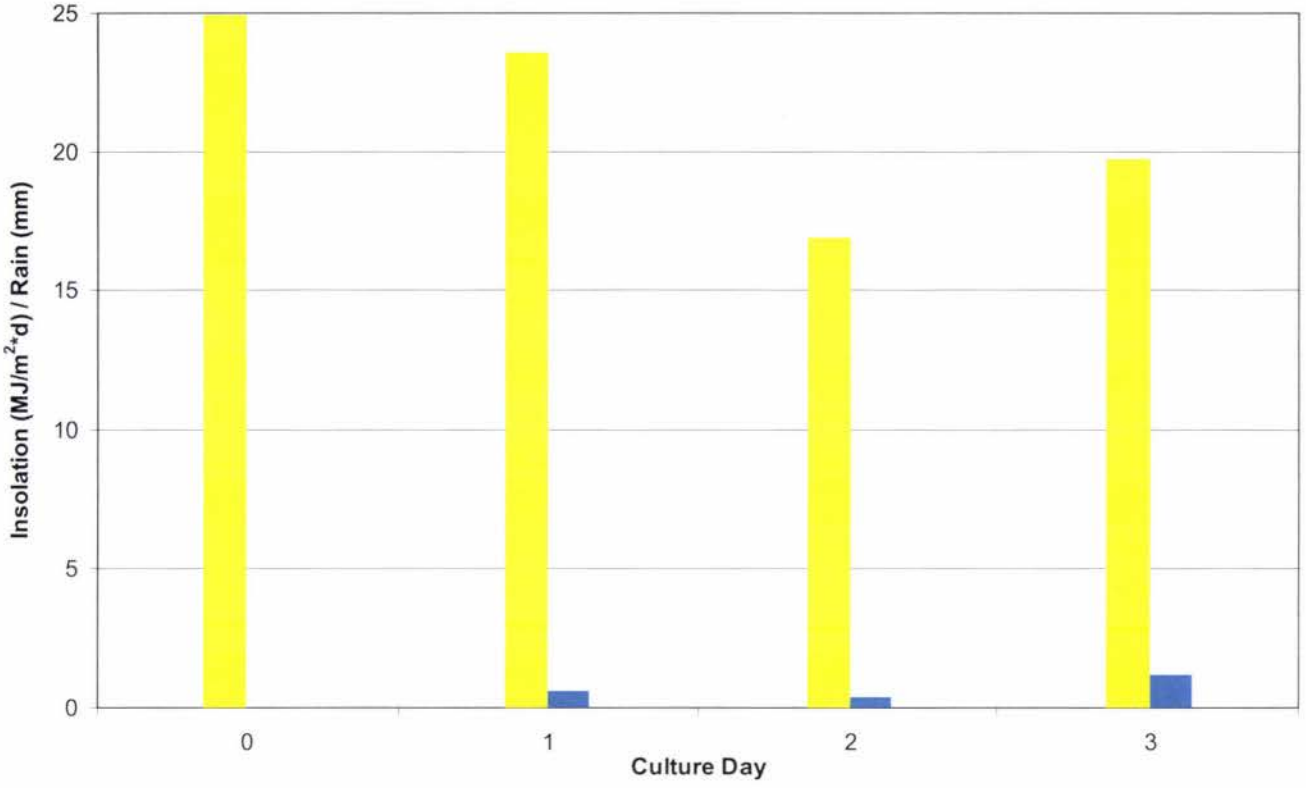

Figure 3.34: Daily insolation (■) and rainfall (■) during the 3-day experiment.

Microscopic analysis of the algae inoculum showed that it was dominated by the colonial algae *Micractinium sp.* with some *Scenedesmus sp.* and smaller single celled algae (Figure: 3.35). *Micractinium sp.* remained dominant but the relative abundance of *Scenedesmus sp.* increased slightly over the 3-day experiment. Numbers of zooplankton (including *Paramecium sp.* and *Brachionus sp.*) were initially low in all 4 mesocosms. Zooplankton numbers (especially *Brachionus sp.*) had increased markedly in all 4 mesocosms by day 3, but unlike many previous experiments, grazing did not decrease the TSS concentrations in these mesocosms (Figure: 3.36).

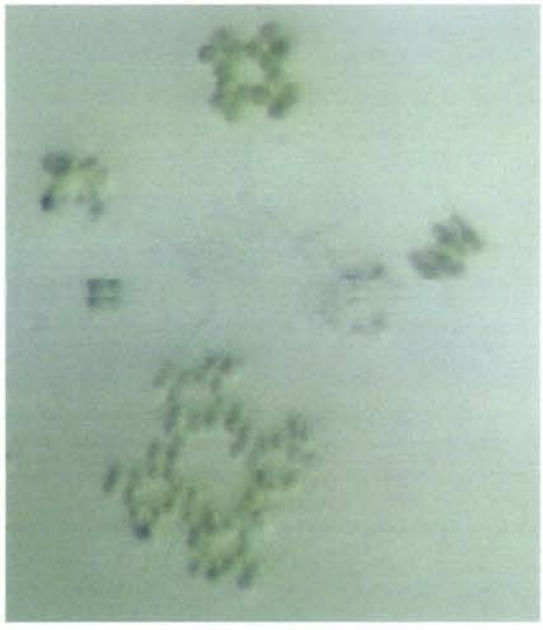

Figure 3.35: Colonies of *Micractinium sp.* (diamonds) and *Scenedesmus sp.* (chains) on day 0 the high $fBOD_5$ experiment with egg material.

Initial TSS levels (~342 mg/L) were high in all mesocosms due to the large volume of the HRAP water inoculum (Figure: 3.36). Total suspended solids levels in all cultures initially increased on day 1 with the largest increase (to ~400 mg/L) in the high egg containing cultures (C1 and A1) compared with (to ~370 mg/L) the low egg containing cultures (C2 and A2) (Figure: 3.36).

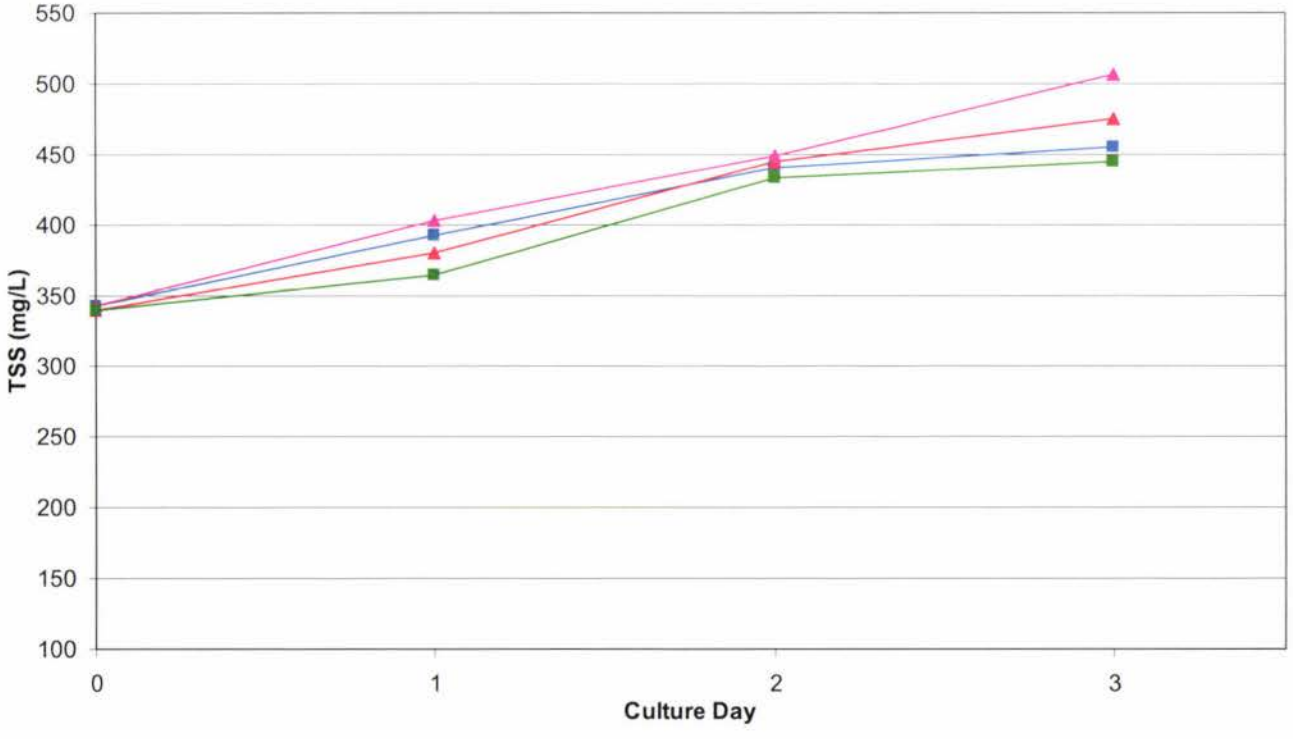

Figure 3.36: TSS concentrations in cultures with CO_2 addition (C1▲ high egg, C2▲ low egg) and algal control cultures without CO_2 addition (A1■ high egg, A2■ low egg) over the 3 day experimental period.

Throughout the remainder of the 3-day experiment TSS levels in all cultures showed a similar increase, with slightly higher growth in the algal cultures with CO_2 addition (maximum TSS >500 mg/L) compared to algal control cultures (~450 mg/L).

The maximum day-time pH of the algal control cultures (A1 and A2) increased almost linearly reaching a maximum pH (~10.8) on day 3 (Figure: 3.37). Carbon dioxide addition restricted the maximum day-time pH level in mesocosms C1 and C2 to between pH 7.24 and 8.33 for the whole experiment. A maximum day-time pH difference of more than 2.5 units was achieved between both of the CO_2 added cultures (C1 and C2) and the control cultures (A1 and A2) during the experiment (Figure: 3.37). The high egg cultures had slightly lower pH than the low egg cultures throughout the experiment.

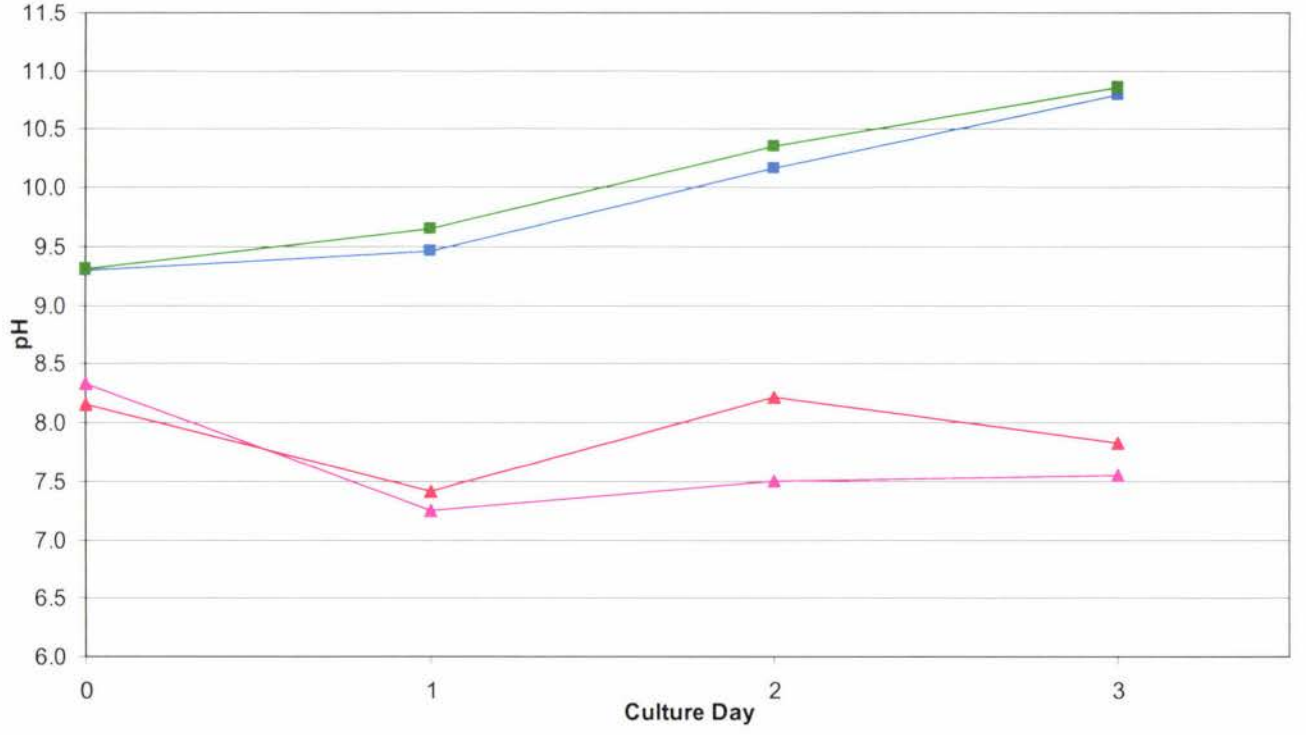

Figure 3.37: Maximum day-time pH in cultures with CO_2 addition (C1▲ high egg, C2▲ low egg) and algal control cultures without CO_2 addition (A1■ high egg, A2■ low egg) over the 3 day experimental period.

Initial NH_4-N concentrations in all mesocosms are not given as the samples were accidentally destroyed (Figure: 3.38). Both of the algal control cultures had a higher NH_4-N removal rate (reduced to 0 mg/L by day 3) than the algal cultures with CO_2 addition, of which the culture with the higher addition of egg solution had slightly lower NH_4-N removal (Figure: 3.38).

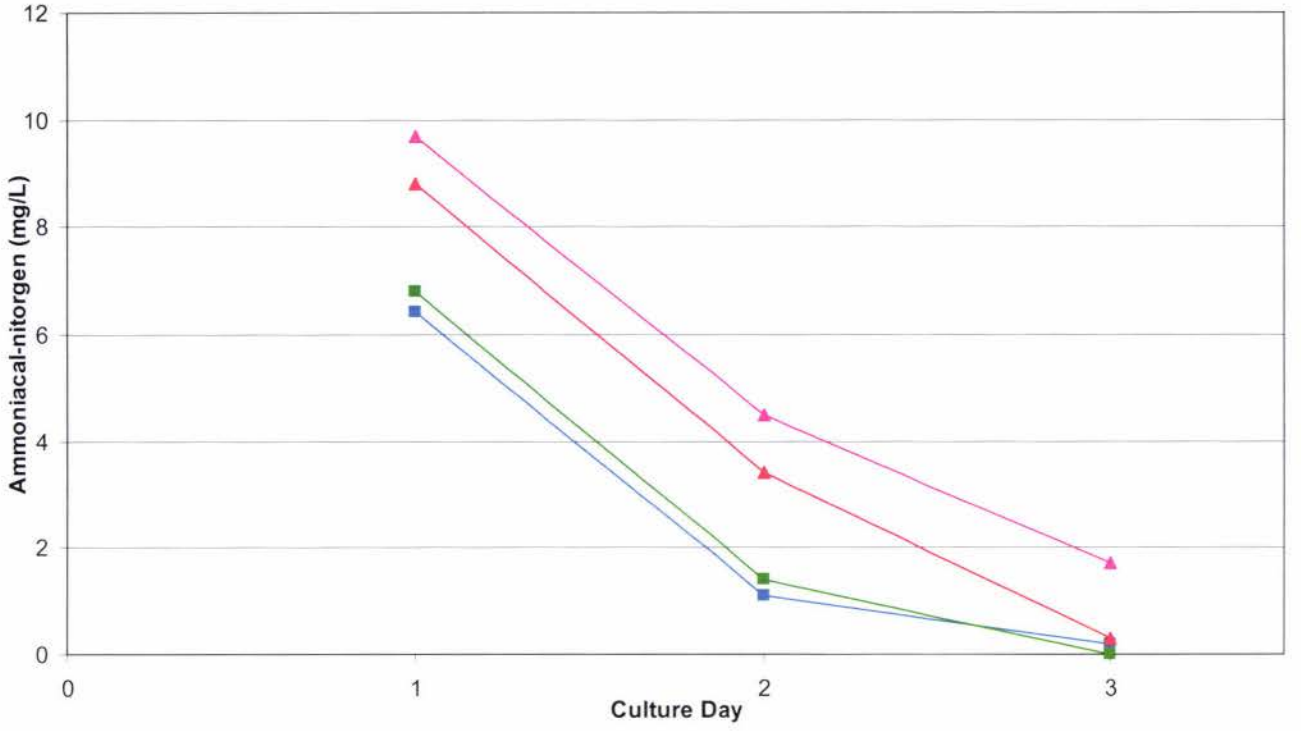

Figure 3.38: Ammoniacal-nitrogen concentrations in cultures with CO_2 addition (C1▲ high egg, C2▲ low egg) and algal control cultures without CO_2 addition (A1■ high egg, A2■ low egg) over the 3 day experimental period.

Initial DRP concentrations in all mesocosms are not given as the samples were accidentally destroyed (Figure: 3.39). The DRP concentrations did not change in the algal cultures with CO_2 addition over the 3-day experimental period, but were reduced to <2.0 mg/L by day 3 in the algal control cultures, of which the culture with the higher addition of egg solution had slightly lower DRP removal (Figure: 3.39).

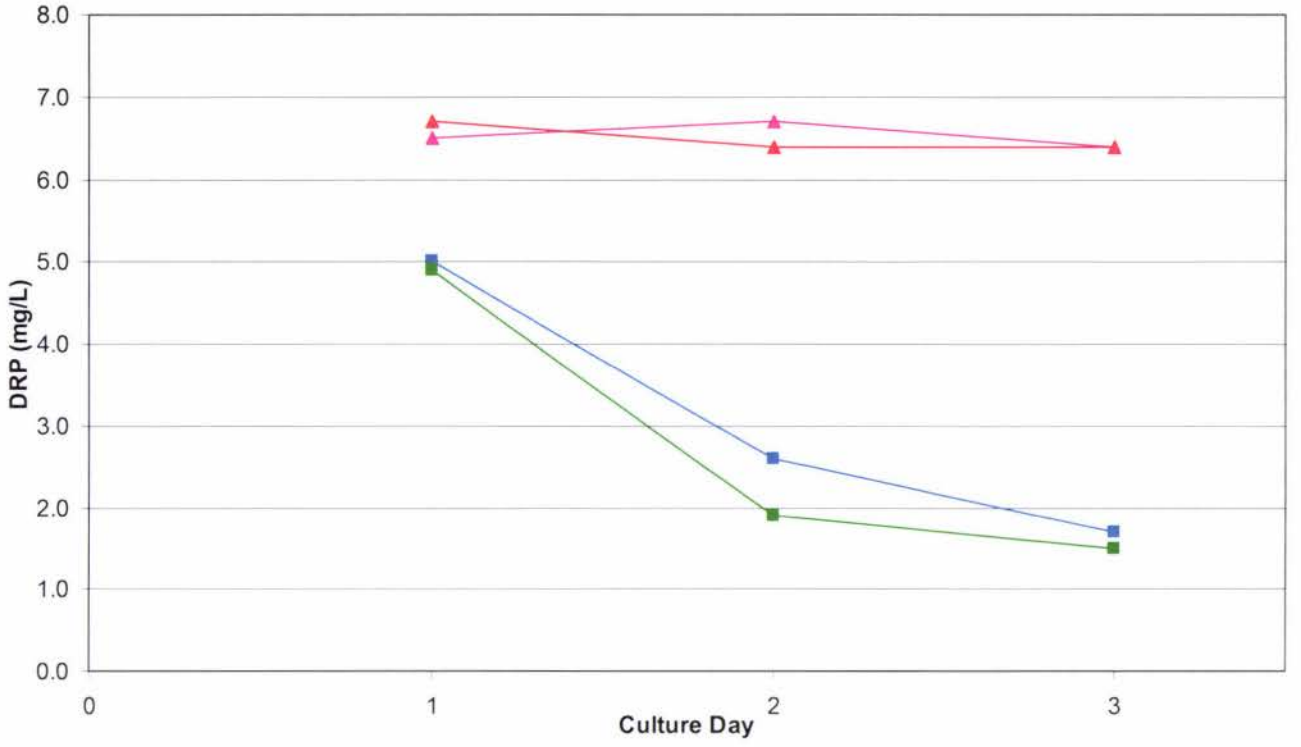

Figure 3.39: Dissolved reactive phosphorus (DRP) concentrations in cultures with CO_2 addition (C1▲ high egg, C2▲ low egg) and algal control cultures without CO_2 addition (A1■ high egg, A2■ low egg) over the 3 day experimental period.

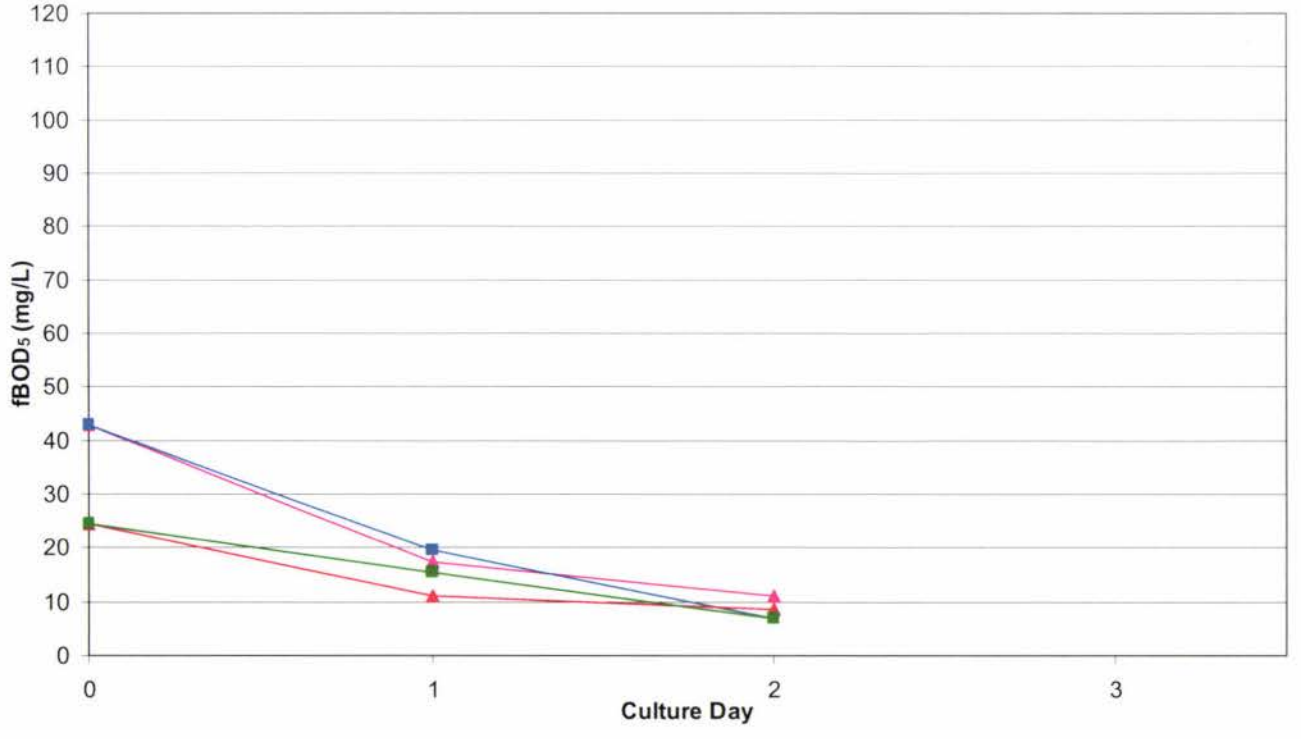

Figure 3.40: $fBOD_5$ concentrations in cultures with CO_2 addition (C1▲ high egg, C2▲ low egg) and algal control cultures without CO_2 addition (A1■ high egg, A2■ low egg) over the 3 day experimental period.

Addition of homogenised egg solution (8 ml to C1 and A1; 4 ml to C2 and A2) raised initial $fBOD_5$ concentrations of the mesocosms to 43 mg/L and 24.5 mg/L respectively, which was only about halve of the target value, indicating that the added material was not completely water soluble. The $fBOD_5$ concentrations were all reduced to ~10 mg/L by day 2 (Figure: 3.40). No difference in $fBOD_5$ removal was detectable between the mesocosms with CO_2 addition (C1 and C2) and the algal control mesocosms (A1 and A2) (Figure: 3.40).

Initial *E. coli* levels (~30,000 MPN/100ml) were rather low compared to previous experiments (Figures: 3.26 and 3.32) as the Ruakura HRAP had pH levels of >10 in the days prior to the inoculum being taken for this experiment. *E. coli* removal rates (4 log removal) in the algal control cultures (A1 and A2) were double those (2 log removal) of the cultures with CO_2 addition (C1 and C2) during the 3-day experiment (Figure: 3.41).

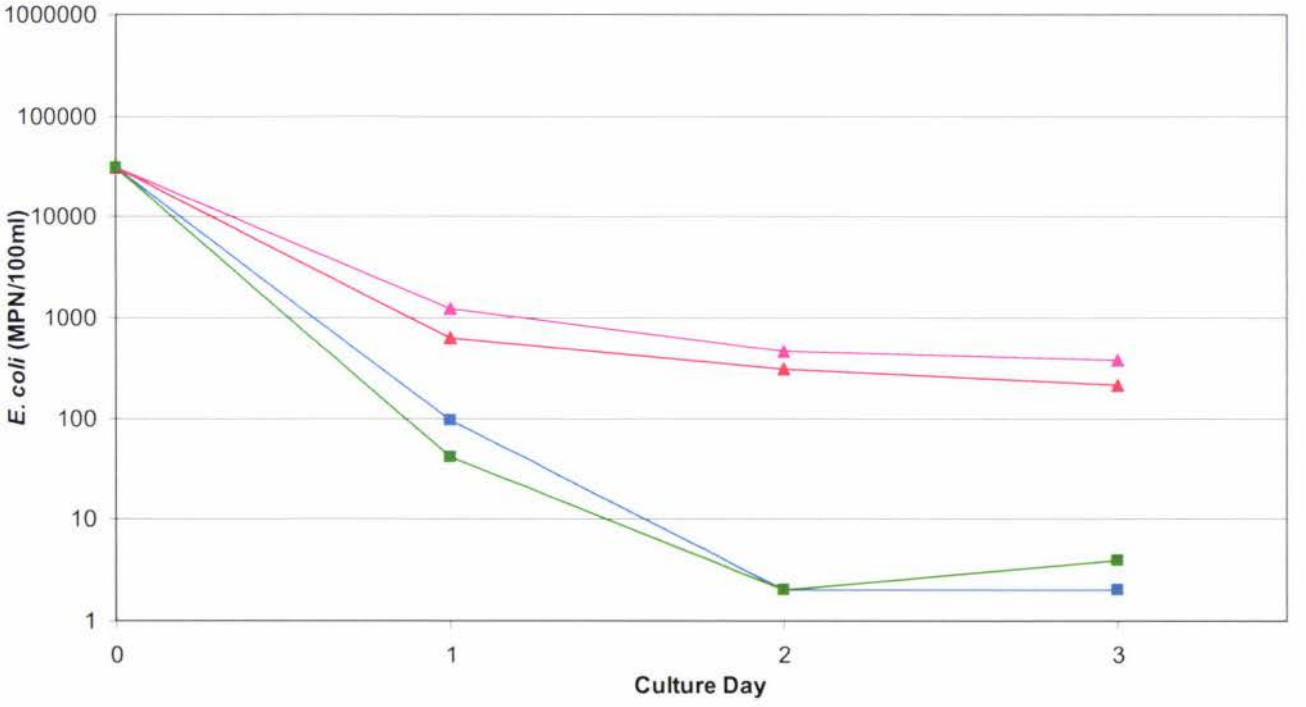

Figure 3.41: *E. coli* numbers in cultures with CO_2 addition (C1▲ high egg, C2▲ low egg) and algal control cultures without CO_2 addition (A1■ high egg, A2■ low egg) over the 3 day experimental period.

3.3 Ruakura Pilot-scale High Rate Algal Ponds

This experiment compared algal growth and wastewater treatment of a HRAP with CO_2 addition (eastern HRAP) to those of a control HRAP without CO_2 addition (western HRAP) over four months (August to December 2005). The CO_2 addition assembly was installed in the eastern HRAP on the 5th of September. Over the experimental period both ponds were monitored at least weekly intervals for pond water temperature, pH, TSS, DRP and NH_4-N, and algae and zooplankton abundance. Three intensive studies of the pond water physical characteristics (temperature, pH and dissolved oxygen) were made using datasondes, firstly from late August to early September (Winter) before the installation of the CO_2 addition to the eastern HRAP, secondly from late September to early October (Spring) and thirdly, from late November to early December (Summer).

Both HRAP had very similar pond water characteristics during the winter intensive study, prior to the installation of the CO_2 addition in the eastern HRAP (Figure: 3.42). Pond water physical characteristics all varied with diurnal changes in insolation. Data from the summer intensive study (November/December 2005) after the CO_2 addition had been installed in the eastern HRAP is shown in Figure 3.43. Both pond water temperature and DO concentration were unaffected by CO_2 addition, except the fact that during the summer intensive study, the decline in DO concentration after the midday peak in the eastern HRAP (with CO_2 addition) occurred about an hour later than that in the control pond.

The CO_2 addition system worked quickly and effectively maintaining the pH of the eastern HRAP below pH 8.3, despite the shallow (~30 cm) depth of the HRAP for CO_2 absorption. This resulted in a maximum pH difference of ~1 pH unit between the eastern and the western HRAP during the day. Control of maximum day-time pH also led to a reduction in night-time pH compared to that of the control HRAP without CO_2 addition (Figure: 3.43). During the winter intensive study day-time pH of both HRAP increased to a maximum value and then declined, however, during the summer intensive study the day-time pH of the HRAP control had two maxima either side of midday with several variations in between (Figures: 3.42 and 3.43).

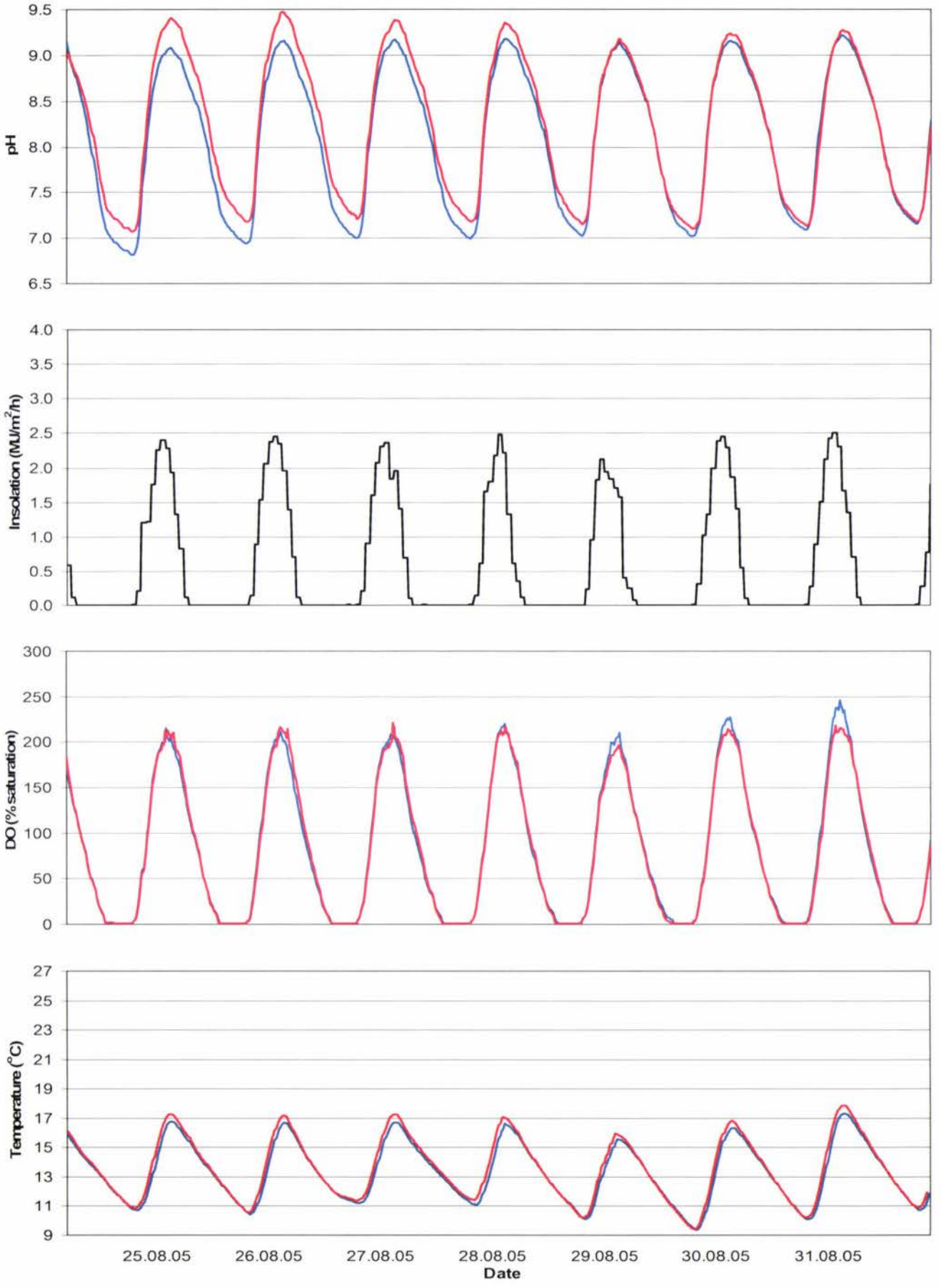

Figure 3.42: pH, DO and temperature of the eastern (■) and the western (■) pilot-scale HRAP in relation to insolation, measured during the winter intensive monitoring period prior to installation of CO_2 addition in the eastern HRAP.

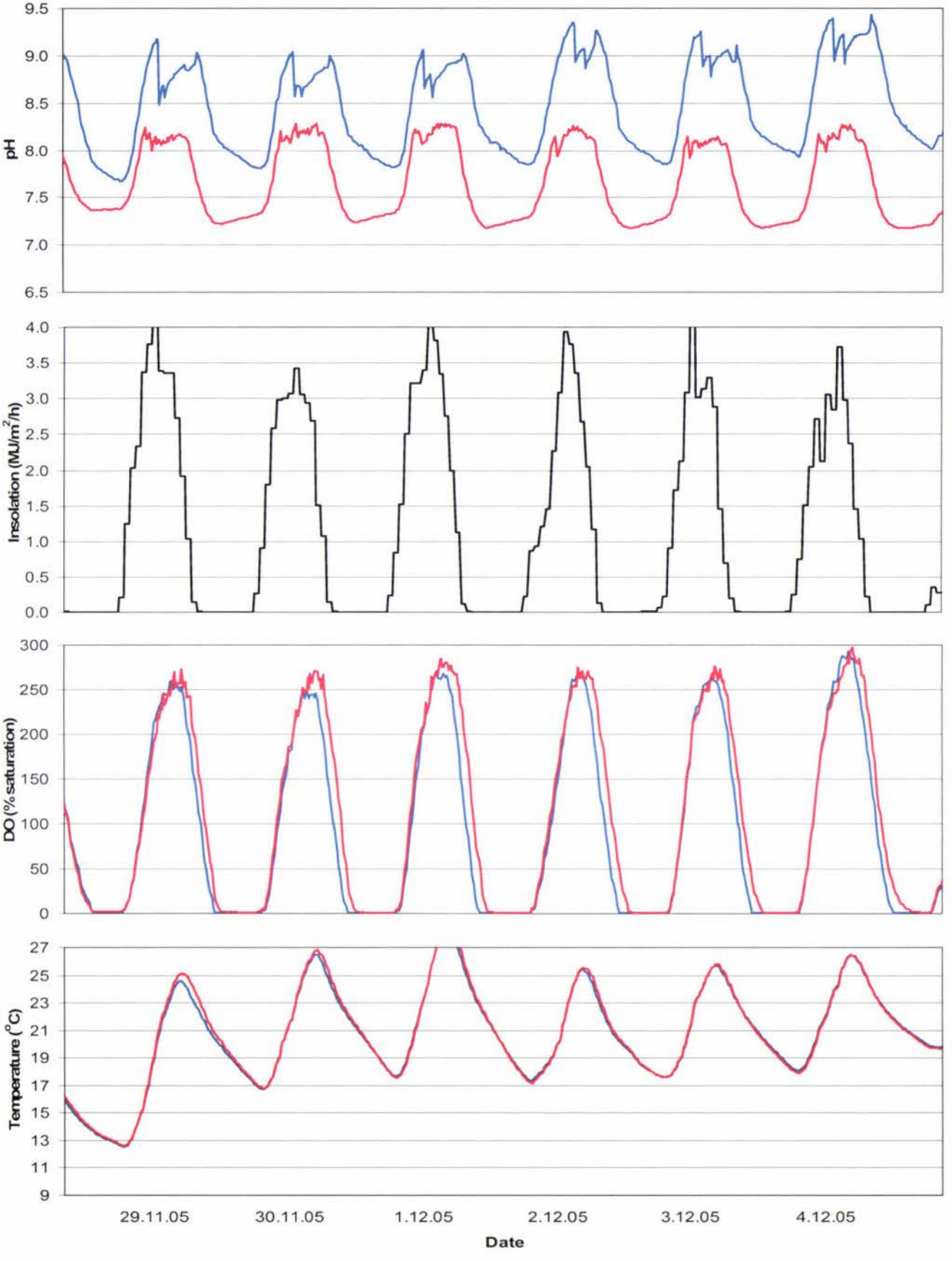

Figure 3.43: pH, DO and temperature of the eastern CO_2 added (■) and the western control (without CO_2 addition) (■) pilot-scale HRAP in relation to insolation measured during the summer intensive monitoring period.

The water temperature of both HRAP was usually higher than that of the influent wastewater (Figure: 3.44) and HRAP water temperature was unaffected by CO_2 addition.

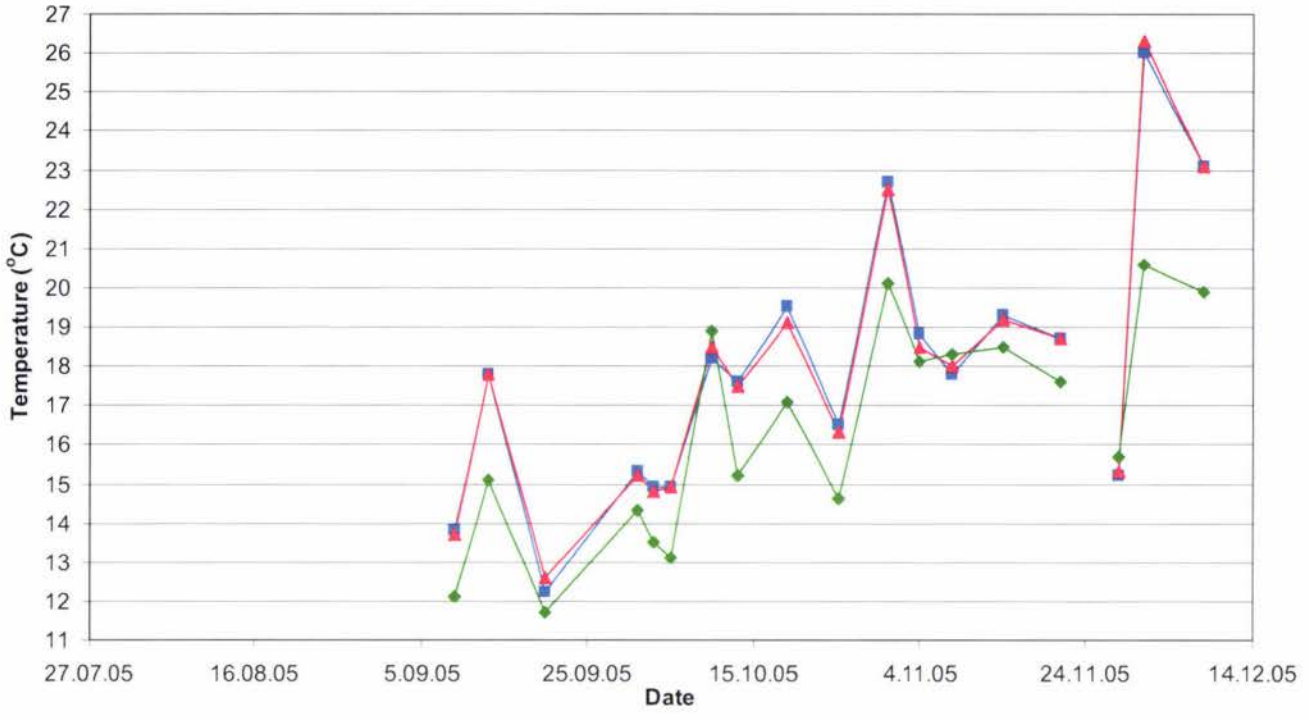

Figure 3.44: Temperature of the influent wastewater (♦), the eastern HRAP with CO_2 addition (▲) and the western control HRAP without CO_2 addition (■) from August to December 2005.

Microscopic analysis of the water from both the eastern (with CO_2 addition) and the western (control) HRAP showed a succession of dominant algae species in both ponds over the experimental period. At the start of the experiment (August 2005) both ponds contained a mixed culture of algae species, including *Dictyosphaerium sp..*, *Actinastrum sp.*, *Ankistrodesmus sp.*, *Scenedesmus sp.*, *Closterium sp.*, and *Pediastrum sp.* The colonial algae *Dictyosphaerium sp.* became the dominant species from the beginning of September until the beginning of October. The *Dictyosphaerium sp.* grew in colonies of >24 in both HRAP, regardless of CO_2 addition unlike observations in the cultures of the winter outside mesocosm experiment and the first laboratory batch experiment (Figures: 3.1 and 3.2). During October, the *Dictyosphaerium sp.* culture in both HRAP died (week 2 for the western (control) HRAP and week 3 for the eastern HRAP (with CO_2 addition)). It took nearly a month, until the end of November for a new algae culture to establish in the HRAP, occurring slightly faster in the eastern HRAP. This new culture was dominated by the single celled algae *Monorapidium sp.* and *Nephroclamis sp.* which dominated the HRAP until the end of the experimental period.

Throughout the experimental period zooplankton including *Paramecium sp., Brachionus sp.* and smaller zooplankton species were present in both HRAP at low levels. Following the death of the *Dictyosphaerium sp.* in October a large population of *Detritus sp.* grazers established in both ponds, but these disappeared as the new culture of *Monorapidium sp.* and *Nephroclamis sp.* developed. In general no difference in zooplankton abundance and activity could be detected between the eastern (with CO_2 addition) and the western (control) pond.

The TSS concentration of the influent wastewater was fairly constant throughout the experimental period ranging between 50 - 100 mg/L (Figure: 3.45). The TSS concentrations in both HRAP were variable, but in general, the eastern HRAP (with CO_2 addition) had a higher TSS concentration (algae biomass) (average 242 mg/L) than the western (control) HRAP (average 202 mg/L) throughout the 4 month experimental period. The October decline in the TSS concentrations of both HRAP coincides with the death of the *Dictyosphaerium sp.* which dominated the ponds at the time. The increase in TSS concentrations in both ponds at the end of October corresponds to the establishment of the *Nephrochlamis sp.* and *Monorapidium sp.* dominated cultures. The algal culture of the eastern HRAP (with CO_2 addition) re-established more quickly than that of the western (control) HRAP. The highest TSS concentrations 338mg/L and 327 mg/L for the eastern (CO_2 added) and the western (control) pond respectively were achieved at the end of the experimental period (summer) (Figure: 3.45) and are similar to the those recorded during the summer outside mesocosm experiment (up to 378 mg/L, Figure: 3.21), but less than half of those measured during the laboratory batch experiments (up to 1050 mg/L, Figure 3.11).

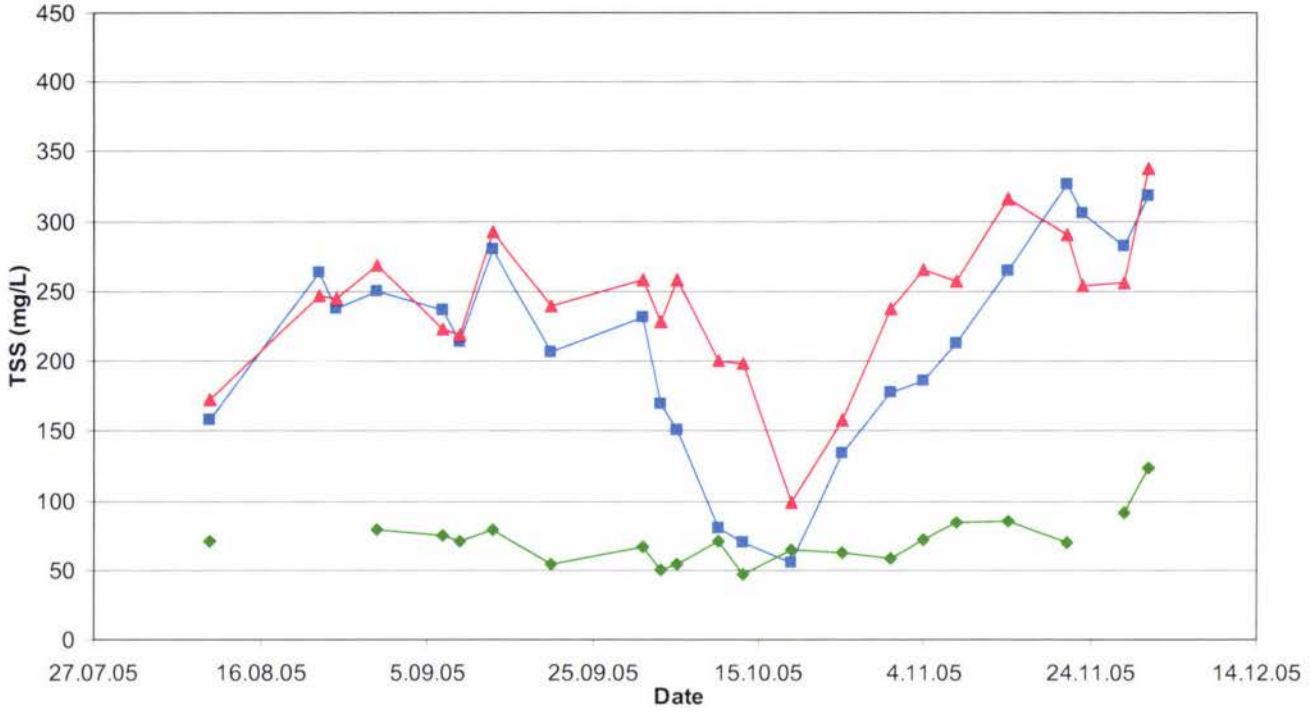

Figure 3.45: TSS concentrations in the influent wastewater (♦), the eastern HRAP with CO_2 addition (▲) and the western control HRAP without CO_2 addition (■) from August to December 2005.

The pH of the influent wastewater was fairly constant throughout the experimental period ranging between pH 6.2 and pH 7.5 (Figure: 3.46). The pH of both HRAP was similar before the installation of CO_2 addition to the eastern HRAP on the 5th of September. The CO_2 addition system maintained the pH of the eastern HRAP below pH 8.5 most of the time, resulting in a maximum day-time pH difference of up to 1 pH units between the eastern and the western HRAP during the day throughout the 4 month experimental period. The high pH of the eastern HRAP (pH 8.94) on the 5th October was a result of the CO_2 bottle running out. The pH of the control western HRAP never attained the high levels (>9.5) achieved in the control algal cultures in previous experiments. The decline in the pH of the western (control) HRAP during October coincides with the death of the *Dictyosphaerium sp.* algae at this time (Figure: 3.45). The increase in the pH of the western HRAP at the end of October corresponds to the establishment of the *Nephrochlamis sp.* and *Monorapidium sp.* dominated culture, but pH levels never returned to those (>9.0) of the *Dictyosphaerium sp.* dominated culture (Figure: 3.46).

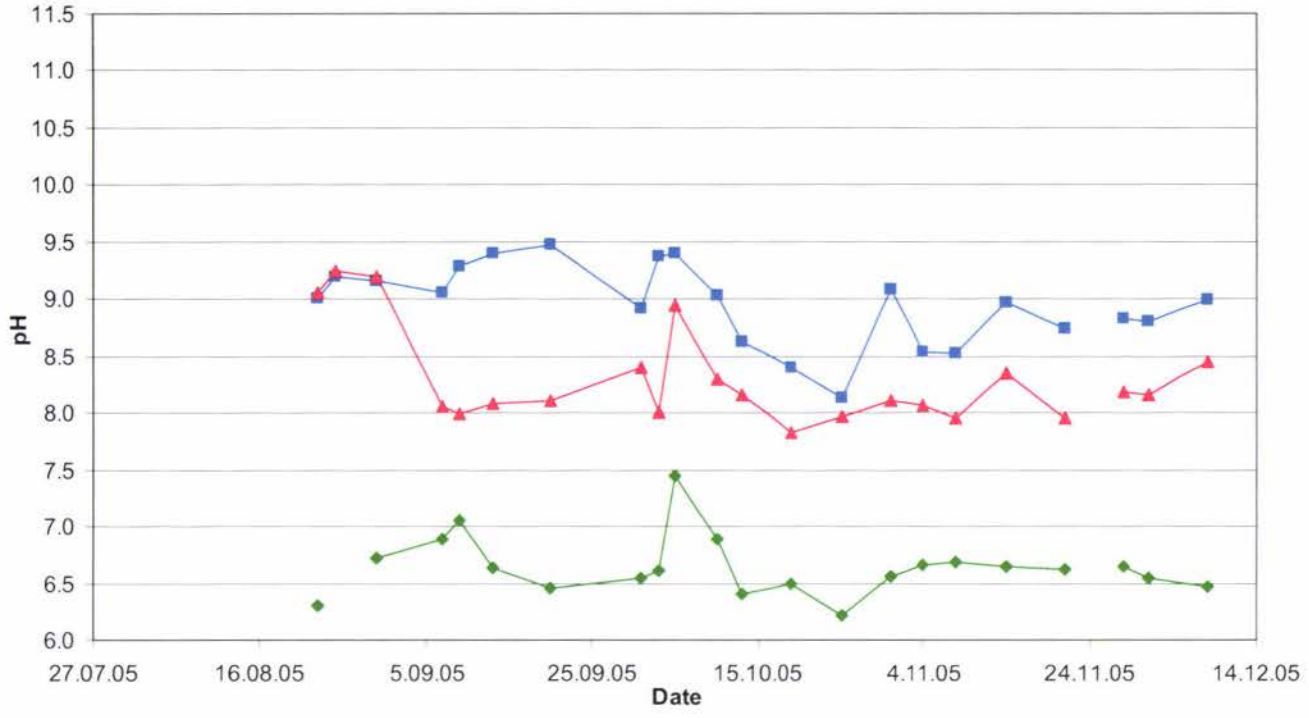

Figure 3.46: Maximum day-time pH in the influent wastewater (♦), the eastern HRAP with CO_2 addition (▲) and the western control HRAP without CO_2 addition (■) from August to December 2005.

The NH_4-N concentration of the influent wastewater was very variable throughout the experimental period ranging from 13 mg/L to 75 mg/L (Figure: 3.47). The NH_4-N concentrations in both HRAP varied directly with the influent concentration.

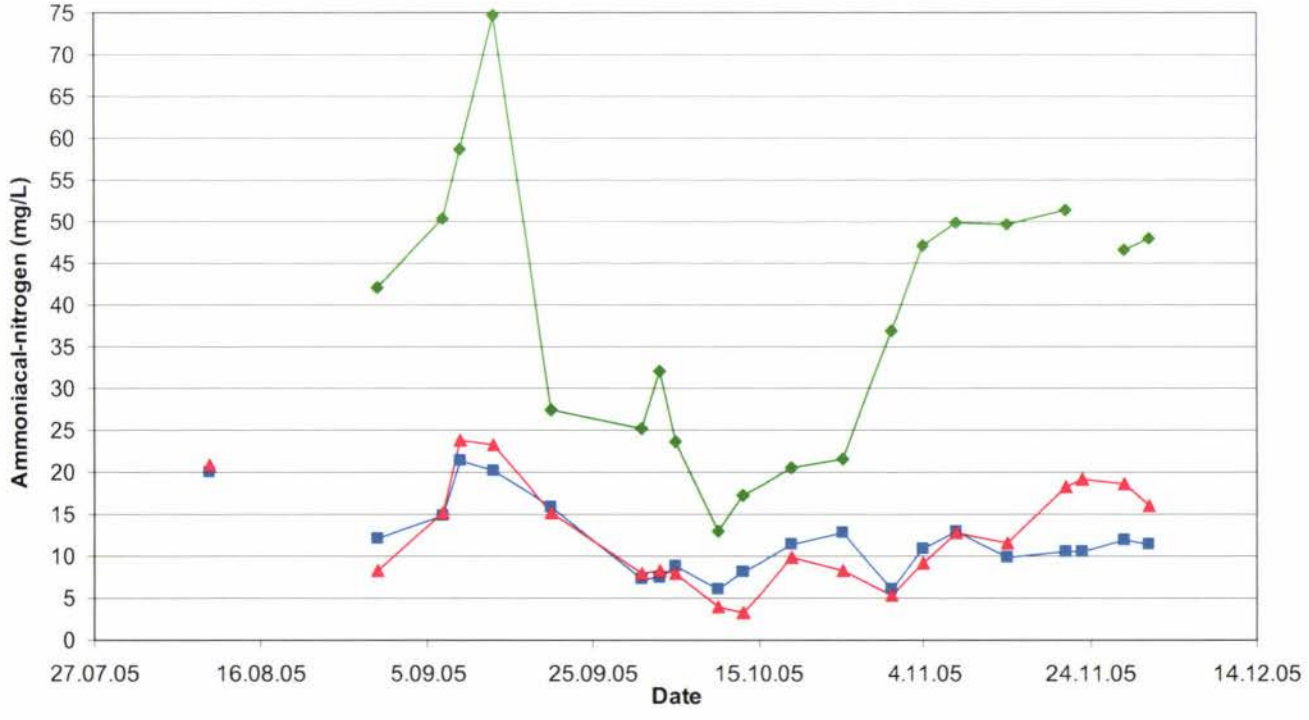

Figure 3.47: Ammoniacal-nitrogen concentrations in the influent wastewater (♦), the eastern HRAP with CO_2 addition (▲) and the western control HRAP without CO_2 addition (■) from August to December 2005.

The DRP concentration of the influent wastewater was very variable throughout the experimental period ranging from 2.8 mg/L to 8.8 mg/L (Figure: 3.48). The DRP concentrations in both HRAP varied directly with the influent concentration, and the eastern HRAP (with CO_2 addition) generally removed more DRP than the western HRAP (control).

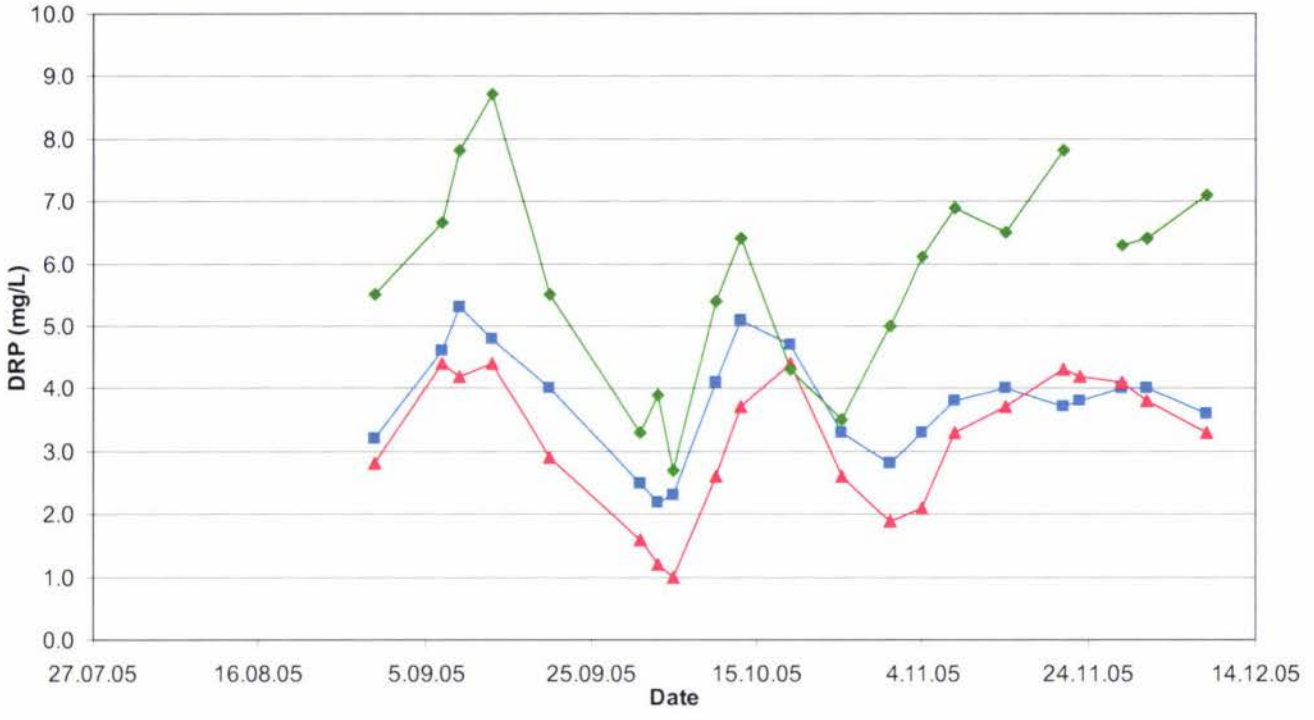

Figure 3.48: Dissolved reactive phosphorus (DRP) concentrations in the influent wastewater (♦), the eastern HRAP with CO_2 addition (▲) and the western control HRAP without CO2 addition (■) from August to December 2005.

4. Discussion

4.1 Effects of CO_2 Addition on Algae Growth (measured as TSS)

In all experiments CO_2 addition to algal wastewater cultures resulted in increased TSS concentrations, compared to the control cultures without CO_2 addition (summarized in Table 4.1).

Table 4.1: Growth promotion (% TSS increase) in cultures with CO_2 addition compared to TSS in control cultures without CO_2 addition.

Experiment	Culture Length (days)	Growth promotion in cultures with CO_2 addition
Laboratory batch experiments (mean of 3 experiments)	4	26%
	8	76%
Outside summer mesocosm experiment	4	17%
	8	53%
Outside winter mesocosm experiment, semi-continuous culture	8 day retention time	19%
Ruakura HRAP experiment, continuous culture	8 day retention time	20%

These results are in agreement with observations made by other authors who found that CO_2 addition to algae wastewater cultures increased algal growth in both laboratory and outside experiments (Fitzgerald and Rohlich 1964; Azov et al. 1982; Conde et al. 1993; Travieso et al. 1993). Increases in algal growth (indicated by TSS) with CO_2 addition found in the present study (17% - 26% after 4 days culture length, 19% - 76% after 8 days culture length (or HRT)) (Table: 4.1) are similar to results reported by Azov et al. (1982) (up to 30%, semi-continuous culture outside experiment, 3 day retention time, Israel) and Goldman et al. (1982) (up to 30%, continuous culture laboratory experiment, 2 day retention time), but are lower than those reported by Fitzgerald and Rohlich (1964) (~69% after 4 days laboratory batch experiment).

Several other authors (Fitzgerald and Rohlich 1964; Azov et al. 1982) have used TSS to indicate algae growth. However, in some instances during the experiments in the present study, TSS might not have accurately represented algal biomass. During the second and third laboratory batch experiments, initial TSS increases in all algal cultures on day 1, were similar to the TSS increase observed in the non-algal control cultures (Figures: 3.7, 3.11) indicating that this TSS increase was possibly due to bacterial growth. However, as the TSS

readings of the non-algal control cultures of the third laboratory batch experiment (Figure: 3.11) dropped again on day 2 and day 3 it would appear that such bacterial blooms were only short lived, and that TSS represented algal biomass rather accurately from day 3 onwards. Bacterial biomass usually represents a minor fraction of the TSS in a field-scale HRAP (Abeliovich and Weisman 1978; Azov et al. 1982), thus, unlike HRAP, initial conditions in the laboratory microcosms stimulated bacterial growth, and were not an exact replication of field conditions.

Initial bacterial growth due to the addition of glucose and egg material to algal cultures during two outside mesocosm experiments (Section: 2.2.2.7 and 2.2.2.8) may also have interfered with the absolute TSS values of the respective cultures. However as both cultures with and without CO_2 addition had equal amounts of glucose or egg material added, the observed differences in TSS levels are likely due to the promotion of algal growth with CO_2 addition.

TSS concentrations measured during the outside batch culture experiments (maxima: from 223 to 506 mg/L) (Figures: 3.21, 3.28 and 3.36) and semi-continuous culture experiments (average: between 202 and 319 mg/L) (Figures: 3.17 and 3.45) are consistent with field data from other authors: Azov et al. (1982) ((100 to 500 mg/L) 250 mg/L, annual average, Israel), Moutin et al. (1992) (188 mg/L, annual average, France) and Green et al. (1995b) (241 mg/L, 2 year average, California). Two clear trends can be identified from the TSS data of the present study. Firstly, the growth promoting effect of CO_2 addition increased with culture length (Figures: 3.7, 3.21). Secondly, the growth promoting effect of CO_2 addition was more pronounced under controlled conditions (laboratory) than under outside conditions (Table: 4.1).

The correlation between increased culture length and increased algal growth promotion through CO_2 addition was also observed by Azov et al. (1982), who attributed this effect to the steady decline in culture water CO_2 concentration over time which ultimately limits the growth of algal cultures.

Algae concentrations (indicated by TSS) from the present study laboratory experiments (Figures: 3.3, 3.7 and 3.11) were higher than those from the outside experiments (Figures: 3.21, 3.28 and 3.45). Several authors (Goldman 1979; Sheehan et al. 1998) reported that algae concentrations achieved under laboratory conditions are, in general, always higher than under outside or field conditions. This may be explained with the fact that laboratory

conditions attempt to optimise and control environmental conditions, whereas outside conditions vary with climate, season and diurnally, leading to suboptimal growth conditions.

During the laboratory experiments of the present study all growth conditions, apart from carbon were controlled and optimised, leading to carbon limitation. Consequently, CO_2 addition led to a substantial increase in algae growth. However, during the outside experiments growth conditions such as temperature and light varied both diurnally and from day to day. Therefore, CO_2 addition probably had less effect on algal productivity as other culture conditions may have also limited algal growth.

Applying Justus von Liebig's law of the minimum to CO_2 in algae wastewater cultures it becomes evident that the total amount of CO_2 available to the algae in the culture, (which is a combination of: CO_2 available through bacterial degradation of organic wastewater solids; CO_2 available by diffusion from the atmosphere; bicarbonate alkalinity in the wastewater (Green et al. 1995); and any CO_2 added), will determine the algal productivity as long as CO_2 is a growth limiting factor in the culture. Consequently the less available one source of carbon, e.g. the CO_2 available from bacterial degradation of wastewater solids, becomes, the more the availability of carbon from another source, e.g. CO_2 addition will determine the maximum algal growth achievable.

Therefore CO_2 addition to a HRAP can correct the nutrient imbalance in terms of carbon and will enhance algal growth as long as carbon is the growth limiting factor. The extent to which this promotion of algal growth will occur is dependent on which, and to what extent, other growth conditions (N, P, temperature etc.) are limiting.

The increase in TSS (algal biomass) resulting from CO_2 addition can have implications for the wastewater quality discharged from a HRAP. Mara (1997) and Abeliovich (2004) have highlighted the problem of many regulatory bodies around the world not distinguishing between the quite different wastewater TSS and algae TSS or wastewater BOD and algae BOD in the effluents discharged by wastewater treatment systems. This has led to many WSP systems being (often unnecessarily) replaced with more capital and energy intensive mechanical treatment systems in recent years, in order to meet tighter regulations (Abeliovich 2004).

However, HRAP always include algae removal, either in algae settling ponds (ASP) or by more sophisticated algae harvesting equipment such as dissolved air flotation with chemical dosing (Oswald 1988; Oswald 1991). The colonial algae that typically dominate HRAP are much more easily removed by gravity settling than the single celled, often motile algae that dominate in classical WSP (Pearson 2005). Therefore simple algae harvesting systems can be used to efficiently reduce algal biomass (TSS) concentrations in the final effluent to low levels. As these algae removal systems have to be designed to cope with (seasonally) fluctuating algal biomass concentrations, the TSS increases achieved through CO_2 addition in the present study should easily be accommodated, thereby preventing the potentially detrimental environmental effects from the discharge of effluent with higher TSS levels. If the algal biomass harvested from the HRAP can be used beneficially (e.g. as a fertiliser, feed or biofuel) an increase in algal biomass (TSS) as a result of CO_2 addition, could also improve the economics of the wastewater treatment operation (Benemann 2003).

4.2 Effects of CO_2 Addition on Algae and Zooplankton Species Composition

A healthy algae culture with good settling characteristics and low numbers of algae grazing zooplankton species is essential for the successful operation of HRAP (Oswald 1988; Nurdogan and Oswald 1995), and producing a final effluent with low TSS levels (as discussed above). It was therefore one of the aims of the present study to determine if CO_2 addition to algae wastewater cultures and the associated reduction in culture pH affects algae and zooplankton species composition, as changes in algae cell size and species composition may both alter the settling characteristics of the algal biomass and susceptibility to grazing by zooplankton.

In the laboratory and outside mesocosm experiments of the present study no major changes in algae species composition and cell size were observed in response to CO_2 addition, except in cultures containing the colonial algae *Dictyosphaerium sp.* The algal inoculum for the cultures of the first laboratory batch experiment (Section: 3.1.1) was dominated by *Dictyosphaerium* sp. (large colonies of >24 cells, Figure 3.1), sourced from the eastern HRAP at Ruakura which was operated with CO_2 addition (~pH 8). During the first laboratory batch experiment the *Dictyosphaerium sp.* in the culture with CO_2 addition (~pH 8) grew as colonies of only 2 to 4 cells or single cells (Figure: 3.2), while *Dictyosphaerium sp.* continued to grow as large colonies in all 3 control cultures at high pH (up to pH 10.9). Similar observations were made with *Dictyosphaerium sp.* dominated cultures during the winter outside mesocosm experiment (Section: 3.2.1). While remaining as large colonies in the control cultures, the *Dictyosphaerium sp.* population of the culture with CO_2 addition changed, and became dominated by small colonies.

In contrast, Goldman and Graham (1981) found that CO_2 addition to laboratory algae cultures increased the cell size of particular algae species. One possible explanation for the results obtained in the present study would be that the higher growth rate in the cultures with CO_2 addition (Figures: 3.3 and 3.17) did not allow sufficient time for large colonies to develop.

Several experiments (3.2.1, 3.2.2, 3.2.3) were influenced by zooplankton grazing, especially by rotifers. However, zooplankton grazing did not follow any particular pattern, and no correlation to CO_2 addition could be established.

In general our experiments indicate that CO_2 addition to algae wastewater cultures has (in most cases) little impact on algae and zooplankton species composition, and consequently on algal settleability. However, the changes observed with the colonial algae *Dictyosphaerium sp.* (which is not commonly found in HRAP) clearly showed that algae wastewater cultures are living systems which are not completely predictable.

4.3 Effects of CO_2 Addition on Ammoniacal-nitrogen

The effects of CO_2 addition to algae wastewater cultures on NH_4-N removal were variable. In some experiments CO_2 addition slowed the reduction of NH_4-N (Figures: 3.9; 3.30), while in other experiments the NH_4-N removal was only slightly effected (Figures: 3.23; 3.38) or did not change (Figure; 3.13; 3.47). However, a comparison of the NH_4-N removal results with the pH of the control cultures in each experiment indicates a relationship between the effect of CO_2 addition on NH_4-N removal and the maximum day-time pH obtained in the control cultures. It appeared that the higher the maximum day-time pH level of the control cultures rose, the greater the reduction in NH_4-N removal due to CO_2 addition was in the respective culture with CO_2 addition (Figures: 3.8 and 3.9: 3.29 and 3.30). Consequently during experiments where the maximum day-time pH levels of the control cultures were only slightly elevated no, or only a slight, reduction in NH_4-N removal was observed in the respective CO_2 added cultures (pH ~8.0) (Figures: 3.46 and 3.47).

Two distinctively different mechanisms, assimilation into algal biomass and ammonia volatilisation, are responsible for the majority of NH_4-N removal in HRAP treating domestic wastewater. Other mechanisms such as nitrification – denitrification play only a minor role (Green et al. 1995b; Green et al. 1996). During the initial days of the second and third laboratory batch experiment (Figures: 3.7, 3.11) and the high $fBOD_5$ experiment with glucose (Figure: 3.28) some NH_4-N removal may also have been associated with bacterial growth. However, as outlined previously, this would have been temporary, and would only occur to a limited extent in a continuously operated field-scale HRAP.

In experiments where the maximum pH of the control cultures was high (e.g. laboratory conditions (Figure: 3.8); outside summer conditions (Figure: 3.29)) a relatively large proportion of the ammoniacal-nitrogen may have been removed from the control cultures by volatilisation. Therefore in the corresponding cultures with CO_2 addition (pH ~8) the decrease in ammonia removal performance indicated that the reduction in ammonia volatilisation could not be offset by increased algal growth and associated nutrient assimilation. For example, during the initial days of the second laboratory batch experiment, the control cultures removed between 4% and 29% more NH_4-N than the CO_2 added culture, despite the algal biomass concentrations in the CO_2 added culture being up to 23% higher (Table: 4.2). This resulted in the control cultures achieving complete NH_4-N removal after 4 days, whereas the culture with CO_2 addition, required 6 days for complete NH_4-N removal(Figure: 3.9).

Table 4.2: Differences in ammoniacal-nitrogen removal performance, algal biomass concentrations (indicated by TSS) and maximum day-time pH of the CO_2 added culture during the first 6 days of the second laboratory batch experiment, based on the mean of the control cultures without CO_2 addition.

	pH	**Algae biomass (TSS)**	**Algae biomass (TSS)**	**NH_4-N removal**	**NH_4-N removal**
	units	mg/L	%	mg/L	%
Day 1	-1.02	-1	-1%	-1.8	-5%
Day 2	-1.16	25	10%	-0.9	-4%
Day 3	-1.73	28	9%	-4.7	-29%
Day 4	-2.87	85	23%	-1.1	-17%
Day 5	-2.69	128	38%	-	-
Day 6	-2.83	135	35%	-	-

However, the high pH levels observed during the laboratory batch experiments (up to pH 10.9) are rarely achievable in the field, where ammonia volatilisation contributes to a smaller and varying extent to the overall ammoniacal-nitrogen removal of a HRAP. For example, Green et al. (1996) found that ammonia volatilisation accounted only for 15% of total NH_4-N removal from a HRAP in California monitored over a 4 month period during summer.

Under conditions where the control culture pH was only slightly elevated, enough to limit algal growth (pH optimum for many common algae <pH 8 (Goldman et al. 1982a; Goldman et al. 1982b)) but not high enough to promote substantial ammonia volatilisation (Green et al. 1996), enhanced algal growth and increased NH_4-N removal through assimilation due to CO_2 addition appeared to offset reduced ammonia volatilisation. For example, during the Ruakura pilot-scale HRAP experiments (Table: 4.3), the maximum day-time pH of the western HRAP without CO_2 addition (control) only reached pH 8.9. At this pH little ammonia volatilisation would be expected (Konig et al. 1987; Green et al. 1996). Therefore CO_2 addition to the eastern HRAP and increased algal growth and assimilation of NH_4-N, were able to offset reduced ammonia volatilisation (Table: 4.3).

Table 4.3: Average ammoniacal-nitrogen removal (compared to influent), algal biomass concentrations (indicated by TSS) and maximum day-time pH values obtained from the Ruakura pilot-scale HRAP over an experimental period of 4 months.

Ruakura HRAP experiments	**Eastern HRAP (with CO_2 addition)**	**Western HRAP (without CO_2 addition)**
Ammoniacal-nitrogen removal compared to influent	69%	67%
Algae biomass concentration (indicated by TSS)	242 mg/L	202 mg/L
Maximum day-time pH	8.1	8.9

Average NH_4-N removal in the eastern HRAP (with CO_2 addition) and the western HRAP (control) (69% and 67% respectively) were within the range of normal operation for these ponds (62% to 79%) (Mandeno 2003), but were lower than values for a field-scale system operating in California reported by Green et al. (1995b) (80 %, 2 year average) and Nurdogan and Oswald (1995) (~85%, 3 month average). These differences may be explained with the instability of our algae cultures and climatic differences. Nevertheless the figures indicate that in general the pilot-scale HRAP replicated a field-scale HRAP to an acceptable degree.

Slightly elevated pH conditions, which restrict algae growth, but are not high enough to promote substantial ammonia volatilisation, regularly occur in HRAP during the New Zealand winter, and result in lower NH_4-N removal compared to summer (Mandeno 2003). Therefore CO_2 addition to HRAP may be particularly useful during winter to improve overall NH_4-N removal through increased algae assimilation.

Compared to NH_4-N removal through ammonia volatilisation, NH_4-N removal by assimilation into algal biomass, augmented through CO_2 addition, is also advantageous in terms of sustainability. Wastewater nitrogen gassed off to the atmosphere, either through nitrification - denitrification or ammonia volatilisation (stripping) is a waste of a resource, whereas nitrogen fixed in algal biomass could be a substitute for synthetic fertiliser (Green et al. 1996; Benemann 2003). Such systems of nutrient reclamation are often ecologically as well as economically sensible (Benemann 2003), and should receive more attention in the future as fossil energy resources and synthetic fertilizer become more expensive.

4.4 Effects of CO_2 Addition on DRP Removal

As with NH_4-N removal, the effects of CO_2 addition on DRP removal were variable. In some experiments CO_2 addition reduced DRP removal (Figure: 3.39), while in other experiments DRP removal was only slightly affected (Figures: 3.14; 3.24) or increased (Figure: 3.48). Again, a relationship between the effects of CO_2 addition on DRP removal and the maximum pH level of the control cultures could be identified. The higher the maximum day-time pH in the control cultures rose, the greater the reduction in DRP removal was in the respective cultures with CO_2 addition (pH ~8) (Figures: 3.8 and 3.9: 3.29 and 3.30). Consequently during experiments where the maximum day-time pH levels of the control cultures were only slightly elevated, no reduction or even an increase in DRP removal was observed in the respective cultures with CO_2 addition (pH ~8.0) (Figures: 3.46 and 3.47).

The removal of DRP in the algae wastewater cultures is generally accomplished by two main removal mechanisms; assimilation into algae biomass and precipitation with various metal cations at high pH (Fitzgerald and Rohlich 1964; Moutin et al. 1992). Phosphorus precipitation may have contributed much to the DRP removal in control cultures at high pH, but could have removed only very limited amounts of DRP in cultures with CO_2 addition, where the maximum culture pH was restricted to pH ~8. DRP removal, like NH_4-N removal, during the initial days of the second and third laboratory batch experiment and the outside mesocosm experiment with glucose was possibly enhanced by bacterial assimilation (as outlined above) which only occurs to a limited extent in continuously operated field-scale HRAP.

In experiments where the control culture maximum pH was high (e.g. laboratory conditions (Figure: 3.8); outside summer conditions (Figure: 3.22)) phosphorus precipitation may have contributed much to the removal of DRP. Therefore in the corresponding cultures with CO_2 addition and restriction of the maximum day-time culture pH to ~8, the reduction in DRP precipitation could not be offset by the increase in algae growth (and associated phosphorus assimilation), leading to an reduction in DRP removal.

For example, once the transformed non-algal control culture of the second laboratory batch experiment was able to attain maximum daytime pH levels significantly above pH 9 between days 5 and 7 (Figure: 3.8), DRP removal in the culture with CO_2 addition was up to 52% less than in the transformed non-algal control culture (Table: 4.4). This occurred despite the algae

biomass concentration of the CO_2 added culture being around twice as high than that of the transformed non-algal control culture without CO_2 addition (Table: 4.4). The rapid reduction of DRP in the 3 control cultures of the same experiment between day 2 to day 4 (when the pH increased substantially above 9) is a further indication of substantial DRP removal by precipitation under the given conditions (Figures: 3.8 and 3.10).

Table 4.4: Differences in DRP removal performance, algal biomass concentrations (indicated by TSS) and maximum day-time pH of the CO_2 added culture between day 2 and day 7 of the second laboratory batch experiment, based on data from the transformed non-algal control culture without CO_2 addition.

	pH	**Algae biomass (TSS)**	**Algae biomass (TSS)**	**DRP removal**	**DRP removal**
	units	mg/L	%	mg/L	%
Day 5	-1.08	273	146%	0.5	9%
Day 6	-2.13	254	94%	-2.5	-45%
Day 7	-2.88	314	111%	-1.4	-52%

In New Zealand, pH levels which promote phosphorus precipitation only occur in HRAP at the height of summer and the efficiency of phosphorus precipitation may also be restricted by a low wastewater metal ion concentrations (Nurdogan and Oswald 1995), resulting in variable phosphorus removal through precipitation.

Under conditions where the control algae wastewater culture pH was only slightly elevated; enough to limit algal growth (pH optimum for many common algae is <pH 8 (Goldman et al. 1982a; Goldman et al. 1982b)) but not high enough to promote substantial phosphorus precipitation (Moutin et al.1992), CO_2 addition and enhanced algal growth (increased DRP removal through assimilation) was able to offset reduced phosphorus precipitation and even increase overall DRP removal. For example during the Ruakura pilot-scale HRAP experiments (Table: 4.5), the maximum day-time pH of the western HRAP without CO_2 addition (control) only reached pH 8.9. At this pH little DRP precipitation would be expected (Moutin et al. 1992). Therefore CO_2 addition to the eastern HRAP, resulting in increased algal growth and assimilation of DRP, was able to offset the reduced phosphorus precipitation and provided higher overall DRP removal.

Table 4.5: Average DRP removal, algal biomass concentrations (TSS) and maximum day-time pH values obtained from the Ruakura plot-scale HRAP over an experimental period of 4 months.

Ruakura HRAP experiments	Eastern HRAP (with CO_2 addition)	Western HRAP (without CO_2 addition)
DRP removal compared to influent	46%	32%
Algae biomass concentration (indicated by TSS)	242 mg/L	202 mg/L
Maximum day-time pH	8.1	8.9

Average DRP removal by the eastern HRAP (with CO_2 addition) and the western HRAP (control) (46% and 32% respectively) were within the range of normal operation for these ponds (25% to 35%) (Mandeno 2003). The figures were also close to values for field-scale systems operating in California reported by Green et al. (1995b) (~47 %, 2 year average) and Nurdogan and Oswald (1995) (~44.2%, 3 month average), indicating an acceptable replication of field conditions in the pilot-scale HRAP.

The average DRP removal in the eastern HRAP with CO_2 addition was 0.6 mg/L higher than that of the western control HRAP, based on an average removal of 2.6 mg/L in the eastern HRAP and 2.0 mg/L in the western control HRAP. This indicates that enhanced algal growth (20% higher TSS) in the eastern HRAP with CO_2 addition more than compensated for the reduced phosphorus precipitation potential (Table: 4.3). The 0.6 mg/L difference in DRP removal between the CO_2 added and control HRAP is surprising, considering that the 20% difference in algal biomass would warrant only a far smaller treatment advantage, even if it is assumed that phosphorus precipitation did not at all occur in the western control HRAP. A possible explanation would be that CO_2 addition not only increased algal growth but also increased the phosphorus content of the algae. Ongoing research by Powell et al. indicates that under certain environmental conditions, algae grown on wastewater are capable of phosphorus luxury consumption (Powell et al. 2005). The possible promotion of phosphorus luxury uptake by algae through CO_2 addition requires more research before general conclusions can be drawn.

The maximum day-time pH levels measured in the control HRAP at Ruakura over the 4 month monitoring period (Figure: 3.46) indicates that under ambient conditions in New Zealand, assimilation into algae biomass can be considered the main mechanism of DRP

removal. During the winter months, when there is likely to be little phosphorus precipitation, and overall phosphorus removal is low (Mandeno 2003), CO_2 addition to the HRAP may be particularly useful for improving the overall DRP removal.

A further benefit of enhanced phosphorus assimilation over phosphorus precipitation would be improved treatment reliability and consistency as some phosphorus precipitates re-dissolve as the HRAP pH declines each night or when culture pH varies between different days, leading to inconsistent DRP removal (Fitzgerald and Rohlich 1964; Hemens and Mason 1968; Oswald 1988; Moutin et al. 1992; Garcia et al. 2006).

4.5 Effects of CO_2 Addition on $fBOD_5$ Removal

One of the aims of the 3 summer outside mesocosm experiments was to confirm the theory proposed by Oswald, that high culture pH (resulting from algae photosynthesis) inhibits bacterial degradation of wastewater BOD (Oswald et al. 1957; Oswald 1960) and whether it can be reversed by CO_2 addition. However, our experiments showed that CO_2 addition and pH control (pH ~8) did not enhance $fBOD_5$ removal compared to control cultures (Figures: 3.25, 3.31 and 3.40).

During the summer outside mesocosm experiment most of the $fBOD_5$ removal (>72%, up to 70 mg/L) occurred on day 1 in all cultures (with and without CO_2 addition) when culture pH was below pH 8 in all 4 cultures (Figures: 3.22 and 3.25). Even in the two experiments with increased initial $fBOD_5$ levels (by glucose or egg material addition) which had high pH in the control cultures from day 1 (Figures: 3.29 and 3.37), no difference in $fBOD_5$ removal was observed between cultures with and without CO_2 addition (Figures: 3.31, 3.40).

Studies with algae cultures grown on synthetic wastewater under laboratory conditions (Pipes 1962) also found no correlation between culture pH and BOD removal. However, the artificial medium (Knops' Solution) used by Pipes (1962) did not contain any ammoniacal-nitrogen, whereas the wastewater algae mixtures used by Oswald (1960) had a moderately high NH_4-N concentration (~30 mg/L). Like Pipes' (1962) cultures, the wastewater algae cultures of the high $fBOD_5$ experiments with glucose and egg material had low initial NH_4-N concentrations (7 mg/L on day 0 and 9.7 mg/L on day 1 respectively).

A possible explanation for the reduction in BOD removal observed by Oswald (1960) would be free ammonia inhibition of the bacteria at both, high culture pH and high NH_4-N levels; assuming that the free ammonia (NH_3) concentrations in our outside cultures were not sufficiently high to inhibit bacterial activity despite the relatively high culture pH.

Henze (1995) and Pearson (2005) state that a great variety of bacteria are involved in aerobic treatment of wastewaters, with the composition being influenced by many factors like the wastewater source, temperature, pH etc. While some aerobic bacteria seem to be sensitive to ammonia toxicity (e.g. 1 mg/L NH_3-N toxicity limit for nitrifying bacteria, Henze 1995), other sources report about successful aerobic treatment of wastewater with free ammonia concentrations more than 10 times higher (Rittstieg et al. 2001; Wichitsathian et al.

2004). Furthermore, studies with anaerobic bacteria have shown that, given sufficient time, bacteria are able to adapt to free ammonia concentrations several times the initial toxicity limit (Siegrist et al. 2005).

Therefore a possible explanation for the differences in BOD removal observed in the present study and the experiments of Oswald (1960) and Pipes (1962), is that some aerobic bacteria (groups) are more sensitive to free ammonia toxicity than others. However more research on this subject is required, before general conclusions can be drawn.

4.6 Effects of CO_2 Addition on Faecal Indicator Bacteria Removal

E. coli removal results from the three outside mesocosm experiments are summarised in Table 4.6. During all three outside mesocosm experiments algae control cultures without CO_2 addition and high pH (Figures: 3.22, 3.29 and 3.37) had higher *E. coli* removal than cultures with CO_2 addition and pH restricted to ~8.0 (Table: 4.6).

Table 4.6: Average *E. coli* removal in 3 summer outside mesocosm experiments.

	Culture	Average Removal (log)	Max. day-time pH
Outside batch experiment	CO_2 addition	2.7	6.5 – 8.2
	Controls	4.1	7.7 rising to 10.5
Outside glucose experiment	Glucose and CO_2 addition*	0.6	6.5 – 7.7
	Glucose control*	0.9	7.2 – 9.4
	CO_2 addition	0.9	6.5 – 7.2
	Control	5.8	8.8 rising to 10.5
Outside egg experiment	High egg and CO_2 addition	1.9	7.2 – 8.3
	High egg control	4.2	9.3 rising to 10.8
	Low egg and CO_2 addition	2.2	7.4 – 8.2
	Low egg control	3.9	9.3 rising to 10.9

* affected by glucose addition

These experiments demonstrated how lowered pH levels resulting from CO_2 addition reduced the removal of *E. coli*. The figures support the conclusions of previous authors (Oswald 1991; Green et al. 1996; Mara 1997; Davis-Colley et al. 2002) that increased pH levels are an important factor for *E. coli* removal in algae based wastewater treatment systems. Higher algal biomass (TSS) concentrations may also have decreased the penetration depth of solar-UV radiation, and further reduced disinfection in the cultures with CO_2 addition (Davies-Colley 2005).

The results in regards to *E. coli* indicate that CO_2 addition to HRAP results in a loss of disinfection performance. Since many regulatory bodies around the world use *E. coli* as the sole indicator for the disinfection performance of wastewater treatment systems (Davies-Colley 2005), the reduced disinfection in terms of *E. coli* removal by HRAP with CO_2 addition would mean that the effluent would require more downstream disinfection to comply with given regulations than effluent from HRAP without CO_2 addition.

In terms of overall disinfection, removal of most other faecal bacteria and all viruses is not influenced by high pH (Davies-Colley 2005), and therefore may not be affected by CO_2 addition and restriction of culture pH. However, independent from pH effects, the increases in algal biomass (TSS) due to CO_2 addition observed in all experiments (section: 4.1) would most certainly lead to a reduction in overall disinfection performance due to a reduced penetration depth of solar-UV radiation. More comprehensive studies, including monitoring of pathogenic bacteria, viruses and protozoa are therefore needed before more general conclusions regarding the effects of CO_2 addition on overall disinfection in HRAP can be drawn.

4.7 Effects of CO_2 Addition on Culture Physical/Chemical Parameters

Continuous monitoring of key HRAP physical/chemical parameters with datasondes enabled observation of subtle differences between the western control HRAP and the eastern HRAP with CO_2 addition. Temperature, DO and pH in both ponds were similar prior to installation of CO_2 addition in the eastern HRAP (Figure: 3.42). After CO_2 addition commenced the main difference between the HRAP was pH, while the parameter DO was only slightly, and temperature not at all, affected. (Figure: 3.43).

During the summer monitoring period the peak day-time pH of the control pond (~pH 9) was low in comparison to both, the winter data (Figure: 3.42) and data obtained previously in December 2002 (Mandeno 2003). This may have been due to the uncommon algae species (*Monorapidium sp.* and *Nephroclamis sp*) that dominated the ponds at this time. The fluctuations in maximum culture pH of the western control HRAP at around midday during this monitoring period were possibly caused by the inflow of fresh wastewater with a high buffer capacity.

Addition of CO_2 to the eastern HRAP, restricting the day-time pH to ~8, resulted in the evening pH decline occurring earlier and reaching lower night-time levels (Figure: 3.43). The lower night-time pH in the eastern HRAP (with CO_2 addition) may be explained by assuming that night-time respiration and CO_2 release are directly proportional to the DO level in the ponds at nightfall, which were similar in both HRAP. Therefore the equal amounts of CO_2 generated through night-time respiration in both ponds would have reduced the pH in the eastern HRAP to a lower level, as the pond pH at nightfall was already lower than that of the western HRAP without CO_2 addition. However the pH of the eastern HRAP with CO_2 addition did not reach acidic levels. This is important in terms of odour emissions, as it can be concluded that even mature and not well maintained HRAP with CO_2 addition should stay alkaline enough to prevent the release of malodours gases like H_2S, from eventually build up bottom sludge layers.

The rate at which DO declined in both HRAP in the evening was similar but occurred about an hour later in the eastern HRAP with CO_2 addition than in the western control HRAP. Since the biomass concentrations in both ponds were almost identical during the summer monitoring period (Figure: 3.45), these differences remain largely unexplained.

5. Conclusions and Recommendations

5.1 Conclusions

The laboratory and outside experiments presented in this thesis indicate that addition of CO_2 to algae wastewater cultures influenced several parameters of wastewater treatment.

1. Addition of CO_2 to algae wastewater cultures increased the TSS concentration by promoting algal growth. The magnitude of this growth increase appeared to be determined by the degree to which algal growth conditions other than carbon were limiting.

2. Monitoring of algae and zooplankton species composition showed that, apart from one exemption, CO_2 addition had little influence on algae species composition, cell morphology, grazing susceptibility or zooplankton abundance. This indicates that CO_2 addition is likely to have little effect on HRAP stability, and consequently operation and management.

3. Carbon dioxide addition to algae wastewater cultures can decrease or increase the removal of NH_4-N and DRP depending on the maximum pH level achievable. Large reductions in nutrient removal were observed under conditions where CO_2 addition led to a large pH difference between the CO_2 added cultures (pH ~8) and the control cultures with highly elevated pH levels. Smaller pH differences between the control cultures and the CO_2 added cultures (pH ~8) resulted in small reductions in nutrient removal, or, in the case of DRP, an increase in treatment performance. Enhanced algae growth with CO_2 addition also enables greater reclamation of both NH_4-N and DRP from the wastewater in the form of algal biomass.

4. Carbon dioxide addition had no effect on $fBOD_5$ removal in the algae wastewater cultures of the present study. However CO_2 addition and restriction of culture pH may potentially improve BOD removal in HRAP treating wastewaters with high NH_4-N concentrations, by alleviating the detrimental effects of ammonia toxicity.

5. Carbon dioxide addition reduced the removal of *E. coli* in the cultures due to a combination of reduced pH and reduced solar-UV penetration as a result of higher TSS levels. Since many regulatory bodies around the world use *E. coli* as sole parameter for assessing the disinfection performance of wastewater treatment systems, effluents from HRAP with CO_2 addition would require additional disinfection to meet regulatory standards.

In summary the experiments conducted in this thesis suggest, that CO_2 addition to a field-scale HRAP will moderately affect several key treatment parameters. As a result of CO_2 addition, increased algae growth would promote reclamation of more nutrients from the wastewater and the production of more, possibly valuable, algae based products per land area. Several other key parameters like $fBOD_5$ removal, algae and zooplankton species composition and nutrient removal performance under low pH conditions appear to be only slightly influenced by CO_2 addition and should therefore hardly affect the treatment performance of a field-scale HRAP. Loss of nutrient removal performance under high pH conditions and reduced *E. coli* disinfection performance are negative effects arising from CO_2 addition. The reduction in nutrient treatment performance during summer, and especially the losses in *E. coli* removal resulting form CO_2 addition may require more sophisticated downstream processing of the HRAP effluent, like increase retention times in subsequent maturation ponds. Such remedial measures have to be evaluated on a case by case basis, and are dependent on the given regulations and discharge regimes of the system. As HRAP are generally part of an integrated system, spare treatment capacity may already be provided by subsequent ponds in some cases, as a pond systems have to be designed to accommodate diurnal, weather dependent and seasonal changes.

This study indicates that in general HRAP can be employed for biogas purification and provide a useful sink for CO_2 rich waste streams. The beneficial effects of CO_2 addition to HRAP do not appear to allow for any design or management changes within the system, while it was indicated that most detrimental effects of CO_2 addition could be accommodated without major alternations, although in some cases significant remedial measures may be required for correcting the losses in disinfection and nutrient removal performance.

5.2 Further Research

This study has identified several areas of further research, which will require more work before biogas scrubbing and CO_2 sequestration in HRAP can be implemented at field-scale.

1. A long term study of more than one year duration, using a pilot-scale or field-scale HRAP, would be required to evaluate the overall gains and losses in nutrient treatment performance originating from CO_2 addition. Such a long term study could also help to identify the implications of seasonal changes on the effects of CO_2 addition to HRAP.

2. One of our experiments indicated that CO_2 addition to HRAP may have the potential to increase the phosphorus content of algal biomass. Studies by other authors have shown that certain environmental conditions can induce luxury consumption of phosphorus by algae. Further studies on algal phosphorus luxury consumption in response to CO_2 addition would be useful to gain a better understanding of the nutrient removal processes in HRAP.

3. All of the experiments presented in this thesis were carried out using pure CO_2 rather than biogas. Therefore the potential effects of other biogas and flue gas contaminants like H_2S, SO_x and NO_x on the treatment performance of HRAP have not been evaluated. Although several studies indicate that the flue gas contaminants SO_x and NO_x should have little effect on HRAP performance, the biogas contaminant H_2S is known to be toxic to algae at low concentrations. Further studies on the effects of H_2S on the algae growth and HRAP treatment performance are therefore required before biogas scrubbing in HRAP can be implemented at field-scale.

4. During our experiments *E. coli* was used as indicator for disinfection, as it is the most widely used indicator for faecal contamination of water bodies around the globe, and many regulations are based on it. However, as CO_2 addition has a direct effect on the pH level of a HRAP and *E. coli* is known to be particularly susceptible to elevated pH levels, monitoring of this indicator alone provided only a partial picture. It would therefore be beneficial to conduct further studies involving wastewater pathogens, viruses and protozoa, to obtain a more comprehensive assessment of the detrimental effects of CO_2 addition on the overall disinfection performance of HRAP.

Appendix A:

Calculations of the carbon flows in a wastewater pond system

Model: Projection of the maximum carbon dioxide load for an algae high rate pond

Principle: the biogas borne carbon has to equal the carbon fixation by algae plus any surplus algae growth induced by carbon dioxide addition, and any carbon dioxide losses through night time respiration, reduced by the amount of inflowing organic carbon.

Wastewater and anaerobic digestion of wastewater in the first treatment step

Assumptions (for municipal wastewater (WW))			Source
raw wastewater VS concentration	250	mg/L	NIWA (2002)
Biogas production rate (VS)	0.3	L/g	Green et al. (1995b)
Carbon dioxide (CO_2) concentration in biogas	35%		Weiland (2003) excluding the
Methane (CH_4) concentration in biogas	65%		N_2 fraction found in WW ponds
Carbon concentration in VS	50%		Metcalf and Eddy (1991)

Numbers from the periodic table:

Molar gas volume	22.4	L/mol
Molar weight C	12	g/mol
Molar weight H	1	g/mol
Molar weight O_2	32	g/mol
Molar weight CH_4	16	g/mol
Molar weight CO_2	44	g/mol

VS content	250	mg/L
Biogas production rate / VS introduced	0.3	L/g
Biogas building rate / m^3 raw WW	**75**	**L/m^3**
Carbon dioxide concentration in biogas	35%	
Methane concentration in biogas	65%	
CO^2 production / m^3 raw WW	26.3	L/m^3
Methane production / m^3 raw WW	48.8	L/m^3
C content CO_2	27%	
C content Methane	75%	
CO_2 - C content raw WW	14.1	mg/L
CH_4 - C content raw WW	26.1	mg/L
Total gaseous C from WW	**40.2**	**mg/L**

VS content	250	mg/L
C concentration in VS	50%	
C content in inflowing WW	125	mg/L
Total gaseous C from 1 m^3 WW	40.2	mg/L
C_{org} content digested WW	**84.8**	**mg/L**

Carbon transformation in a HRAP

Assumptions (for HRP)			Source
Retention time	8	days	NIWA Ruakura monitoring
Depth	0.33	m	Craggs et al. (2002a)
Algae carbon content:	50%		Oswald (1988)
Algae productivity	10	$g/m^2/d$	Goldman (1979); NIWA (2002)
Surplus production through CO_2	3	$g/m^2/d$	Based on Goldman et al. (1982a)

Calculated algal C uptake		
Algal C fixation	**5**	**$g/m^2/d$**
Surplus algal C fixation	**1.5**	**$g/m^2/d$**

Night time carbon dioxide losses from the HRAP

Principle:
No anaerobic activity is present in the HRAP
Atmospheric oxygen diffusion into HRAP at night is negligible
Subsequently the carbon dioxide amount lost every night is directly proportional to the DO concentration in the HRAP liquid at nightfall (conversion 100%)

Assumptions (for C losses)			Source
DO concentration at nightfall	14	mg/L	NIWA Ruakura monitoring
C content CO_2	27%		From previous page

Numbers from the periodic table:

Molar gas volume	22.4	L/mol
Molar weight O_2	32	g/mol
Molar weight CO2	44	g/mol

Molar concentration DO	0.44	mmol/L
Production potential for CO_2	0.44	mmol/L/day
Production potential for CO_2	19.25	mg/L/day
Potential losses expressed as C	5.25	mg/L/day
Area night time C losses	**1.73**	**$g/m^2/d$**

Area availability of Carbon dioxide C and organic wastewater C

C_{org} content digested effluent	**3.50**	**$g/m^2/d$**
CO_2 - C content / m^3 raw WW	**0.58**	**$g/m^2/d$**

The maximum carbon dioxide loading for a HRAP

Equation (1):

$$C_{max.ut.} = (C_{algaefix.} + C_{algaesurp.} + C_{vol.}) - C_{org.in.}$$

Max.Potential for Biogas C scrubbing	=	Algae C uptake
	+	Surplus algae growth
	+	Night time C losses
	-	Influent C_{org} load

	Algal C fixation	**5**	**g/m²/d**
+	**Surplus algal C fixation**	**1.5**	**g/m²/d**
+	**Area night time C losses**	**1.73**	**g/m²/d**
-	**C_{org} content digested WW**	**3.5**	**g/m²/d**
=	**Maximum potential for C uptake**	**4.73**	**g/m²/d**
Biogas CO_2-C available at 250 g/m³ VS:		0.58	g/m²/d
Utilisation ratio, expressed as % of max.:		12%	

Maximum potential for C uptake	4.73	g/m²/d	
equals			
Maximum potential for CO_2 uptake	8.84	L/m²/d	
equals			
Maximum biogas scrubbing potential	25.25	L/m²/d	at 35% CO_2 in biogas

The ideal loading rate of the system at a given algae growth rate

Principle: The sum of the carbon being contained in the organic wastewater compounds entering the HRAP and the carbon dioxide fraction of the biogas stream obtained in the antecedent anaerobic treatment step have to equal the carbon demand of the high rate pond algae

Equation (2)

	$C_{algaefix.} + C_{algaesurp.} + C_{vol.} = C_{biogasCO2} + C_{org.in.}$			
	Algae C uptake			
+	Surplus algae growth	=		Influent C_{org} load
+	Night time C losses		+	Biogas CO_2 - C

	Algal C fixation	**5**	**g/m²/d**
+	**Surplus algal C fixation**	**1.5**	**g/m²/d**
+	**Areal night time C losses**	**1.73**	**g/m²/d**
=	**Sum**	**8.23**	**g/m²/d**

From above it can be seen that anaerobic digestion of sewage solids splits the carbon stream into three carbon streams (organic wastewater C, biogas carbon dioxide C and biogas methane C at a ratio of :

68%	residual organic C
21%	methane C
11%	carbon dioxide C

As the carbon contained in the methane is not utilisable for the algae, and for the both remaining carbon streams a transformation and utilisation efficiency of 100% is assumed the carbon available for the algae in the HRP can be expressed as a fraction of the VS content of the raw sewage in the following way.

Influent C_{org}	+	Biogas CO_2 - C	=	79% VS - C (raw wastewater)

therefore:	8.23	g/m^2*d	=	79% VS - C (raw wastewater)
		VS - C	=	10.41 $g/m^2/d$

At the pond depth and detention times mentioned above this amount of VS - C is equal to

10.41 $g/m^2/d$ x 8 days detention time ÷ 0.33 m pond depth ÷ 50% VS - C content

= **504.6 mg/L** **VS concentration (raw wastewater)**

Appendix B:

Raw Data Tables

First Microcosm Batch Experiment

13. October till 21. October 2005

	Culture Alpha with CO_2					Control culture median				
	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L
Day 0	20.3	82	7.01	17.4	5.6	20.3	82	7.01	17.4	5.6
Day 4	20.7	298	7.50	0.5	3.0	20.3	208	10.79	0.3	1.8
Day 5	20.6	355	7.99	0.8	1.4	20.2	218	10.81	1.0	2.5
Day 6	20.8	433	7.34	0.3	0.5	20.1	200	10.32	1.2	3.7
Day 7	20.8	447	7.85	0.8	0.4	20.2	220	10.30	0.1	3.0
Day 8	20.9	595	7.80	0.5	0.4	20.2	245	10.94	0.8	3.3

	Control culture Beta					Control culture Gamma					Control culture Delta				
	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L
Day 0	20.3	82	7.01	17.4	5.6	20.3	82	7.01	17.4	5.6	20.3	82	7.01	17.4	5.6
Day 4	20.3	212	10.80	0.4	1.8	20.3	212	10.78	0.3	1.7	20.3	200	10.78	0.1	1.8
Day 5	20.3	228	10.80	0.8	2.5	20.1	222	10.81	1.1	2.4	20.2	204	10.81	1.1	2.5
Day 6	20.3	202	10.12	1.4	3.9	19.9	208	10.68	1.1	3.3	20.1	190	10.17	1.2	4.0
Day 7	20.3	223	10.20	0.1	2.9	20.2	208	10.08	0.1	3.5	20.1	228	10.61	0.1	2.7
Day 8	20.4	254	10.96	0.2	3.0	20.0	244	10.92	0.7	3.0	20.1	236	10.94	1.4	4.0

Second Microcosm Batch Experiment

8. November till 25 November 2005

	Culture Alpha with CO_2					Control culture mean					Sewage blank				
	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L
Day 0	18.5	133	6.99	39.8	6.1	18.5	133	6.99	39.8	6.1	18.5	87	6.99	49.8	6.9
Day 1	20.6	230	7.63	26.1	3.9	20.2	231	8.65	24.3	4.1	20.0	134	7.74	39.8	5.8
Day 2	20.6	272	7.95	18.6	3.2	20.2	247	9.11	15.9	4.0	19.9	157	8.05	35.4	5.5
Day 3	20.7	335	7.99	13.8	2.8	20.1	307	9.72	6.4	1.8	20.3	160	8.18	34.9	5.4
Day 4	20.7	448	7.87	8.7	1.9	20.2	363	10.74	0.2	0.8	19.9	165	8.53	31.7	5.6
Day 5	20.6	460	8.07	4.4	1.4	20.2	332	10.76	0.3	1.2	20.0	187	9.15	24.8	5.6
Day 6	20.5	524	7.82	0.6	1.0	20.1	389	10.65	0.0	0.8	19.8	270	9.95	9.3	2.7
Day 7	20.7	596	8.02	1.1	0.8	20.1	442	10.71	0.0	0.4	19.9	282	10.90	0.7	1.1
Day 8	20.8	643	7.94	0.1	0.3	20.2	474	10.70	0.0	0.8	20.0	335	10.88	0	1.1
Day 9	21.0	700	7.87	0.1	0.1	20.3	528	10.69	0.0	0.4	20.3	398	10.86	0	1.0
Day 10	20.9	703	8.15	0	0.2	20.2	585	10.69	0.0	0.6	19.9	447	10.83	0	1.1
Day 11	20.8	743	7.84	0	0.1	20.0	637	10.57	0.0	0.3	19.8	507	10.77	0	1.1
Day 12	20.9	813	7.99	0	0.1	20.1	716	10.65	0.0	0.2	20.2	547	10.86	0	0.9
Day 13	20.8	857	7.87	0	0.1	20.0	814	10.51	0.0	0.1	19.6	657	10.78	0	0.7

	Control culture Beta					Control culture Gamma					Control culture Delta				
	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L
Day 0	18.5	133	6.99	39.8	6.1	18.5	133	6.99	39.8	6.1	18.5	133	6.99	39.8	6.1
Day 1	20.1	234	8.81	24.5	4.0	20.3	230	8.57	24.7	4.1	20.2	230	8.57	23.6	4.1
Day 2	20.1	252	9.21	14.2	4.1	20.3	244	9.07	15.6	4.0	20.3	246	9.06	17.9	4.0
Day 3	20.0	316	9.97	4.5	1.3	20.3	313	9.61	6	2.0	20.1	293	9.57	8.8	2.1
Day 4	20.0	365	10.74	0.1	0.8	20.3	365	10.74	0	0.8	20.2	360	10.73	0.6	0.9
Day 5	20.0	337	10.77	0.2	1.2	20.3	330	10.77	0.1	1.3	20.2	330	10.75	0.7	1.2
Day 6	19.9	399	10.65	0	0.7	20.3	380	10.64	0	0.8	20.0	387	10.65	0	0.9
Day 7	20.0	467	10.73	0	0.4	20.3	420	10.70	0	0.4	20.1	440	10.70	0	0.4
Day 8	20.1	480	10.71	0	0.7	20.5	480	10.70	0	0.8	20.1	463	10.70	0	0.9
Day 9	20.1	533	10.70	0	0.5	20.5	527	10.69	0	0.4	20.2	523	10.68	0.1	0.4
Day 10	20.1	617	10.69	0	0.6	20.5	560	10.68	0	0.6	20.1	577	10.70	0	0.7
Day 11	19.9	685	10.52	0	0.3	20.3	563	10.62	0	0.4	19.8	663	10.57	0	0.2
Day 12	19.9	737	10.53	0	0.2	20.5	720	10.74	0	0.3	19.9	690	10.67	0	0.1
Day 13	19.8	847	10.40	0	0.1	20.3	793	10.60	0	0.2	19.9	803	10.53	0	0.1

Third Microcosm Batch Experiment

29. November till 9. December 2005

	CO_2 added algal culture					Control algal culture					Non - algal control (dark)					Non - algal control (light)				
	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L
Day 0	20.1	174	7.36	28.6	4.0	20.1	174	7.36	28.6	4.0	19.8	144	6.83	33.2	4.4	19.8	144	6.83	33.2	4.4
Day 1	21.0	260	7.87	17.4	1.1	20.8	254	8.28	17.1	1.2	24.9	194	7.77	21.3	1.4	20.4	218	7.48	21.6	1.4
Day 2	21.0	344	7.91	9.0	0.2	20.7	336	9.41	9.7	0.8	24.9	177	7.77	24.6	1.8	20.5	180	7.68	20.8	1.7
Day 3	20.9	423	7.91	5.0	0.1	20.6	373	10.84	4.1	0.5	24.6	167	7.85	25.1	1.8	20.1	162	7.79	21.3	1.7
Day 4	21.0	418	7.80	0.2	0.1	20.6	372	10.95	1.0	0.5	24.9	105	7.48	28.1	3.3	20.3	103	7.45	24.7	3.1
Day 5	20.9	528	7.60	0.3	0.1	20.6	440	10.97	0.8	0.4	25.0	102	7.90	29.6	4.0	20.2	93	7.70	25.9	3.7
Day 6	21.0	604	7.89	0.3	0.1	20.5	473	10.86	0.6	0.3	24.8	80	8.05	29.0	4.3	20.0	75	8.00	25.9	4.1
Day 7	21.0	708	8.10	0.0	0.1	20.6	528	10.80	0.5	0.2	25.0	78	7.99	34.3	4.6	20.2	88	8.10	26.4	4.2
Day 8	20.9	863	8.04	0.0	0.1	20.5	577	10.81	0.3	0.2	24.9	75	8.11	33.1	4.7	20.2	93	8.43	23.3	4.4
Day 9	21.0	960	7.88	0.0	0.1	20.5	695	10.81	0.5	0.3	25.0	90	8.20	31.1	4.9	20.2	125	8.81	17.5	4.3
Day 10	21.0	1050	8.03	0.3	0.1	20.5	730	10.84	0.2	0.2	25.2	88	8.2	34.1	5.1	20.2	220	9.23	9.8	4.0

Winter Outside Mesocosm Experiment

29. August till 28. September 2005

Date	Day	Minimum air temp. °C	Maximum air temp. °C	Insolation MJ/m^2/day	Rain mm	A1 control pH	A1 control TSS mg/L	C1 CO_2 from day 5 pH	C1 CO_2 from day 5 TSS mg/L	C2 CO_2 from day 5 pH	C2 CO_2 from day 5 TSS mg/L	A1 control pH	A1 control TSS mg/L
29-Aug	0	1.3	16.1	13.0	0	9.93		9.93		9.93		9.93	
30-Aug	1	8.8	15.7	15.7	0	7.42	164	7.99	196	7.62	174	8.05	200
31-Aug	2	13.0	16.3	15.9	0	7.95	177	8.75	210	8.26	200	8.80	206
1-Sep	3	1.9	18.5	16.9	0	8.13	210	8.74	237	8.35	223	8.90	238
2-Sep	4	2.4	17.9	16.1	0	9.15	233	9.63	270	9.23	258	9.75	285
3-Sep	5	1.7	18.4	16.5	0	9.27	266	9.68	276	9.19	270	9.86	288
4-Sep	6	4.5	18.1	9.4	0	9.23	282	9.60	307	8.58	288	9.78	302
5-Sep	7	3.9	16.9	10.4	0	9.18	298	9.51	338	7.97	305	9.69	315
6-Sep	8	6.3	15.5	9.6	1.4	9.90	258	10.70	280	7.75	283	10.25	265
7-Sep	9	5.6	17.2	11.0	1.2	9.49	298	9.91	318	7.95	310	9.75	260
8-Sep	10	2.4	15.6	13.8	0	9.33	290	7.81	303	7.99	323	9.70	255
9-Sep	11	5.1	15.6	9.9	0.2	9.65	285	7.70	320	7.87	328	9.88	303
10-Sep	12	8.7	15.3	11.0	0.8	9.50	300	8.95	283	7.80	355	9.75	305
11-Sep	13	8.5	14.6	11.6	0	9.49	313	7.60	333	7.81	383	9.66	310
12-Sep	14	10.0	17.6	12.9	0	9.48	288	8.18	323	7.75	375	9.59	295
13-Sep	15	7.2	20.1	12.6	0	9.39	338	8.08	368	7.76	405	9.48	325
14-Sep	16	10.6	18.4	9.2	0	9.40	328	8.08	328	8.10	390	9.48	300
15-Sep	17	10.2	16.8	17.0	9.6	9.36	338	8.00	380	7.85	410	9.49	340
16-Sep	18	6.2	15.8	8.8	0.6	9.13	285	8.18	345	8.47	373	9.33	305
17-Sep	19	10.1	14.3	14.5	20.8	9.67	293	8.15	353	7.90	358	9.30	288
18-Sep	20	10.2	15.9	5.7	39.2	9.34	266	8.47	314	8.33	321	9.29	264
19-Sep	21	7.7	15.3	10.6	19.6	9.00	238	8.79	275	8.75	283	9.28	240
20-Sep	22	3.0	11.9	19.2	5.8	9.28	248	7.31	290	6.70	310	9.43	265
21-Sep	23	-0.1	12.2	15.9	0.2	9.61	233	7.63	250	7.64	285	9.70	243
22-Sep	24	3.8	13.6	17.0	2.2	9.75	250	9.26	295	7.78	305	9.95	245
23-Sep	25	7.2	14.2	17.6	14.6	10.00	210	8.80	285	7.57	285	9.91	215
24-Sep	26	5.0	16.6	19.1	0	10.24	235	7.85	348	8.14	303	9.85	225
25-Sep	27	4.8	15.5	8.3	0	10.22	232	7.88	321	7.82	276	9.83	215
26-Sep	28	6.8	16.2	11.7	0	10.19	228	7.90	293	7.50	248	9.80	205
27-Sep	29	9.6	17.2	10.3	0	9.81	195	7.70	278	7.78	215	9.45	200
28-Sep	30	12.3	17.5	6.6	2.8	9.66	198	6.70	323	7.71	203	9.33	210

Summer Outside Mesocosm Experiment

23. January till 31. January 2006

					C1 with CO_2							C2 with CO_2						
Day	Max. air temp °C	Min. air temp °C	Insolation MJ/m²/day	Rain mm	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	$BODf_5$ mg/L	E coli MPN/100 ml	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	$BODf_5$ mg/L	E coli MPN/100 ml
0	25.4	11.2	23.72	4.4	23.3	155.6	7.71	41.6	5.8	86	12996500	23.3	155.6	7.71	41.6	5.8	86	12996500
1	24.4	17.2	2.74	38	18.9	111.4	7.64	36.2	5.6	21	14136000	18.8	142.9	7.61	35.6	5.3	21	15530700
2	23.2	18.7	15.11	24	22.4	164.3	7.07	30.6	4.5	12	18416000	22.6	187.1	6.45	30.4	4.7	11	24066200
3	24.4	16.2	23.95	0	25.6	162.9	7.53	28.4	4.2	15	12262000	26.8	198.6	6.51	25.6	3.8	4	14540000
4	23.9	13.8	19.63	0	24.5	178.6	8.00	25.2	4.1	6	1814000	24.9	202.9	6.82	23.0	3.5	8	3338000
5	25.1	15.8	24.69	0	29.6	250.0	6.50	18.5	3.5	7	402000	30.3	260.0	8.04	16.0	3.3	10	480000
6	27.6	19.2	20.15	0	26.2	277.1	8.04	13.7	3.3		98000	26.4	331.5	7.53	11.3	2.9		97000
7	27.0	19.4	18.23	0	30.4	295.7	8.10	9.5	3.2		41000	31.1	378.3	7.95	6.9	2.8		86000
8	27.9	17.8	16.83	0	26.7	252.1	8.17	12.0	3.2		31000	27.7	376.0	8.10	6.2	2.6		20000

					A1 control culture							A2 control culture						
Day	Max. air temp °C	Min. air temp °C	Insolation MJ/m²/day	Rain mm	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	$BODf_5$ mg/L	E coli MPN/100 ml	Temp °C	TSS mg/L	pH	NH_4^+ mg/L	DRP mg/L	$BODf_5$ mg/L	E coli MPN/100 ml
0	25.4	11.2	23.72	4.4	23.3	155.6	7.71	41.6	5.8	86	12996500	23.3	155.6	7.71	41.6	5.8	86	12996500
1	24.4	17.2	2.74	38	18.8	127.1	7.77	35.8	5.0	8	17328700	18.8	150.0	7.77	35.7	5.2	24	15530700
2	23.2	18.7	15.11	24	22.4	154.3	7.77	30.2	4.6	17	22397000	22.6	142.9	7.71	30.6	4.5	29	20924800
3	24.4	16.2	23.95	0	25.8	152.9	8.22	27.1	4.4	4	17328000	26.8	160.0	8.20	26.1	4.4	3	18416000
4	23.9	13.8	19.63	0	24.5	168.6	8.75	20.5	4.5	9	40000	25.0	158.6	8.69	20.6	4.3	7	4620000
5	25.1	15.8	24.69	0	29.6	191.4	9.39	12.8	4.4	7	20000	30.4	187.1	9.49	10.8	4.1	7	40000
6	27.6	19.2	20.15	0	25.9	212.9	9.89	5.0	3.4		10000	26.3	217.1	9.93	3.1	2.9		10000
7	27.0	19.4	18.23	0	30.4	258.3	10.52	1.1	1.9		<1000	30.9	222.9	10.44	1.1	2.3		<1000
8	27.9	17.8	16.83	0	26.9	218.6	9.82	1.6	3.3		<1000	27.5	191.4	9.59	1.6	3.6		<1000

High $fBOD_5$ Experiment with Glucose

16. February till 20. February 2006

C1 (CO_2 added culture with glucose)

Day	Insolation	Rain	Temp	TSS	pH	NH_4^+	DRP	$fBOD_5$	*E. coli* MPN /	Temp	TSS	pH	NH_4^+	DRP	$fBOD_5$	*E. coli* MPN /
	$MJ/m^2/d$	mm	°C	mg/L		mg/L	mg/L	mg/L	100ml	°C	mg/L		mg/L	mg/L	mg/L	100ml
0	16.88	0	23.9	307	7.20	7.1	1.9	109.0	8164000	24.4	307	8.80	7.1	1.9	109.0	8164000
1	19.79	0	27.7	370	7.21	0.3	0.5	12.0	<24192000	26.7	402	8.73	0.2	0.9	13.5	14136000
2	20.71	0	30.4	406	7.71	0.3	0.8	2.5	11198500	29.3	384	9.39	0.3	1.1	3.5	2613000
3	16.49	0	23.8	376	6.52	0.6	1.0	8.5	9208000	23.1	376	7.22	0.7	1.2	6.3	1178000
4	23.82	0	28.0	398	7.17	0.0	1.0		2247000	26.6	390	7.32	0.0	1.3		1092000

A1 (control culture with glucose)

C2 (CO_2 added culture without glucose)

Day	Insolation	Rain	Temp	TSS	pH	NH_4^+	DRP	$fBOD_5$	*E. coli* MPN /	Temp	TSS	pH	NH_4^+	DRP	$fBOD_5$	*E. coli* MPN /
	$MJ/m^2/d$	mm	°C	mg/L		mg/L	mg/L	mg/L	100ml	°C	mg/L		mg/L	mg/L	mg/L	100ml
0	16.88	0	23.8	303	7.21	7.3	2.0	19.0	6131000	23.9	303	8.81	7.3	2.0	19.0	6131000
1	19.79	0	26.6	322	7.10	4.9	1.6	8.4	1334000	26.4	320	9.37	3.9	2.1	7.8	860000
2	20.71	0	28.9	324	6.50	3.8	1.5	6.0	1112000	29.7	324	9.94	2.1	1.5	4.7	<10000
3	16.49	0	23.1	308	6.51	4.4	1.7	4.3	554000	22.9	332	9.58	0.6	1.9	4.2	10
4	23.82	0	26.9	332	7.12	3.3	1.7		712000	26.8	374	10.53	0.0	0.8		<10

A2 (control culture without glucose)

Mean CO_2 added cultures

Day	Insolation	Rain	Temp	TSS	pH	NH_4^+	DRP	$fBOD_5$	*E. coli* MPN /	Temp	TSS	pH	NH_4^+	DRP	$fBOD_5$	*E. coli* MPN /
	$MJ/m^2/d$	mm	°C	mg/L		mg/L	mg/L	mg/L	100ml	°C	mg/L		mg/L	mg/L	mg/L	100ml
0	16.88	0	23.85	305	7.21	7.2	2.0	64.0	7147500	24.2	305	8.81	7.2	2.0	64.0	7147500
1	19.79	0	27.15	346	7.16	2.6	1.1	10.2	12763000	26.6	361	9.05	2.1	1.5	10.6	7498000
2	20.71	0	29.65	365	7.11	2.1	1.2	4.3	6155250	29.5	354	9.67	1.2	1.3	4.1	1311500
3	16.49	0	23.45	342	6.52	2.5	1.4	6.4	4881000	23.0	354	8.40	0.7	1.6	5.2	589005
4	23.82	0	27.45	365	7.15	1.7	1.4		1479500	26.7	382	8.93	0.0	1.1		546005

Mean control cultures

High $fBOD_5$ Experiment with Egg Material

1. March till 4. March 2006

C1 (CO_2 added culture with high egg dose)

Day	Radiation	Rain	Temp	TSS	pH	NH_4^+	DRP	$BODf_5$	*E. coli*	Temp	TSS	pH	NH_4^+	DRP	$BODf_5$	*E. coli*
	$MJ/m^2/d$	mm	°C	mg/L		mg/L	mg/L	mg/L	MPN/100ml	°C	mg/L		mg/L	mg/L	mg/L	MPN/100ml
0	24.90	0.0	22.2	343	8.33			43.0	31000	22.0	340	8.15			24.5	31000
1	23.54	0.6	21.8	403	7.24	9.7	6.5	17.3	1210	21.3	380	7.41	8.8	6.7	11.0	630
2	16.90	0.4	21.0	449	7.50	4.5	6.7	11.0	467	21.2	445	8.22	3.4	6.4	8.6	305
3	19.70	1.2	20.9	506	7.55	1.7	6.4		379	21.7	475	7.82	0.3	6.4		213

C2 (CO_2 added culture with low egg dose)

A1 (control culture with high egg dose)

Day	Radiation	Rain	Temp	TSS	pH	NH_4^+	DRP	$BODf_5$	*E. coli*	Temp	TSS	pH	NH_4^+	DRP	$BODf_5$	*E. coli*
	$MJ/m^2/d$	mm	°C	mg/L		mg/L	mg/L	mg/L	MPN/100ml	°C	mg/L		mg/L	mg/L	mg/L	MPN/100ml
0	24.90	0.0	21.9	343	9.30			43.0	31000	21.7	340	9.31			24.5	31000
1	23.54	0.6	20.9	393	9.46	6.4	5.0	19.5	97	21.1	365	9.65	6.8	4.9	15.5	41
2	16.90	0.4	20.9	441	10.16	1.1	2.6	6.9	2	21.2	433	10.35	1.4	1.9	7.0	2
3	19.70	1.2	20.9	455	10.79	0.2	1.7		2	21.2	445	10.86	0.0	1.5		4

A2 (control culture with low egg dose)

Mean CO_2 added cultures

Day	Radiation	Rain	Temp	TSS	pH	NH_4^+	DRP	$BODf_5$	*E. coli*	Temp	TSS	pH	NH_4^+	DRP	$BODf_5$	*E. coli*
	$MJ/m^2/d$	mm	°C	mg/L		mg/L	mg/L	mg/L	MPN/100ml	°C	mg/L		mg/L	mg/L	mg/L	MPN/100ml
0	24.90	0.0	22.1	342	8.24			33.8	31000	21.8	342	9.31			33.8	31000
1	23.54	0.6	21.6	392	7.33	9.3	6.6	14.2	920	21.0	379	9.56	6.6	5.0	17.5	69
2	16.90	0.4	21.1	447	7.86	4.0	6.6	9.8	386	21.1	437	10.26	1.3	2.3	7.0	2
3	19.70	1.2	21.3	491	7.69	1.0	6.4		296	21.1	450	10.83	0.1	1.6		3

Mean control cultures

Ruakrua pilot-scale HRAP

10. August till 8.December 2005

		Western control HRAP					Eastern HRAP with CO_2 addition					Anaerob. digested influent sewage				
Date	Insolation	Temp	TSS	pH	NH_4^+	DRP	Temp	TSS	pH	NH_4^+	DRP	Temp	TSS	pH	NH_4^+	DRP
	$MJ/m^2/d$	°C	mg/L		mg/L	mg/L	°C	mg/L		mg/L	mg/L	°C	mg/L		mg/L	mg/L
10.08.05	9.2		158					172					71			
23.08.05	7.2		263	9.00				247	9.05					6.31		
25.08.05	15.5		237	9.19				245	9.25							
30.08.05	15.7		250	9.15	12.0	3.2		269	9.19	8.3	2.8		79	6.72	42.1	5.5
7.09.05	11.0		236	9.05	14.8	4.6		223	8.05	15.1	4.4		75	6.89	50.4	6.7
9.09.05	9.9	13.8	214	9.28	21.4	5.3	13.7	219	7.99	23.8	4.2	12.1	71	7.05	58.7	7.8
13.09.05	12.6	17.8	280	9.39	20.2	4.8	17.8	292	8.08	23.2	4.4	15.1	79	6.63	74.7	8.7
20.09.05	19.2	12.2	206	9.47	15.8	4.0	12.6	239	8.11	15.1	2.9	11.7	54	6.46	27.5	5.5
1.10.05	7.3	15.3	231	8.92	7.2	2.5	15.2	258	8.40	7.9	1.6	14.3	66	6.55	25.1	3.3
3.10.05	8.5	14.9	169	9.37	7.5	2.2	14.8	228	8.00	8.3	1.2	13.5	50	6.61	32.1	3.9
5.10.05	13.8	14.9	150	9.40	8.8	2.3	14.9	258	8.94	8.0	1.0	13.1	54	7.44	23.6	2.7
10.10.05	17.7	18.2	80	9.03	6.1	4.1	18.5	200	8.30	4.0	2.6	18.9	70	6.89	13.0	5.4
13.10.05	11.7	17.6	69	8.62	8.1	5.1	17.5	198	8.15	3.2	3.7	15.2	47	6.40	17.3	6.4
19.10.05	14.4	19.5	55	8.39	11.4	4.7	19.1	99	7.83	9.8	4.4	17.1	64	6.49	20.6	4.3
25.10.05	26.9	16.5	134	8.13	12.7	3.3	16.3	158	7.96	8.3	2.6	14.6	62	6.22	21.5	3.5
31.10.05	23.5	22.7	177	9.1	6.1	2.8	22.5	237	8.10	5.4	1.9	20.1	58	6.6	36.9	5.0
4.11.05	14.2	18.8	186	8.5	10.8	3.3	18.5	265	8.06	9.2	2.1	18.1	72	6.7	47.1	6.1
8.11.05	20.0	17.8	213	8.52	12.9	3.8	18.0	257	7.95	12.7	3.3	18.3	84	6.68	49.8	6.9
14.11.05	18.8	19.3	264	8.96	9.9	4.0	19.2	316	8.34	11.5	3.7	18.5	85	6.7	49.7	6.5
21.11.05	10.5	18.7	327	8.74	10.5	3.7	18.7	290	7.95	18.3	4.3	17.6	69	6.6	51.3	7.8
23.11.05	28.0		306		10.5	3.8		254		19.1	4.2					
28.11.05	15.8	15.2	282	8.83	11.9	4.0	15.3	256	8.18	18.6	4.1	15.7	91	6.7	46.5	6.3
1.12.05	32.2	26.0	318	8.80	11.3	4.0	26.3	338	8.16	16.0	3.8	20.6	123	6.6	47.9	6.4
8.12.05	23.5	23.1		8.99		3.6	23.1		8.45		3.3	19.9		6.5		7.1

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