ALGAL AND BACTERIAL DYNAMICS IN WASTE STABILIZATION PONDS AND WASTEWATER STORAGE AND TREATMENT RESERVOIRS

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Abstract

This study was carried out in northeast Brazil, a region of semi-arid climate with severe water shortage. The treatment of wastewater can save clean water for other purposes and provide safe effluent to be used in agriculture, which represents an economy in artificial fertilizers.

Waste stabilization ponds (WSP) are considered an efficient and cheap method of treating wastewater, especially when agricultural reuse is considered. More recently, the technology of Wastewater storage and treatment reservoirs (WSTR) has also been considered for treatment and reuse of wastewater. The treatment process in both systems relies on bacterial and algal activities in the treatment unit. This study describes the algal and bacterial dynamics in WSP and WSTR.

The experiments on WSP showed that faecal coliform removal was faster in shallow than in deep WSP. However deep ponds represent a land-saving alternative in comparison to shallow ponds. Therefore, whether WSP are built deep or shallow will depend on financial considerations. With respect to organic matter removal, deep ponds are definitively a better solution than shallow ponds, as they require the same hydraulic retention time to reach the same degree of treatment (in terms of BOD_5 removal) with less area requirement. In relation to nutrient removal, shallow WSP gave the best results.

The recent technology of WSTR proved to be a viable alternative to WSP for wastewater treatment and reuse, providing good quality effluents similar to those from WSP but with higher nutrient concentrations (N and P) but an absence of pathogens.

In the WSP, chlorophyll *a* reached the highest values in the shallowest ponds studied. In the WSTR system, the highest chlorophyll *a* concentrations were found in the top 200 cm during hours of high light intensity (10:00 – 16:00h).

The algal population in the WSTR showed strong similarity with that found in the WSP systems. In general, the algal genera *Euglena, Oscillatoria, Chlamydomonas* and *Pyrobotrys* showed resistance to high organic loading, whereas *Ankistrodesmus* and *Scenedesmus* exhibited sensitivity to it. This resistance of some algal genera to organic loading enables a wide range of loading to be used at the design stage of both WSP and WSTR.

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Abbreviations

APE – Anaerobic pond effluent;

BOD_5 – Biochemical oxygen demand over 5 days;

CEC – Council of European Communities;

COD – Chemical oxygen demand;

DO – Dissolved oxygen;

FC – Faecal coliform;

TS – Total solids

SS – Suspended solids

RW – Raw wastewater;

HRT – Hydraulic retention time;

WHO – World Health Organization;

WSP – Waste stabilization ponds;

WSTR – Wastewater storage and treatment reservoirs.

1. INTRODUCTION AND LITERATURE REVIEW

1.1. General introduction to WSP

Waste stabilisation ponds (WSP) are man-made basins, generally shallow (1-5m deep) and rectangular, with earth embankments or occasionally retaining walls. The WSP can be designed for treating domestic or industrial wastewater, pre-treated or not. In WSP, wastewater is treated biologically, mainly, by means of the action of micro-organisms such as bacteria and algae. WSP present some advantages over conventional wastewater treatment, especially in tropical and subtropical countries, although these systems can be found all over the world (Silva and Mara, 1979). Nowadays, ponds are used not only in small communities of developing countries but wherever sufficient low-cost land is available.

Many authors (Mara, 1976; Silva, 1982; Arthur, 1983; Mara and Pearson, 1986; de Oliveira, 1990) have pointed out the main advantages of ponds systems, over other methods of wastewater treatment, such as high efficiency, system flexibility and simplicity and costs.

a) Effectiveness of treatment: in contrast to all other secondary treatment processes which are inefficient at removing excreted pathogens (virus, bacteria, protozoa and helminth) and require a tertiary treatment if their effluents are to be reused for irrigation, WSP can achieve a high degree of removal of these pathogens and organic matter.

b) Flexibility: WSP are extremely robust due to their long hydraulic retention time. They can withstand both organic and hydraulic shock loads.

c) Simplicity: WSP systems are simple to construct, operate and maintain

d) Costs: Ponds are considered the cheapest method of wastewater treatment because the costs involved in construction, operation and maintenance are actually very low (Arthur, 1983). Their energy comes from the sun and from chemical reactions, without need of electricity.

The main disadvantage of these systems is the high land requirements. However the investment made in land for ponds can always be realised later.

WSP systems comprise three main types of ponds: anaerobic, facultative and maturation.

Anaerobic ponds

They are generally 2-5 m deep, which represents a land saving alternative in relation to other pond types, and have no dissolved oxygen. BOD removal is achieved by sedimentation of settleable solids and subsequent anaerobic digestion in the sludge layer. Above 15°C digestion is so intense that the pond literally bubbles with the release of biogas, around 70% methane and 30% carbon dioxide (Mara and Pearson, 1998).

Despite the fact that anaerobic WSP receive high organic loadings, odour is not a problem if they are well designed. The odour problems are mainly caused by the presence of hydrogen sulphide in the wastewater. Sulphide can be generated by sulphur reduction in aquatic environments through three biochemical processes (de Oliveira, 1990): 1) assimilatory sulphur reduction, 2) desulfuration and 3) dissimilatory reduction of sulphur. In the last two processes, which are applicable to anaerobic ponds, the sulphide produced (by the proteolytic bacteria in the desulfuration and by the sulphate reducing bacteria in the dissimilatory reduction of sulphur is released in the wastewater which can cause odour.

In aqueous solution, sulphide can be present as hydrogen sulphide gas (H_2S), bisulphide ions (S^{2-}) and sulphide ions (HS^-). The relative proportions of H_2S, HS^- and S^{2-} vary with pH. At low pH ($\approx$5) H_2S predominates, while at pH values of 8 and above, most of the reduced sulphur exists in solution as HS^- and S^{2-} ions, and the amount of free H_2S is so small that odour problems do not occur (Sawyer *et al.*, 1994).

Organic matter removal is mediated by acidogenic and methanogenic bacteria. In order to obtain good performance in anaerobic ponds, it is necessary to keep the equilibrium between the metabolism of these groups of bacteria. The acidogenic bacteria convert the products of hydrolysis into simple acids like acetic and propionic and other products like carbon dioxide, ammonia as well as others. The methanogenic bacteria are classified in two groups. Both groups produce methane, one from acetate and the other from the reduction of carbon dioxide. These bacteria work more efficiently at temperatures higher than 15 °C and pH conditions higher than 6, because they are sensitive to acidity. In addition, these bacteria grow much more slowly than the acid-producing bacteria. Acidic wastewater thus requires neutralising prior to treatment in anaerobic ponds.

Facultative ponds

Facultative ponds are generally 1-2m deep and can be the first pond in a series or be preceded by an anaerobic pond. In the first case, it is called a primary facultative pond, receiving raw wastewater, and the second type, a secondary facultative pond, receiving pre-treated sewage, usually effluent from anaerobic ponds. Their superficial organic loading varies between 100 – 400 kgBOD_5/ha.d (Mara & Pearson, 1998).
Three zones exist in a facultative pond: a surface zone where aerobic bacteria and algae exist in a symbiotic relationship; an anaerobic bottom zone in which accumulated solids are decomposed by anaerobic bacteria; and an intermediate zone that is partly aerobic and partly anaerobic, in which the decomposition of organic wastes is carried out by facultative bacteria (Metcalf & Eddy, 1991).
The main algae present in facultative ponds are the motile genera such as *Chlamydomonas*, *Pyrobotrys* and *Euglena*, which can move easily according to the incident light intensity. The ability of *Euglena* to utilise acetate and butyrate in the light and in the dark and not be inhibited by proprionate may partially explain the dominance of this genus in many facultative ponds where turbidity will reduce light penetration through the water column and thus algal photosynthesis (Pearson *et al*, 1987). However, the concentration of algae can change according to the organic loading and temperature. In a healthy facultative pond chlorophyll *a* is usually in the range 500 – 2000 μg/l per litre (Pearson, 1996).In ponds with high organic loadings and where mixing of the water by the wind is minimal, the motile algae are often observed to form a dense narrow band at, or some centimetres below, the water surface and move vertically in the water column during daylight in response to changes in the incident light intensity. Under such circumstances photosynthesis is largely restricted to the top 20 or 30 cm since little photosynthetically active radiation (PAR) penetrates below the algal band (Pearson *et al*, 1987). The concentration of oxygen also varies throughout the day. The highest values occur in the mid-afternoon and the lowest at night. The pH also varies because when algal activity is very

intense, more hydroxyl ions are present in the solution as a result of displacement in the carbonate/bicarbonate equilibrium.

The algae-bacteria interaction in facultative ponds, which occurs in the surface layers, is very important to good performance of the pond. Algae, through photosynthesis, produce most of the oxygen present in the pond. This oxygen is used by the bacteria in the aerobic degradation of organic matter. The nutrients and carbon dioxide released in this degradation are, in turn, used by the algae.

Sulphide and ammonia are two products of anaerobic digestion in anaerobic ponds or in the anaerobic zone of facultative ponds. These products can be toxic to algae switching off photosynthesis and thus oxygen production, having a negative effect in pond performance. Primary facultative ponds receiving excessive loadings of organic material (but lower than those appropriate to anaerobic ponds) or inorganic sulphur, or both, change colour from green to red due to proliferation of purple sulphur bacteria and reduction in algal populations. These bacteria remove sulphides from the pond surface, but they cannot prevent the release of hydrogen sulphide at night, so situations where they proliferate should be avoided by redesign (Houghton and Mara, 1992).

Different algae show different levels of tolerance to both ammonia and sulphide (Pearson, 1990). The toxicity effects depend on the concentrations of the unionised forms, which in turn depend on the pH of the water. Ammonia and sulphide are more toxic to algae in the unionised form as these pass more easily across the algal cell membranes (Pearson *et al.*, 1987). Therefore, ammonia toxicity increases with increasing pH, while sulphide toxicity increases with decreasing pH.

It was observed by Abeliovich and Azov (1976) that longer exposure (>5h) to a given NH_4Cl concentration at a fixed pH led to significant reductions in photosynthesis, thus leading these researchers to suggest that exposure time is a critical factor in defining the relationships between NH_3 concentration and inhibition of photosynthesis. There is also evidence that both sulphide and ammonia can affect algal speciation in habitats through differences in tolerance between algal genera.

Pearson *et al.* (1987) studying the effect of ammonia and sulphide toxicity to algae, found that algae were capable of recovery from exposure to a 8.5 mg/L total sulphide solution for at least 8h at pH 7.25. However, recovery tended to be slower the longer the exposure period. The observed tolerance sequence to sulphide was *Chlamydomonas* > *Chlorella* > *Scenedesmus* > *Euglena*. Investigating the toxicity of

ammonia to the same algae at pH of 8.0, they noticed the following sequence of tolerance to ammonia: *Chlorella* > *Scenedesmus* > *Chlamydomonas* > *Euglena*.
König *et al.* (1987) found that as with sulphide, *Euglena* species were the most sensitive to ammonia inhibition, while *Chlorella* species were observed to have inherent tolerance to ammonia, and that they can survive under ammonia concentrations several times greater than the concentrations normally experienced by them in ponds from which they were isolated. So, as pointed out by Freund *et al.* (1993) this algal genus is important in terms of photosynthetic oxygen production under environmental stress conditions.

Anoxic ponds

These may be defined as ponds loaded at a level between the organic loading creating totally anaerobic conditions and that accepted as producing facultative pond conditions; that is between 15 and 100 $gBOD_5/m^3.d$. (Pescod, 1996). As in anaerobic ponds, BOD is removed by sedimentation of settleable solids and subsequent anaerobic digestion in the sludge layer.
Almasi and Pescod (1996) studied the presence of algal species in the water column of anoxic ponds and found that *Euglena* and *Chlamydomonas* predominated in the surface layers, producing oxygen and therefore preventing odour release.

Maturation ponds

Most usually, the depth of maturation ponds in a pond series is made the same as that of the associated facultative pond, i.e. 1 – 1.5m (Mara, 1976). Maturation ponds are not deep in order to obtain good results in terms of pathogen and nutrient removal. In these ponds, BOD removal also occurs, but with less intensity than in facultative and anaerobic ponds. The main factors, which contribute to biological desinfection, are time, temperature, high pH (> 9 due to rapid algal photosynthesis), high light intensity and photoactivated humic substances. Light wavelengths of 425-700 nm can damage faecal bacteria by being absorbed by the humic substances ubiquitous in wastewater. These then, enter an excited state for long enough to damage the cell. This process also depends on the presence of oxygen, and is considerably enhanced at high pH (Pearson, 1996).

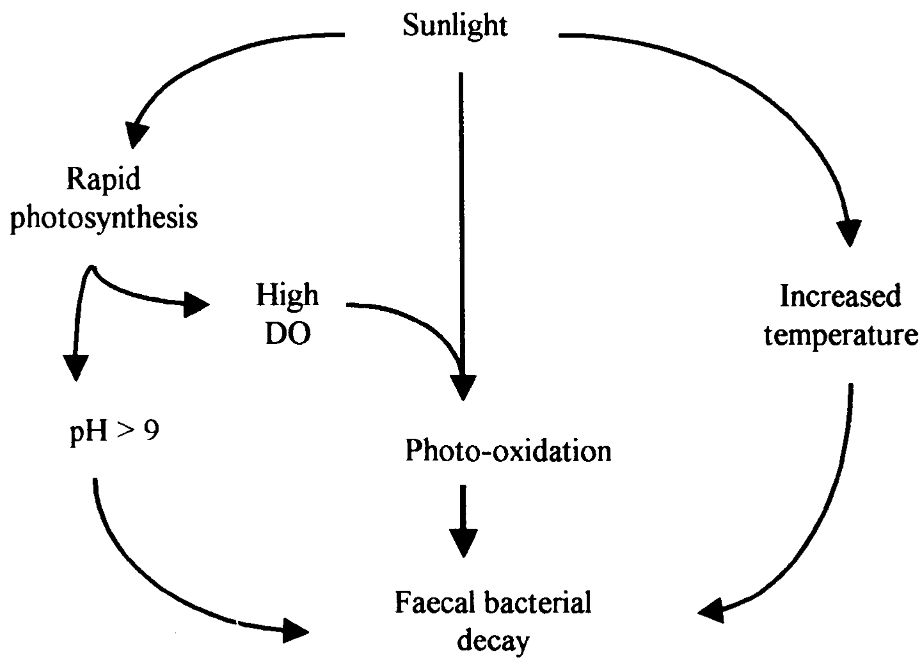


Figure 1.1. Combined effect of sunlight, pH and dissolved oxygen (DO) on faecal bacterial decay. Source: Curtis *et al.* (1992)

Maturation ponds are very rich in DO (dissolved oxygen), very often completely aerobic (Mara, 1976; Pearson *et al.*, 1987; de Oliveira; 1990), as a consequence of two main causes:

• they are less turbid than other ponds, so light can penetrate more deeply permitting algal photosynthesis to produce oxygen to greater depths;

• their organic loading rates are lower than those applied to facultative ponds. Therefore less oxygen is required and thus consumed for aerobic degradation of the organic matter.

Nutrient removal occurs through incorporation into algal cells and sedimentation. In facultative and maturation ponds ammonia is mainly removed when incorporated into algal cells. In maturation ponds, where pH is generally high, some of the ammonia leaves the pond by volatilisation. Total nitrogen removal in WSP systems can reach 80 % or more, and ammonia removal can be as high as 95 % (Pearson, 1996). Phosphorus removal occurs due to three main mechanisms: sedimentation of the organic matter, phosphorus precipitation at high pH levels and incorporation into cells of microorganisms, which settle out of the water column later (Araujo, 1993).

In maturation ponds, the population of algae is more diverse, since the organic loading is less than in the previous pond types. There is also more light penetration, which enables the presence of non-motile algae to be distributed at all depths.

Maturation ponds are also susceptible to thermal stratification during daylight hours, although showing a less defined thermocline than facultative ponds (Silva, 1982; de Oliveira, 1990).

1.1.1. Basic process design principles of WSP

Individual anaerobic ponds should be kept small (< 1.5 ha) to minimise surface mixing and the ingress of air into the pond in windy conditions. Length to breadth ratios < 1:3 should be used to encourage in-pond mixing of the contents to ensure good contact between the microorganisms and their metabolic substrates in line with general anaerobic reactor design (Pearson, 1996).

Anaerobic ponds are designed on the basis of volumetric BOD loading (λ_v, g/m^{3}d) mainly for BOD removal. λ_v should lie between 100 and 400 g/ m^3.d. The performance of anaerobic ponds increases significantly with temperature with the ambient range even found in the tropics where water temperatures may exceed 28^{0}C. (Mara and Pearson, 1998).

Facultative ponds, like anaerobic ponds, are also designed for BOD removal but on a surface organic loading basis (λ_s, 100 to 400 kg BOD_5/ ha.d) rather than volumetric loading to ensure the development of a healthy algal population that produces oxygen (Mara & Pearson, 1998). The permissible design value of λ_s increases with temperature. Maturation ponds are usually designed for excreted pathogen and nutrient removals. The effluent take-off from maturation ponds should be close to the surface (5 cm) where the pH is high and photo-oxidative processes contribute to bacterial die-off. The depth of the pond is also important. Shallow maturation ponds might be more efficient at bacterial removal than deeper ones.

1.2. General Introduction to Wastewater Storage and Treatment Reservoirs

WSP are operated as continuous flow systems releasing effluent throughout the year, but the water demand for irrigation, for instance, increases more during the dry season, resulting in some wastage of treated sewage in the wet season. Stabilization reservoirs solve the problem of treated effluent wastage in the wet season, by storing and treating wastewater during the whole year.

The treatment capacity of stabilization reservoirs relies on the same degradation and photosynthesis processes found in WSP. However, stabilization reservoirs are not merely deep stabilization ponds with a longer mean residence time. Ponds are designed as steady-state flow reactors with constant volume and loading, while stabilization reservoirs have an annual empty-full-empty cycle which stems from the seasonal character of irrigation: wastewater enters the reservoir continuously during the whole year, while outflow occurs only during the irrigation season. The mean residence time of the effluents (MRT), the percentage of fresh effluents within the reservoir (PFE) and the organic and hydraulic loading of the reservoir change during the year as the reservoir's depth and volume change. The non-steady-state flow characteristic of these reservoirs is the main factor affecting the reservoir's performance, removal efficiencies and effluent quality (Juanico and Shelef, 1994).

These reservoirs were first used in the early 1970's in Israel, with the purpose of storing effluents released during the wet season to be used in the dry season (Juanico and Shelef, 1991), but it was noticed that the effluent was also treated and these reservoirs were called stabilisation reservoirs. Nowadays, these reservoirs have a new and more suitable name, **Wastewater Storage and Treatment Reservoirs - WSTR** (Pearson, 1996). Herein, the last term (WSTR) will be used.

Although the use of WSTR is already common practise in many countries, studies regarding their performance as sewage treatment unit are still very scarce, most of them being reported from Israel.

In Israel there are about 130 WSTR in operation. Their storage capacities vary from 50,000 to 6 million m^3 and depths from 5.5 to 15 m (Juanico and Shelef, 1994). Full scale WSTR are also in operation in the USA (Porcella *et al*, 1971; Ayers and Westcot, 1985; Fuog *et al*, 1995), Mexico, Tunisia (Mara and Cairncross, 1989), Spain (Moreno *et al* 1984; Soler *et al*, 1988, 1991; Mujeiriejo and Sala, 1991) and Germany (Felgner and Sandring, 1983).

The surface organic loading was suggested by Juanico and Shelef (1994) as the main parameter determining the oxygen regime in a WSTR. Reservoirs receiving 30 kg BOD_5/ha.d were fully aerobic or facultative, whereas those which received a higher loading (150 kg BOD_5/ha.d) were anaerobic most of the time, giving rise to strong emissions of offensive odour. An annual mean of 30-40kg BOD_5/ha.d was recommended for the design of WSTR in Israel by these authors.

Stratification is a phenomenon also suggested as affecting WSTR performance (Juanico, 1995). Since WSTRs are generally deep, stratification in their vertical profile is more likely to occur than in shallow water bodies as the differences in temperatures between the upper and lower layers are greater. Milstein *et al.* (1994a), in a study on some shallow WSTRs in Israel, reported that in WSTRs of up to 5 meters deep, seasonal stratification was absent, whereas when studying a deep (8m) reservoir (Milstein *et al*, 1994b) under the same climatic conditions, stratification was found.

In a hypertrophic system, such as an WSTR, stratification can greatly affect the chemical, bacterial and planktonic processes as no oxygen, necessary to the aerobic degradation of the organic matter, can be transported to the deeper layers. In addition, the biological degradation rate of the organic matter is slowed in the deeper layers due to lower temperatures (Dor *et al.*,1987 and Dor *et al*, 1987b; Fattal *et al.*,1993).

According to Dor and Raber (1990) in a study on 12 WSTRs in Israel, it was found that reservoirs receiving effluents with high BOD values (> 40-50 mg/l) tended to stratify because high BOD values result in higher turbidity and the consequent trapping of solar radiation within the upper water layers, resulting in stratification.

Moreno *et al.*(1984) studying a 13.5 m deep stabilisation pond operated on a batch regime, found pronounced stratification.

Previous work on WSTR indicated that the performance of the reservoir as a treatment unit (i.e. removal of BOD, COD and FC) depends more on the operational cycle than on the seasonal climatic one (Juanico and Shelef, 1991;1994; Liran *et al.*, 1994; Juanico, 1994).

Liran *et al.* (1994) studied faecal coliform removal in WSTR and found that the MRT was the most important parameter affecting FC removal efficiency during a filling-resting phase. It is worth noting that during this phase, as the reservoir worked in a batch regime, the PFEm (percentage of fresh effluent) were zero. No environmental parameters were found to correlate with FC removal in this phase.

Pearson (1996) noticed that the use of WSTR ensures that a greater proportion of the nutrient value of the raw wastewater is conserved for crop fertilisation. Nitrogen losses in WSTR systems (40-50 percent) are much reduced as compared with WSP systems (80-90 percent), and phosphorus loss in WSTR was negligible compared with around 30 percent in WSP.

1.2.1. WSTR basic operational regimes

In Israel, the WSTR are operated under different regimes, depending on the effluent usage and demand. The WSTR operational regime determines the performance of the reservoir in terms of treatment capacity, outflow volumes, organic loading and oxygen regime, etc (Juanico, 1995).

WSTR are generally operated in a fill-rest-use cycle, comprising three phases:

- Filling phase: is the period when the reservoir is only receiving sewage;
- Resting phase: is the period when the reservoir neither receives nor releases liquid;
- Emptying phase: is the period of time when the reservoir is only releasing effluents.

Despite the existence of these three distinct phases, in some operational regimes, as pointed out by Athayde Júnior (1999), more than one of these phases can occur simultaneously, and the resting phase may not exist.

The most common WSTR operational regimes are:

(a) Continuous flow regime (continuous input)

The reservoirs operated in this regime receive wastewater continually and the resting phase does not exist. The discharge can be continuous or not depending on the crop irrigated. This treatment does not produce effluent of good quality, because fresh effluent is also released.

(b) Batch regime

In the batch regime, the reservoir stops receiving influent before its outlet is opened. This regime confers a higher quality to the effluent, but represent some wastage of treated sewage when the inlet of the reservoir is closed.

(c) Sequential batch regime

In this regime, a set of reservoirs receives effluents during all the year, but the reservoir which releases effluent stops receiving inputs before its outlet is opened. This regime was proposed by Mara and Pearson (1992) with the aim of achieving the WHO (1989) requirements for unrestricted irrigation. Sequential batch reservoirs have the advantage over the continuous flow because the presence of the resting phase results in an effluent of improved quality.

(d) Quasi-sequential batch regime

In this operational regime, the input of wastewater is stopped when the maximum volume of the reservoir is reached and then the release of effluent begins. In this case, the resting phase does not exist. If two or more reservoirs are operated in parallel, they become more economic because there is always input of wastewater in the system (while one is releasing effluent the other is filling).

1.2.2. Basic process design principles of WSTR

According to Juanico and Shelef (1994) WSTR are relatively new, and there are no engineering tools for their design and operation. In Israel, they are designed based on trial and error and very few empirical data. The storage capacity (maximum volume) of the reservoir is computed by making a balance between the gains (inflow and rain) and the losses (evaporation and seepage) during the non-irrigation season. The outlet is made of a pipe suspended from a raft at about 1m below the water surface in order to take the effluents from the oxygen-rich epilimnion.

Deep reservoirs with a small area/volume relationship are recommended in semi-arid regions where evaporation losses may account for more than 15% of inputs to the reservoir and increase the salinity of the remaining effluents (Juanico and Shelef, 1994).

The organic loading of the reservoir is the main parameter determining its oxygen regime. Pearson (1996) states that WSTR can be loaded at rates up to those used for facultative ponds; i.e. up to 350 kgBOD_5/ha.d at 25°C, and since, despite their depth, they behave as facultative ponds, they should be designed on the basis of aerial, rather than volumetric, loading.

WSTR are designed to receive either anaerobic pond effluent or raw wastewater, however, the use of anaerobic pond reduces WSTR volume requirements in the same way as it reduces WSP area requirements.

1.3. Basic microbiological process in WSP and WSTR

The presence of carbon dioxide, nitrogen in various forms, phosphorus, and other trace elements creates conditions suitable for the development of algae in wastewater. The properties of an algal cell – that is, its form; shape; size; density; color; rate of growth and photosynthesis; respiration rate; vitality; content of carbohydrate, fat, ash and content of various mineral ions; etc depend upon the environment to which the cell is exposed.

The types of algal cells contained in WSP effluents are principally young cells, producing much more oxygen than they use in respiration (Harvey *et al*, 1951). This may also be valid for WSTR.

The phosphorus requirement is greatest for the youngest and most rapidly growing cells, indicating that phosphorus utilization is closely associated with photosynthesis.

Nitrogen is essential in protein and chlorophyll *a* synthesis by algae. For most algae, ammonia followed by nitrate are the preferred nitrogen sources (Ip *et al*, 1982), although some Cyanobacteria are able to fix nitrogen from the atmosphere (Ip *et al*, 1982). The algae assimilate these nutrients for their growth and at the same time contribute to their removal. The intensive algal growth in ponds often causes an increase in pH which then leads to ammonia stripping and phosphate precipitation, which further enhances the nutrient removal efficiency in these algal systems (Lau *et al*, 1995).

The cyanobacteria were previously called blue-green algae until their prokaryotic structure was determined. Since the cyanobacteria possesses an algal type of photosystem, the photosynthetic pigment chlorophyll *a* and release oxygen during photosynthesis this group has been included in the algal study (Canter-Lund and Lund, 1995).

According to Dor *et al* (1987) hypertrophic conditions like those found in wastewater reservoirs lead to the development of a close relationship between algae and bacteria combining competition, mutual suppression and mutual support. These authors suggest that, under these conditions, the sensitivity of the community to environmental changes declines.

In this symbiotic relationship, between bacteria and algae, the latter perform oxygenic photosynthesis providing the majority of the oxygen required by aerobic and facultative bacteria for degrading organic compounds. A small part of the oxygen in the water comes from the atmosphere. To perform oxygenic photosynthesis the algae require energy (sunlight), carbon dioxide and mineral salts, the last two supplied by the heterotrophic bacteria resulting from the aerobic decomposition of the organic matter. Uhlmann (1980) considered this algae/bacteria relationship oversimplified because bacteria also utilize dissolved organic substances originating from the photosynthetic processes of phytoplankton. On the other hand, most of the phytoplankton species predominating in WSP are mixotrophic and thus at times can also use dissolved organic material. Seretaki (1996) also mentions that some algae need organic matter to promote growth, and so they could possibly be used for the degradation of refractory substances, e.g. substances which are more resistant to biodegradation. This author adds that no data exist concerning the COD level in wastewater treatment plants and its influence on algal coenoses formation. The COD level is important since it includes not only the easily biodegradable components present in sewage but also refractory components.

In fact this process is very complex indeed and it is increasingly clear that the microalgae are not just the providers of oxygen but are directly implicated in other processes like organic carbon and nutrient removal, and most importantly in the process of biological desinfection (Pearson, 1996).

The algae convert solar energy to the energy in their cellular matter and heat. The efficiency of conversion of solar energy to plant tissue often reaches 5%, and the efficiency of conversion of solar energy to heat apparently reaches in excess of 90 %. The heat so produced accelerates both aerobic and anaerobic microbiological waste oxidation and reduction and hastens the death of pathological organisms (Oswald, 1973).

The release of oxygen by the algae and the increase in pH also contribute to pathogen removal (Pearson *et al*, 1987) and light activated humic acids also play a role in the desinfection process (Curtis *et al.*, 1992). Although, Liran *et al* (1994) state that among the several parameters which describe algae concentration and activity (Chlorophyll, TSS, dissolved oxygen, pH and others) pH was the only one with significant correlation with coliforms, indicating that pH itself and not algal activity *per se* is responsible for faecal coliform die-off.

Algal growth and productivity are affected by many factors, such as solar radiation, temperature, organic loading and nutrient availability, although different algal species respond in different ways to these factors (Llorens *et al.*, 1993). Wiedeman (1965) stated that certain algae may require high organic loading while others are inhibited by it.

Palmer (1969), combined the findings of 165 field workers who had studied the presence of algae in water polluted with organic matter throughout the world, to determine the impact of pollution load on the presence of genera and species. Palmer concluded that organic pollution tends to influence the algal flora more than other factors in aquatic environment, such as water hardness, light intensity, pH, dissolved oxygen, rate of flow, size of water body, temperature and other types of pollutants. He stated that the 7 genera of most pollution-tolerant algal genera are *Euglena, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicurla,* and *Stigioclonium* and the genus *Oscillatoria* of the cyanobacteria .

Llorens *et al* (1993) studied primary productivity and production measurements and their relationship to the variations of nutrients (N and P), CO_2, pH, temperature, dissolved oxygen and solar radiation in a 8m deep stabilization pond. This author agrees with Palmer (1969) that organic loading is the main factor influencing phytoplankton communities in sewage depuration lagoons, however Llorens and his colleagues state that when the organic loading decreases algae like *Scenedesmus* and *Chlorella* appear.

König (1984) working on pilot-scale ponds in Northeast Brazil noted that as the organic loading increased, flagellate algae tended to become dominant, particularly the genera *Euglena*, *Chlamydomonas* and *Pyrobotrys*. Based on frequency data, this author noticed that the number of genera decreased down to about 9 when surface organic loadings of more than 100 kg BOD_5/ha.d were applied to the maturation ponds. A decrease in the number of genera also occurred in primary facultative ponds when there was an increase in the organic loadings.

In any aquatic environment, the changes in algal biomass and diversity are a result of the changes in physico-chemical and biological characteristics of the water. These changes in algal biomass and diversity can be determined by frequency and chlorophyll *a* analysis. Chlorophyll *a* determination is the most common method used to obtain the concentration of algal biomass, because of its simplicity and speed (König, 1990). These results can give information about the prevailing organic loading and the treatment efficiency achieved in the system.

Under conditions of very heavy loading resulting in extended anaerobic periods, *Chlamydomonas* is the only algal genus to thrive (de Noyelles, 1967). Pearson *et al* (1987) associated the predominance of *Chlamydomonas* with overloaded conditions in facultative ponds, attributing to it a high degree of tolerance to sulphide toxicity. This may explain, in part, the occurrence of this genus, the largest one with over 400 species (Lund and Lund, 1995) even in certain anaerobic ponds at times (de Oliveira, 1990).

Chlorella, especially *C. pyrenoisa*, appears to be the most successful organism under conditions of heavy loading resulting in pH ranges near neutrality and moderate to high BOD levels but with no extended anaerobic periods. The availability of organic carbon may influence the presence of *Chlorella* since this organism is unable to utilize bicarbonate carbon (de Noyelles Jr, 1967). *Scecenedesmus* and *Ankistrodesmus* are found in environments where the levels of organic matter have been reduced from those favouring *Chlamydomonas* and *Chlorella*. *Scenedesmus* shows some tolerance to high organic conditions similar to those favouring *Chlorella*, whereas *Ankistrodesmus* appears to be inhibited by extended exposure to such levels. Both *Scenedesmus* and *Ankistrodesmus* are able to use bicarbonate-C, which may partially explain why they replace *Chlorella* at low organic levels and accompanying pH ranges above 8 (de Noyelles Jr, 1967).

De Oliveira (1990), in his study on the performance of deep WSP in Northeast Brazil, noticed that mean concentrations of *Euglena* tended to be inversely related to the organic loadings throughout his experiments and that this observation is in accordance with the remarks of Patil *et al* (1975) who concluded euglenoids to be well adapted to conditions of low BOD and nutrient concentrations. These workers therefore disagreed with Palmer (1969) for whom *Euglena* heads a list of the most pollution tolerant genus of algae. Canter-Lund and Lund (1995) stated that Euglenophytes are common in water rich in organic matter with *Phacus, Euglena* and *Trachelomonas* genera being the commonest. Even these algal genera which can photosynthesise, they need organic supplements and, hence their abundance in places rich in decaying and plant matter. It is often difficult to grow Euglenophytes in the laboratory in the absence of bacteria, which, presumably, produce the nutrients they need.

Pyrobotrys is another genus of algae that can grow at high organic loadings and tolerate anaerobic periods (de Oliveira, 1990).

Pearson *et al.* (1987), investigating the utilization of organic substrates, the significance of light and the inhibitory effects of sulphide and ammonia on some WSP algae, emphasises the importance of light to the growth of these algae. They noticed that light is probably the key factor controlling algal speciation in many situations.

The dependence of algal photosynthesis on light penetration through the water column would also appear to bestow a competitive advantage on motile light-seeking algal species such as the flagellate forms particularly in ponds with high organic loads, which will be high in suspended solids and thus turbidity.

Water temperature also affects dissolved oxygen concentration, especially at the lower concentrations. As the temperature rises, the solubility of oxygen in water decreases. Temperature also interferes with algal growth. Since the higher the temperature the lower the CO_2 solubility thus affecting algal biomass concentration and the primary production rate (Ip *et al*, 1982).

Various algae can have different effects in the O_2 concentration in ponds. For example, a bloom of floating, mat – forming cyanobacteria (e.g. *Oscillatoria* and *Anacystis*) has a marked effect on dissolved oxygen. Since with the presence of these genera the sunlight can penetrate only a short distance, thereby decreasing photosynthetic oxygen release (Wiedeman, 1965). On the other hand, Oswald *et al*

(1953) noticed that due to the higher rate of growth, and perhaps due to its smaller size, *Chlorella* produces an excess of oxygen greater than that produced by *Euglena*. König (1984) noticed that higher concentrations of dissolved oxygen in facultative and maturation ponds occurred between 12 and 16 h in the afternoon and changed according to the photosynthetic activity of the algae.

Temperature and light intensity also interfere in the movement of the algae in the pond water column. The flagellate algae move within the water column in order to find the best conditions in the pond resulting in algal stratification, specially in facultative ponds. In maturation ponds algal stratification is less pronounced due to the more homogeneous penetration of light and the predominance of non–flagellate genera. Stratification can lead to an increase in algae in the pond effluent and as a result in an increase in effluent BOD, COD and SS.

Recent observations in deep ponds also confirm that the motile algae (e.g. *Euglena*) of facultative ponds move to the pond bottom sediments at night and metabolise chemoorganotrophically (Pearson, 1996).

If the phytoplankton population finds favourable conditions it may develop and as a consequence, cause an increase in the concentration of suspended solids (Toms *et al*, 1975; Saqqar and Pescod, 1996). Ellis (1983) considers the high concentration of suspended solids in WSP effluents results from algae as one disadvantage of these systems.

Algae can contribute up to 70% of the BOD of an unfiltered effluent from a maturation pond. The COD and BOD contributions by the algae differ to some degree with species. However, whereas the entering sewage solids are highly putrescible and hazardous to the public health, the algal cells in the effluent, called "algal BOD", are highly stable (Harvey *et al.*, 1951) and considered less damaging on receiving waters than conventional sewage BOD (Pearson, 1996).

It is well recognised that the presence of algae in ponds provides other advantages besides treating the wastewater. They can be harvested and due to their high nutritional values, can be used for animal feed. They also have a great potential for producing various chemicals of industrial importance such as oils and β-carotene (Guterman and Ben-Yaakov, 1987).

1.4. Nutrients in wastewater

The use of treated wastewater for crop irrigation is one alternative when the rainy season is very short. The use of this water for irrigation releases more clean water for use in potable supplies (e.g. for cooking, baths, etc). The treated wastewater provides another advantage to agriculture in the form of nutrients, such as nitrogen and phosphorus, which reduces the amount of fertilizers that need to be added to the soil to support good crop production

Although nitrogen is very important to the development of plants (and animals) (Metcalf-Eddy, 1991), excessive quantities of it can cause problems to certain crops and also provoke eutrophication in receiving water bodies. The Brazilian environmental law (CONAMA, 1986) states that 5mg/L of ammonia is the maximum concentration allowed in the effluent from wastewater treatment plants for discharge into aquatic water bodies so as to avoid eutrophication of aquatic reservoirs.

According to Ayers and Westcot (1995) the value of total nitrogen is very important due to the frequent bacterial transformations that occur so changing the forms of nitrogen present. According to these authors, concentrations of total nitrogen of less than 5 mg/L are safe. Nitrogen is present in domestic wastewater in the following forms: ammonia (12-50mg/L), organic nitrogen (8-35mg/L), nitrite and nitrate, very low concentrations (Metcalf & Eddy, 1991). Since the maximum concentration that does not have a negative environmental impact is 5 mg/L, it is necessary to remove part of the nitrogen from the wastewater during treatmernt. Ammonia is the form of nitrogen most commomly used to characterize nitrogen content in sewage (Reis, 1995).

In general, up to 80% of ammonia can be removed in WSP (Silva,1982; Silva *et al*, 1997). The main mechanisms are:

• Volatilization

This mechanism is considered by many authors to be the main mechanism of nitrogen removal in WSP. High pH (> 8.0) and temperature are the key parameters driving the process (Pano and Middlebrooks, 1982).

• Assimilation by the algal biomass

One of the main forms of recycling nitrogen in WSP is the assimilation of ammonia by algae. When these organisms die, the heterotrophic bacteria degrade a proportion of the algal cell nitrogen , releasing it as ammonia back into the water column. The non-biodegradable part stays in the pond sediments (Ferrara and Avci, 1982; Arceivala, 1986).

• Processes of nitrification and denitrification

Although these mechanisms are mentioned in the literature, it is suggested by Pano and Middlebrooks (1982) as not being significant in WSP.

In relation to phosphorus, there is no evidence in the literature that high concentrations can cause problems to crop productivity. The soluble phosphorus form, orthophosphate, is the form of phosphorus easily assimilated by aquatic organisms and plants (Sawyer *et al*, 1994). When the concentration of orthophosphate is less than 0.005mgP/L it can limit the development of algae (WPCF,1993).

The main mechanism of phosphorus removal in WSP is the precipitation of orthophosphate at high pH resulting in the formation of hydroxyapatite. Orthophosphate is also removed through the assimilation by the algae in the ponds.

1.5. Introduction to current work and objectives

The experimental waste stabilisation ponds (WSP) and wastewater storage and treatment reservoirs (WSTR) systems at EXTRABES provide a unique opportunity to study the microbiological processes important in wastewater treatment in these systems since unlike full scale systems, they can be manipulated at will.

In the pond study, the data were collected in four independent primary facultative ponds working under two depth regimes, 1.25 and 2.30m and in three series of five ponds with depth varying from 0.81m to 2.20m. In the independent primary facultative ponds an attempt was made to:

- Link faecal coliform (FC) and BOD_5 removal to organic loading on the ponds and pond depth;

- Link algal biomass (expressed as chlorophyll *a*) to surface organic loading, total ammonia, total sulphide and its unionised forms (NH_3 and H_2S respectively) at the two different depths

The data collected from the three five pond series, of different depths, will be analysed for:

- FC, BOD_5 and nutrient (ammonia and orthophosphate) removal efficiency in relation to depth;
- Chorophyll *a* concentration (i.e.algal biomass) in relation to surface organic loading, total ammonia, total sulphide and their unionised forms NH_3 and H_2S respectively at different depths;
- Algal genera diversity and cell numbers and their relation to surface organic loading but only in the shallowest series.

In the eight experiments performed in the 6.5m WSTR, an attempt will be made to determine:

- Diurnal (24h profile) variations in pH, DO, temperature and chlorophyll *a* within WSTR water column;
- The algal biomass (expressed as chlorophyll *a*) fluctuations in the filling and resting phases of the WSTR and its relation to total ammonia, orthophosphate, NH_3 and H_2S concentrations;
- Algal diversity and biomass concentration in relation to organic loading in the filling and resting phases of the WSTR.

In this way it is hoped to expand our understanding and knowledge of the process microbiology of WSP and WSTR systems with a view to improving their design.

2. MATERIALS AND METHODS

2.1. Research station

The data collected in this study was obtained at EXTRABES (Federal University of Paraíba Wastewater Treatment Experimental Station) situated in the city of Campina Grande (7° 13' 11" S, 35° 52' 31"W, 550 m above m.s.l), Paraíba State, northeast Brazil. There are only two seasons in Campina Grande, the rainy season that lasts for four or five months, between May and August and the dry season is from seven to eight months, between September and April. The monthly mean air temperature is within the range 20-30°C, the annual rainfall is about 760 mm (Silva *et al.*, 1987) and the annual evaporation (Class A tank) is 2200-3000mm (Governo do Estado da Paraíba, 1992).

The experiments were carried out at different times of the year but they are largely comparable, due to the small variation in the ponds and WSTR's water temperatures (24-28 °C, with a mean water temperature of 25 °C), as shown by previous studies: Silva (1982); König (1984); Arridge (1997). EXTRABES was set up in March 1977 to study the performance of different types of biological wastewater treatment processes under different operational conditions. The aim was to define the optimum design parameters for these treatment processes in tropical regions. The research has been centered on various types and configuration of waste stabilization ponds (WSP) and more recently on Wastewater Storage and Treatment Reservoirs (WSTR). The experimental systems used in this study are described below.

2.2. Experimental systems

2.2.1. System A

a) Description

System A comprises four independent primary facultative ponds (F_2, F_3, F_4, F_5). Each of these four ponds was studied under two different HRT and surface organic loadings, making a total of eight different operational conditions. These ponds were monitored for 27 and 18 months in experiment I and II respectively. Subsequently, these four ponds had their depths increased from 1.25 to 2.30 m. During this second phase, only one set of experiment was performed in each pond with a length of 18 months. Table 2.1 shows the physical and operational characteristics of these ponds during the three sets of experiments and Figure 2.1 shows the pond layout and x-sections.

Table 2.1- Physical and operational characteristics of system A

Experiments	Ponds	Length (m)	Width (m)	Depth (m)	Flow (m^3/d)	HRT (d)
I	F_2	25.70	7.50	1.25	20.33	11.80
	F_3	26.40	7.40	1.25	20.33	12.00
	F_4	25.70	7.40	1.25	25.03	9.50
	F_5	25.70	7.30	1.25	12.43	18.90
II	F_2	25.70	7.50	1.25	32.16	7.50
	F_3	26.40	7.40	1.25	39.00	6.30
	F_4	25.70	7.40	1.25	34.82	6.80
	F_5	25.70	7.30	1.25	31.32	7.50
III	F_2	25.40	7.15	2.30	24.22	17.30
	F_3	25.50	7.15	2.30	33.44	12.60
	F_4	25.50	7.10	2.30	36.20	11.50
	F_5	25.40	7.10	2.30	29.64	13.80

b) Wastewater pumping

The wastewater was pumped from a wet well adjacent to a 900mm trunk sewer running along EXTRABES to a constant level tank (CLT) near the ponds, by a 1.2 HP 3380 rpm submersible pump (Dynapac Equipamentos Industriais Ltda., São Paulo, Brazil) from where the required flow was pumped by a peristaltic pump(Netzch, model NE30, Pomerode, Santa Catarina, Brazil) to each primary facultative pond. The excess flow in the constant level tank returned by gravity to the wet well.

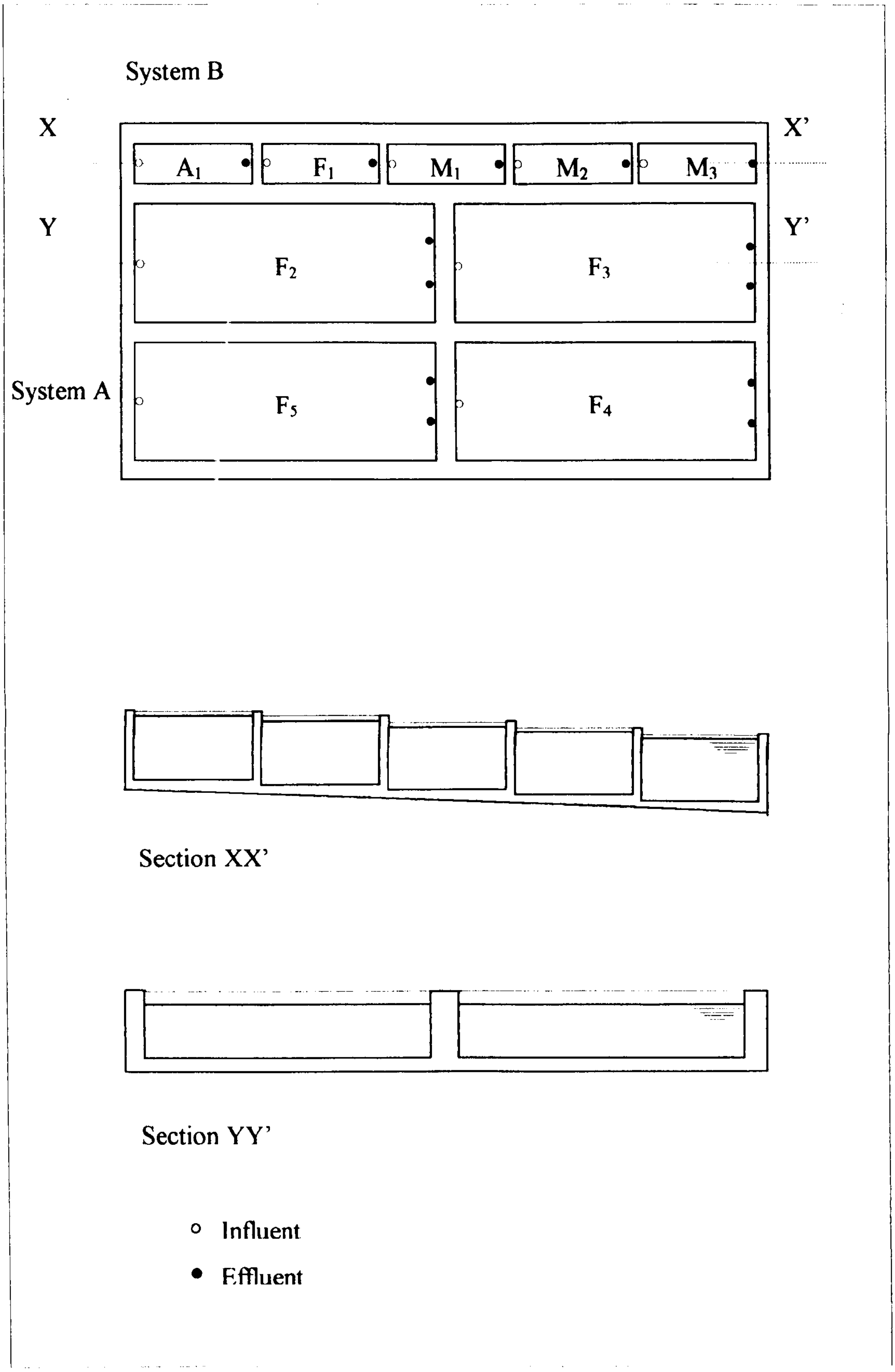


Figure 2.1- Layout and x-sections of systems A (independent facultative ponds "F") and System B (series A_1 to M_3).

2.2.2. System B

a) Description

System B is situated beside system A and comprises five ponds in series: one anaerobic pond (A_1), one facultative pond (F_1) and three maturation ponds (M_1, M_2, M_3). This system was studied under three different operational conditions (ie. hydraulic retention time and organic loading). The five-pond series were monitored for 28, 18 and 12 months in experiments I, II and III respectively. As with system A, the ponds in system B also had their depths increased; in this case from 1.00 to 2.20 m and 2 additional sets of experiments, with different operational characteristics were performed. Experiments IV and V lasted for 18 and 12 months respectively. Table 2.2 shows the physical and operational characteristics of these ponds during the five sets of experiments while Figure 2.1 shows the layout and x-sections of the system B series.

b) Wastewater pumping

The wastewater was pumped in the same way as in System A, until the CLT. From this tank the wastewater was pumped by a variable speed heavy duty peristaltic pump (Watson-Marlow, model HRSV, Falmouth, Cornwall, England) to the anaerobic pond (A_1). From the anaerobic pond, the wastewater followed by gravity along the series. The final effluent was discharged in the trunk sewer.

2.2.3. System C

a) Description

This innovative system of WSP was situated on the site of the municipal treatment station (Estação da Catingueira), 10 km away from the center of Campina Grande. System C comprised two parallel anaerobic ponds (A_9 and A_{10}), five parallel secondary facultative ponds of different geometry (F_{21} to F_{25}), one primary maturation pond (M_{15}), five parallel secondary maturation ponds of different geometry (M_{16} to M_{20}), and four parallel tertiary maturation ponds (M_{21} to M_{24}).

Table 2.2 – Physical and operational characteristics of system B.

Experiments	Ponds	Length (m)	Width (m)	Depth (m)	Flow (m^3/d)	HRT (d)
I	A_1	10.00	3.35	1.25	6.17	6.80
	F_1	10.00	3.35	1.00	6.17	5.50
	M_1	10.00	3.35	1.00	6.17	5.50
	M_2	10.00	3.35	1.00	6.17	5.50
	M_3	10.70	3.35	1.00	6.17	5.80
II	A_1	10.00	3.35	1.25	21.24	2.00
	F_1	10.00	3.35	1.00	21.24	1.60
	M_1	10.00	3.35	1.00	21.24	1.60
	M_2	10.00	3.35	1.00	21.24	1.60
	M_3	10.70	3.35	1.00	21.24	1.70
III	A_1	10.00	3.35	1.25	10.56	4.00
	F_1	10.00	3.35	1.00	10.56	3.20
	M_1	10.00	3.35	1.00	10.56	3.20
	M_2	10.00	3.35	1.00	10.56	3.20
	M_3	10.70	3.35	1.00	10.56	3.40
IV	A_1	10.00	3.35	2.20	14.74	5.00
	F_1	10.00	3.35	2.20	14.74	5.00
	M_1	10.00	3.35	2.20	14.74	5.00
	M_2	10.00	3.35	2.20	14.74	5.00
	M_3	10.0	3.35	2.20	14.74	5.00
V	A_1	10.00	3.35	2.20	9.22	8.00
	F_1	10.00	3.35	2.20	9.22	8.00
	M_1	10.00	3.35	2.20	9.22	8.00
	M_2	10.00	3.35	2.20	9.22	5.00
	M_3	10.70	3.35	2.20	9.22	8.00

The pond M_{24} was divided by baffles, resulting in a 0.44 m wide and 64 m long channel. The physical and operational characteristics of this system are shown in table 2.3, while figure 2.2 shows the system scheme. System C was studied under two different operational conditions (i.e. hydraulic retention time and organic loading). The first experiment lasted for 13 months and the second one for 10 months.

The wastewater entering at the Catingueira treatment plant comes from Campina Grande and therefore has the same characteristics as the wastewater entering EXTRABES.

b) Wastewater pumping

Two NETZSCH pumps (NE 30A, POMERODE, Santa Catarina, Brazil) pumped raw wastewater to the anaerobic ponds. Every two weeks the flows were adjusted, and no variations above 2% of the projected flow were observed.

The ponds in series with A_9 and A_{10} were fed by gravity. The effluent of the anaerobic ponds was collected and combined through PVC tubes and then the flow divided into five parts via a flow splitting box (FSB_1). Each part fed one facultative pond. The effluents from facultative ponds were collected and combined to feed the primary maturation pond (M_{15}). The effluent of M_{15} was divided into eight parts via FSB_2, from which five of them fed the five secondary maturation ponds (M_{16} to M_{20}) and the others fed three biological filters: BF_2, BF_3, BF_4 (which were not part of this study).

The wastewater flow to each pond was through PVC tubes. Tubes collected the effluents of the secondary maturation ponds M_{16}, M_{17} and M_{18} conducted them to the FSB_3 where the flow was divided into four parts. Each part fed one tertiary maturation pond (M_{21} – M_{24}). The effluents from the secondary maturation ponds M_{19} and M_{20}, as well as the effluents from the tertiary maturation ponds (M_{21} to M_{24}) were collected and discharged via an inspection box as shown in Figure 2.2. The final effluent was conducted to the municipal wastewater treatment plant (Estação da Catingueira).

Table 2.3- Physical and operational characteristics of System C.

Experiments	Ponds	Length (m)	Width (m)	Depth (m)	Flow (m^3/d)	HRT (d)
	A_9	4.90	1.65	2.50	20.00	1.0
	A_{10}	4.90	1.65	2.50	20.00	1.0
	F_{21}	12.00	2.00	1.00	8.00	3.0
	F_{22}	12.00	2.00	1.33	8.00	4.0
	F_{23}	12.00	2.00	1.67	8.00	5.0
	F_{24}	12.00	2.00	2.00	8.00	6.0
	F_{25}	4.90	4.90	2.00	8.00	6.0
I	M_{15}	17.35	8.80	1.00	40.00	3.8
	M_{16}	10.40	3.75	0.90	5.00	7.0
	M_{17}	10.40	3.75	0.64	5.00	5.0
	M_{18}	10.40	3.75	0.39	5.00	3.0
	M_{19}	10.40	3.75	0.39	5.00	3.0
	M_{20}	10.40	1.30	0.39	5.00	1.0
	M_{21}	8.45	3.70	0.60	3.75	5.0
	M_{22}	8.45	3.70	0.60	3.75	5.0
	M_{23}	8.45	3.70	0.60	3.75	5.0
	M_{24}	8.45	3.70	0.60	3.75	5.0
	A_9	4.90	1.65	2.50	40.00	0.5
	A_{10}	4.90	1.65	2.50	40.00	0.5
	F_{21}	12.00	2.00	1.00	16.00	1.5
	F_{22}	12.00	2.00	1.33	16.00	2.0
	F_{23}	12.00	2.00	1.67	16.00	2.5
	F_{24}	12.00	2.00	2.00	16.00	3.0
	F_{25}	4.90	4.90	2.00	16.00	3.0
	M_{15}	17.35	8.80	1.00	80.00	1.9
II	M_{16}	10.40	3.75	0.90	10.00	3.5
	M_{17}	10.40	3.75	0.64	10.00	2.5
	M_{18}	10.40	3.75	0.39	10.00	1.5
	M_{19}	10.40	3.75	0.39	10.00	1.5
	M_{20}	10.40	1.30	0.39	10.00	0.5
	M_{21}	8.45	3.70	0.60	7.50	2.5
	M_{22}	8.45	3.70	0.60	7.50	2.5
	M_{23}	8.45	3.70	0.60	7.50	2.5
	M_{24}	8.45	3.70	0.60	7.50	2.5

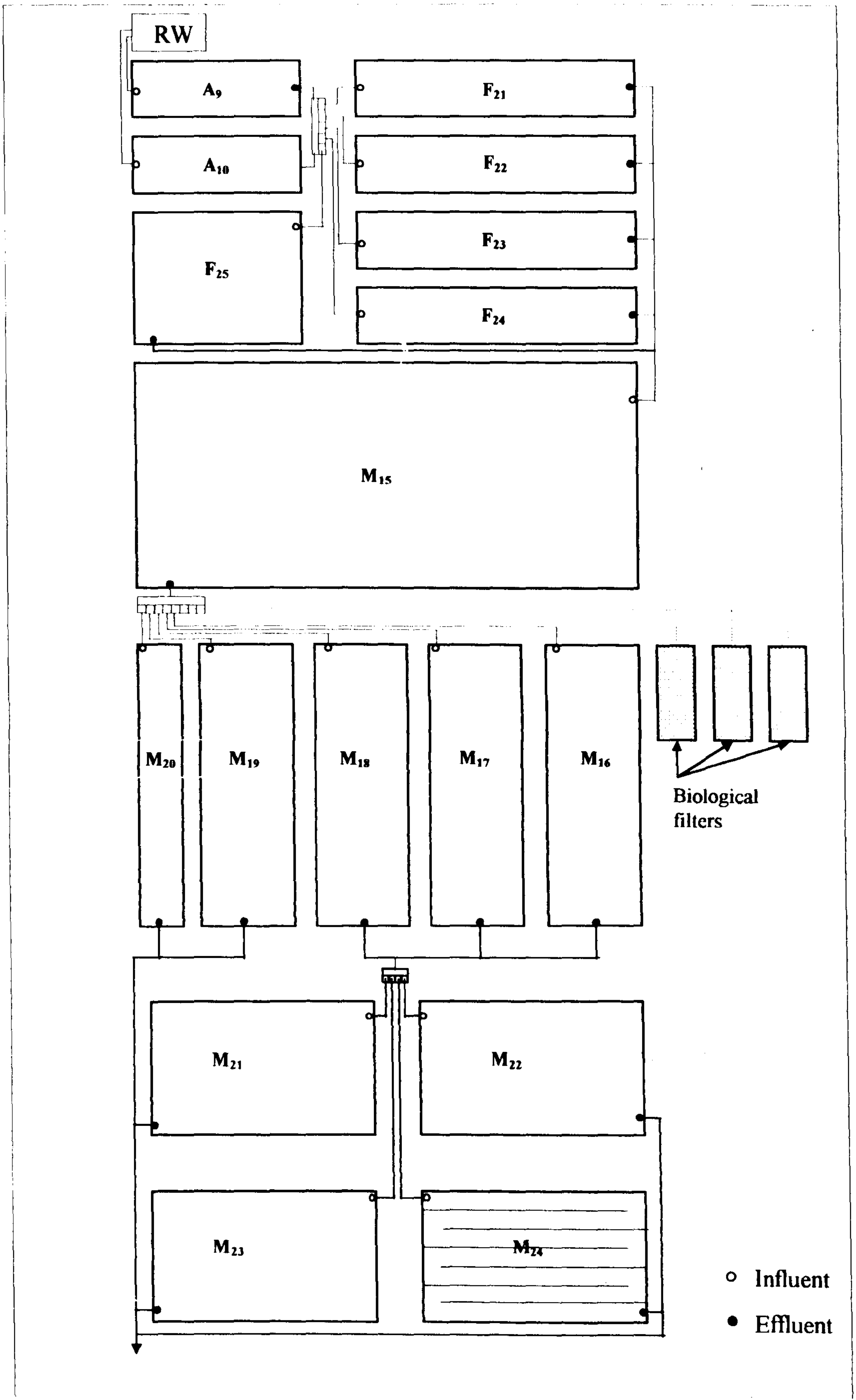


Figure 2.2- Layout of system C

2.2.4. System D

a) Description

The Wastewater Stabilization and Treatment Reservoirs (WSTR) pilot-scale system at EXTRABES, comprised three WSTRs ($WSTR_1$, $WSTR_2$, and $WSTR_3$) and one anaerobic WSP (A_{12}). The physical and operational characteristics of the reservoirs are shown in table 2.4 and the layout in figures 2.3 and 2.4

The $WSTR_1$ and $WSTR_2$ were constructed by renovating and extending the 15 m diameter primary and secondary sedimentation tanks, respectively, and were fed with anaerobic pond effluent. $WSTR_3$ resulted from the adaptation of the secondary digester of the former municipal wastewater treatment plant and was fed with raw wastewater.

b) wastewater pumping

Domestic raw wastewater was pumped from a wet well adjacent to a 900 mm diameter trunk sewer running along EXTRABES, to a constant level tank (CLT_1) using a 1.2 hp/2280 rpm submersible pump (Dynapac Equipamentos Industriais Ltda, São Paulo, Brazil). From the CLT_1 the wastewater was pumped to the anaerobic waste stabilization pond by a peristaltic pump (NETZSCH model NE 30 A) and then the effluent of this pond was discharged in another CLT (CLT_2) from where the effluent was pumped to $WSTR_1$ or $WSTR_2$ by a peristaltic pump (NETZSCH model NE 30 A). To feed $WSTR_3$, the wastewater was conducted by gravity from the CLT_1 to the reservoir external wall, on the ground, from where it was pumped to the reservoir by a peristaltic pump (Watson – Marlow; Falmouth, Cornwall, England). All the reservoirs received the influent wastewater near the wall, 50cm above the bottom.

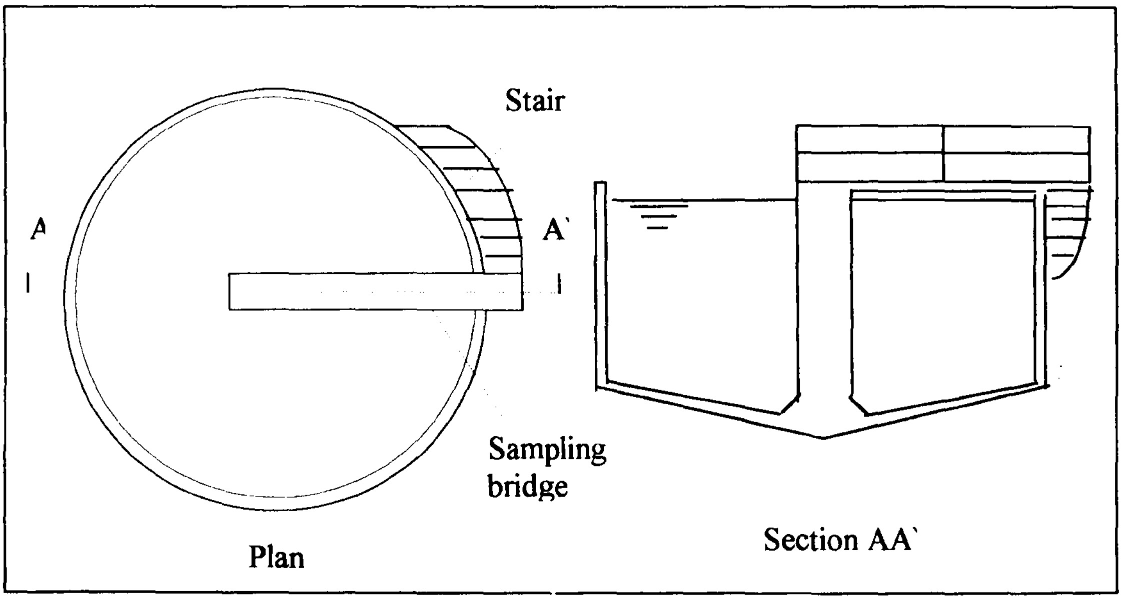


Figure 2.3 - $WSTR_1/WSTR_2$ scheme, in plan and section

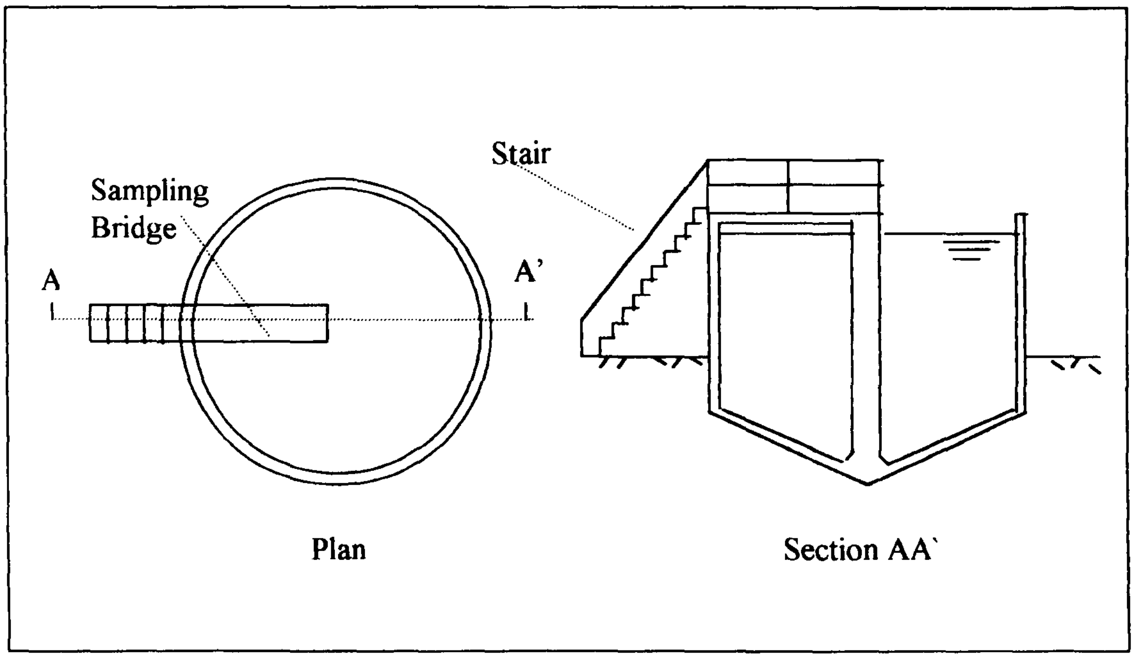


Figure 2.4-. $WSTR_3$ scheme, in plan and section

Table 2.4 – Physical and operational characteristics of system D

Reservoirs	Experiment	Internal diameter (m)	Mean depth (m)	Superfice area (m^2)	Volume (m^3)	Influent	Influent flow rate (m^3/day)	Filling time (days)	Resting time (days)
$WSTR_1$	I	15.15	6.50	180.27	1,150.72	Anaerobic pond effluent (HRT=24h)	32.84	35	90
	II	15.15	6.50	180.27	1,150.72	Anaerobic pond effluent (HRT=12h)	63.86	18	52
	III	15.15	6.50	180.27	1,150.72	Anaerobic pond effluent (HRT=12h)	32.84	35	54
$WSTR_2$	I	15.15	6.50	180.27	1,150.72	Anaerobic pond effluent (HRT=12h)	28.03	41	91
	II	15.15	6.50	180.27	1,150.72	Anaerobic pond effluent (HRT=12h)	76.63	15	105
$WSTR_3$	I	10.65	6.50	89.08	568.63	Domestic raw wastewater	76.61	74	89
	II	10.65	6.50	89.08	568.63	Domestic raw wastewater	19.42	29	39
	III	10.65	6.50	89.08	568.63	Domestic raw wastewater	14.82	38	124

2.3. Sampling

2.3.1. Sampling in the WSP

In systems A and B samples of the raw wastewater and of the effluents of the ponds were collected between 08:00 and 09:00 a.m., weekly, for physico-chemical and microbiological analysis. Besides the effluent samples, column samples were collected for the analyses of Chlorophyll *a* to check if there was any difference in concentrations between the effluent and the entire pond water column sample.
The column sample was obtained using a water column sampler (Fig. 2.5) made of sections of acrylic tubing, which could be screwed together according to the depth of the pond. The water column sampler was introduced with the cap open into the pond near the outlet to sample the entire pond depth taking care to avoid collecting the sludge layer. After introduced into the pond, the water column sampler was closed and removed. This operation was repeated until the desired effluent volume was collected. In system C, raw wastewater was collected between 08:00 and 09:00 a.m. twice a week. However, in the ponds of system C only column samples were collected for the analyses of all parameters.

2.3.2 Sampling in the WSTR

During the filling phase, the WSTR were monitored every time the depth of the water column increased by a meter. During the resting phase, samples were collected either weekly, twice a week or fortnightly at 08:00 a.m. Depths sampled were: 5; 25; 50; 75; 100; 150; 200; 300; 400; 500 and 600 cm (down from the water surface). Samples were collected near the reservoir wall, using a WATSON MARLOW 604 S peristaltic pump and a polythene hose attached to two 25 cm in diameter disks, separated by 5 cm from each other. The sampler device is shown in figure 2.6. During each filling phase, the anaerobic pond effluent and or the raw wastewater were also sampled. The WSTR were monitored from the beginning of the filling phase until FC had reduced to 10^2 cfu/100 mL, which is one order of magnitude less than the WHO (1989) bacteriological standard suggested for unrestricted irrigation.

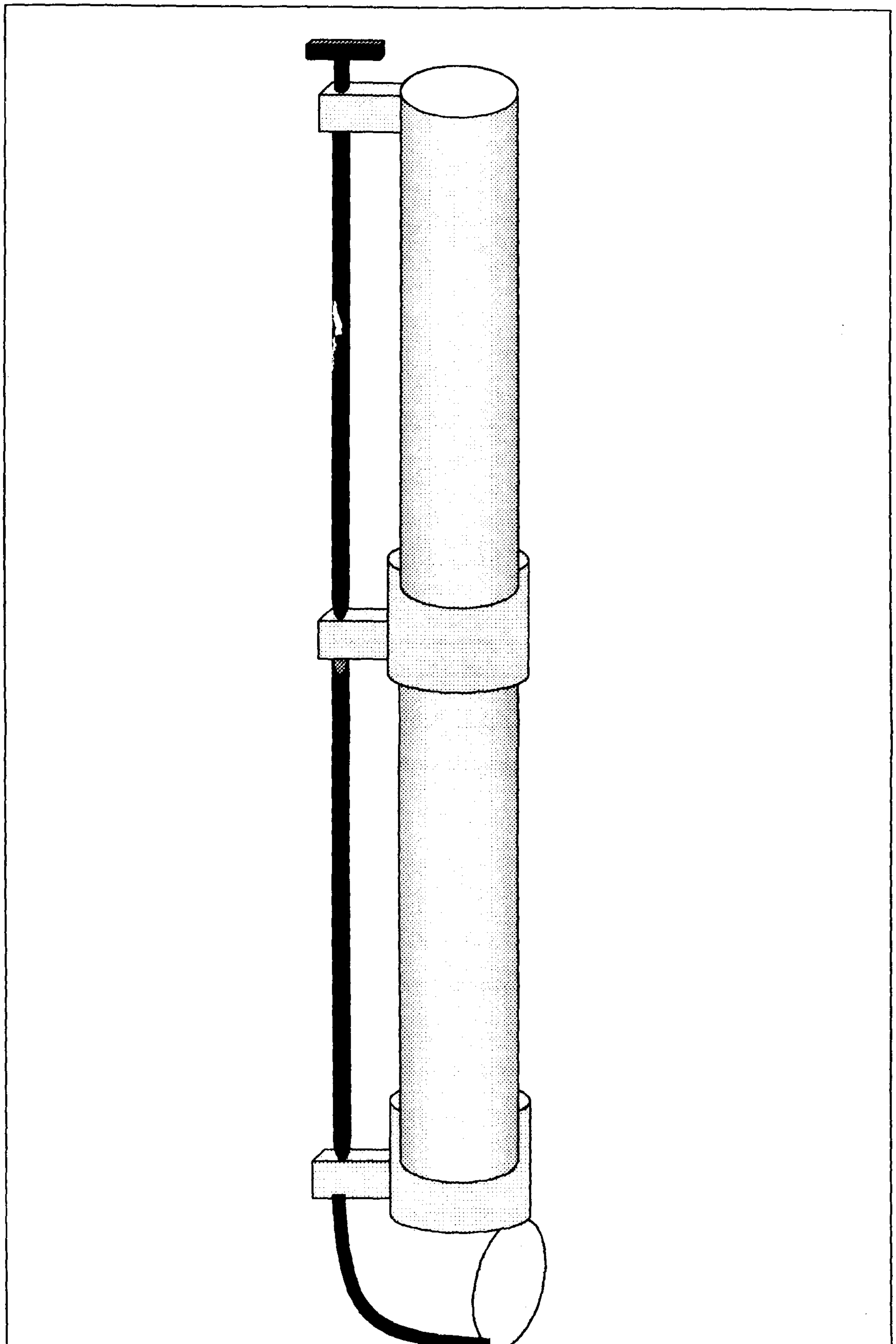


Figure 2.5 - Water column sampler

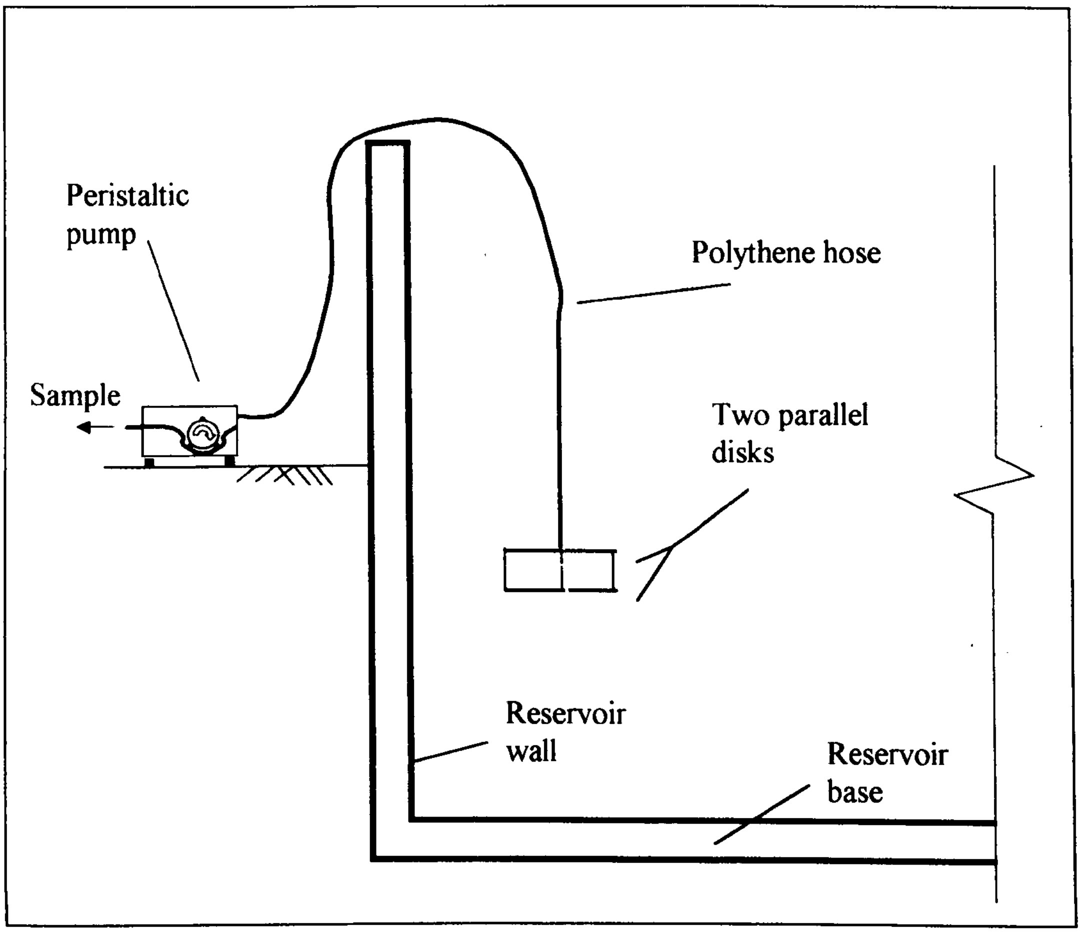


Figure 2.6 – Device used to collect sample from specific level of the WSTR.

2.4. Measurements and analyses

The physicochemical parameters were determined according to the procedures described in APHA *et al.* (1975, 1980 and 1992) unless otherwise stated. The measurements and analyses in systems A and B, system C and system D followed APHA *et al.* 1975, 1980 and 1992, respectively.

• Chlorophyll *a* concentration was initially determined by the technique of extraction with 90% acetone. The samples were then read in a spectrophotometer PYE-UNICAM model SP6-500 at 663 nm and 750 nm. Afterwards, it was noticed that when the main algae present in the sample were Chlorella, the chlorophyll *a* pigment was not extracted well. The technique was changed to extraction with 100% methanol (Jones, 1979). The extracts were measured at 665 and 750 nm in a PHARMACIA LKB NOVASPEC – II spectrophotometer;

• Algal identification was made by microscope examination using either a Wild Heerbrugg (M12) or an Olympus (model FHT 202027) microscope;

• Algal counts were estimated using an Improved Newbauer Haemocytometer chamber (depth 0.1, 1/400mm^2) in the same microscopes used for algal identification;

• BOD_5 was determined according to the standard BOD bottle procedure;

• COD was determined initially through the tritimetric procedure and later through the dichromate closed reflux method;

• Total ammonia was, in the beginning of the study, measured using an Orion model 407 A specific ion meter fitted with an Orion ammonia electrode (model 95-12). Later on, the direct nesslerization method was used on supernatant samples pre-treated with zinc sulphate and sodium hydroxide. The samples were read at 450 nm in a PHARMACIA LKB NOVASPEC – II spectrophotometer using 1 cm cuvette;

• Nitrate: colorimetric method of the chromotrophic acid at 410 nm in a PHARMACIA LKB NOVASPEC – II spectrophotometer;

• Nitrite: colorimetric method of the diazotzation at 543 nm in a PHARMACIA LKB NOVASPEC – II spectrophotometer;

• Total sulphide was measured using the Iodometric method, including pre-treatment by precipitation to remove interfering substances, and afterwards the analyses followed the Methylene blue technique;

• Orthophosphate was determined by the colorimetric ascorbic acid method at 880 nm in a PHARMACIA LKB NOVASPEC – II spectrophotometer;

• FC enumeration was initially done through the membrane filtration method using Millipore filters (type HAWG 04751) and M-FC broth (Difco). Due to the high price of the M-FC broth, tests were made using the medium Lauryl sulphate broth. As the two medium gave comparable results, later on the M-FC broth was replaced by the Lauryl sulphate broth (Ayres and Mara, 1996), with incubation at 44.5 °C for 24h;

• Temperature was determined during sample collection using a mercury-filled thermometer Inconterm;

• Dissolved oxygen was determined electrometricclly with an oxygen sensitive membrane probe (YSI model 5720 A) connected to a YSI model 54 A DO meter;

• pH was measured by the electrometric method using initially a pH meter Radiometer model 29; later on a Pye Unicam model PW 9418 pH meter with a combined pH electrode (Pye Ingold model 401E07) and a temperature compensator probe (Pye unicam PT 100) and subsequentialy a Jenway 3030 pH meter combined with a Russel BNC electrode and Jenway PCT 121 temperature compensation probe.

Chapter 3. System A – Four primary facultative ponds

In this chapter the interrelationships between various parameters relevant to pond performance were studied in four independent primary facultative ponds receiving different organic loadings. The same sewage source was used and during the experiments the ponds were operated at depths of either 1.25 or 2.30 m with surface organic loading varying between 208 to 555 kgBOD$_5$/ha.d. In all the figures shown in this chapter, the value plotted for each pond is the mean of a minimum of 76 samples.

3.1. The effect of surface organic loading on effluent BOD

There was a positive correlation between surface organic loading and BOD$_5$ effluent concentration (figure 3.1), so that as pond loading increased effluent BOD$_5$ increased and thus effluent quality decreased. There was a similar relationship when the ponds were made deeper (2.30m) (figure 3.1). In fact it would seem that at least until the maximum value used of 338 kgBOD$_5$/ha.d for the deep ponds, there was no difference in pond performance between the two depths, because they can be plotted as a continuum (figure 3.1a) which is statistically significant at the level of 5%.

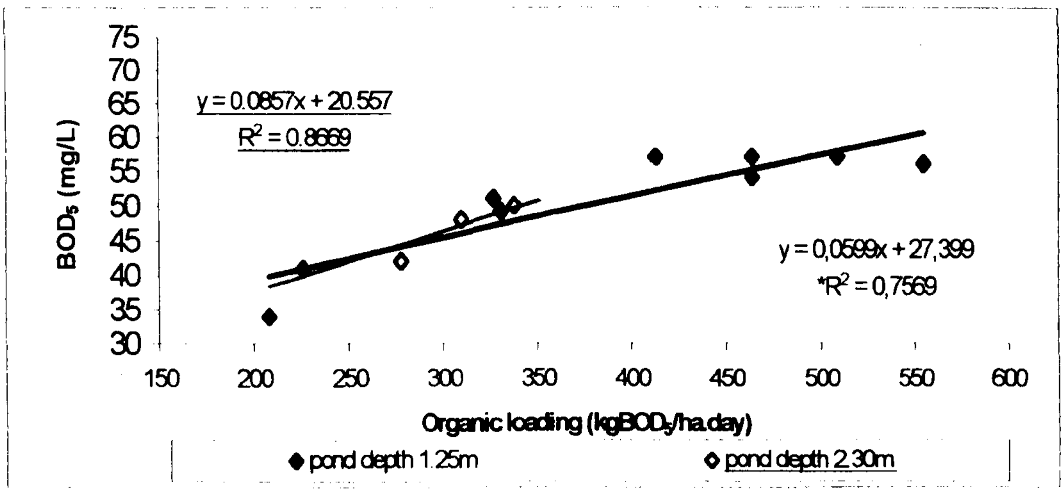


Figure 3.1. Effluent BOD$_5$ plotted against surface organic loading in the primary facultative ponds when they were 1.25m deep and when they became 2.30m deep.

* The regression was significant at the level of 5% in this and all subsequent figures unless otherwise indicated.

N.B. The regression equation for the ponds which depth was 2.30m is underlined in this and all subsequent figures unless otherwise indicated.

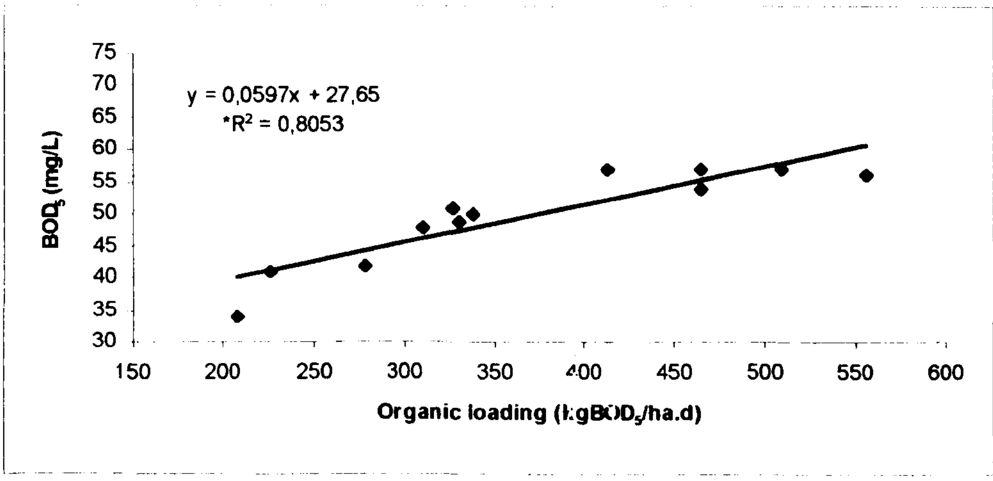


Figure 3.1a. Effluent BOD_5 plotted as a continuum against surface organic loading in the primary facultative ponds.

3.2. The impact of surface organic loading on ammonia and sulphide concentrations

A positive linear regression was found between surface organic loading against total ammonia concentration for both the deep and shallow ponds although the correlation for the deep ponds was not significant at the level of 5%, possibly due to the reduced number of points. This increase in total ammonia with organic loading can be explained by the presence of proteinaceous material being mineralised. In contrast to the results for effluent BOD_5 where depth made no difference the effluent total ammonia concentration was higher in the deeper ponds than in the shallow ones over the same range of organic loading (figure 3.2) and increased with organic loading. This might be explained by the higher pH's found in the shallow ponds compared to the deeper ponds for the same surface organic loadings resulting from algal photosynthetic activity (figure 3.3).

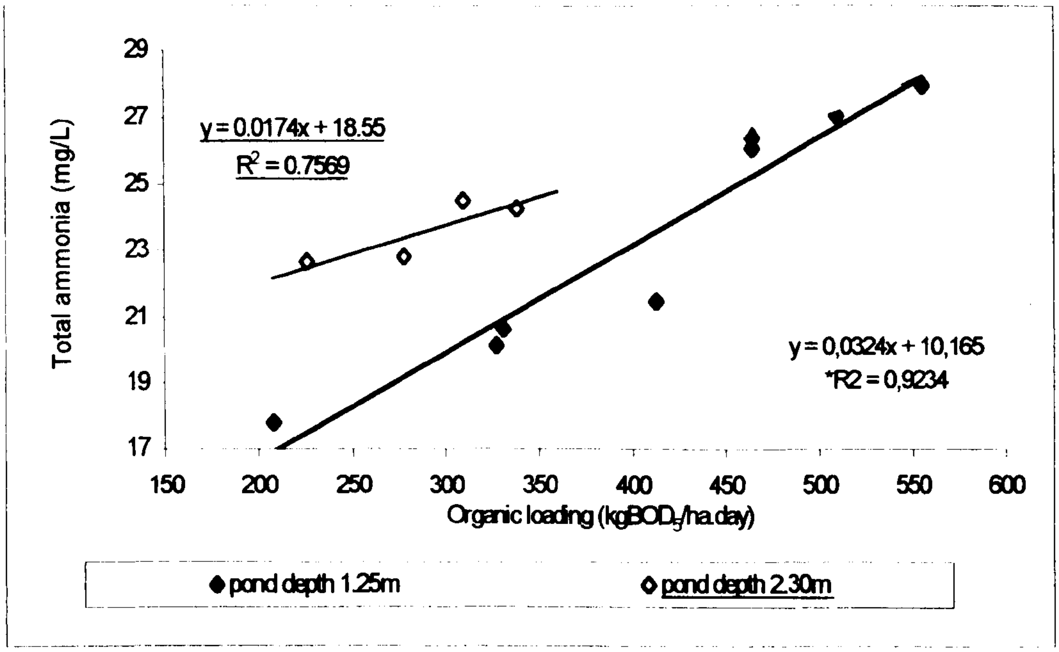


Figure 3.2. Total ammonia plotted against surface organic loading in the primary facultative ponds.

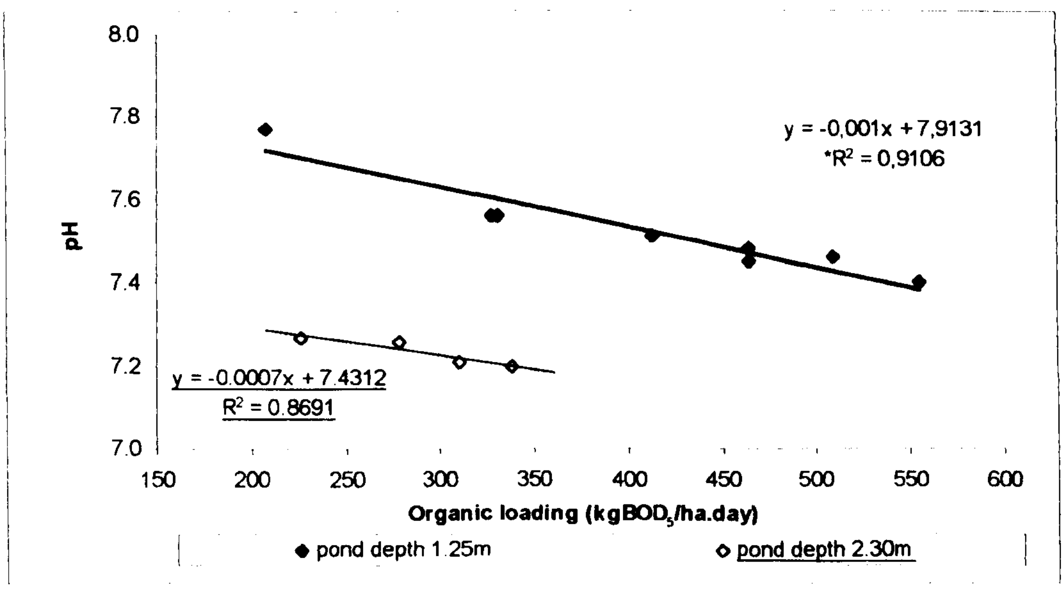


Figure 3.3. pH plotted versus surface organic loading in the primary facultative ponds.

Thus the loss of volatile ammonia (NH_3) was greater from the shallow ponds than the deep ones. This is supported by the data presented in fig 3.4 which showed that NH_3 values were higher in the shallow ponds than in the deep ponds and that NH_3 was highest at lower surface organic loadings which correlated with the high pH at these loadings. NH_3 was calculated using the equation 1 (Emerson et al., 1975):

$$NH_3 = \frac{TotalAmmonia}{1 + 10^{(9.2464 - pH)}} \quad \text{(Equation 1)}$$

As might be predicted total sulphide also increased in the facultative ponds with increased surface organic loading and it was also much higher in the deep ponds than in the shallow ones (fig 3.5). Unfortunately in this set of experiments sulphide was not measured in the shallow ponds at the lower organic loading range but the difference between the two curves was very clear.

Since the sewage entering the ponds was not septic, sulphide concentration was a function of organic loading and anaerobic activity in the ponds. The higher concentration of sulphide in the deep ponds probably reflected larger anaerobic zone in these ponds.

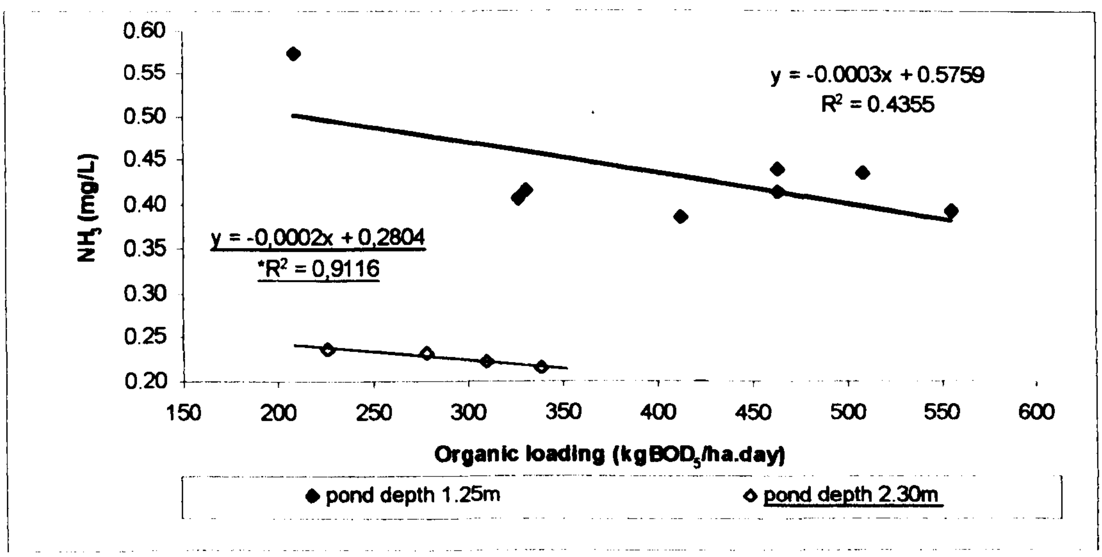


Figure 3.4. NH_3 plotted against surface organic loading in the primary facultative ponds.

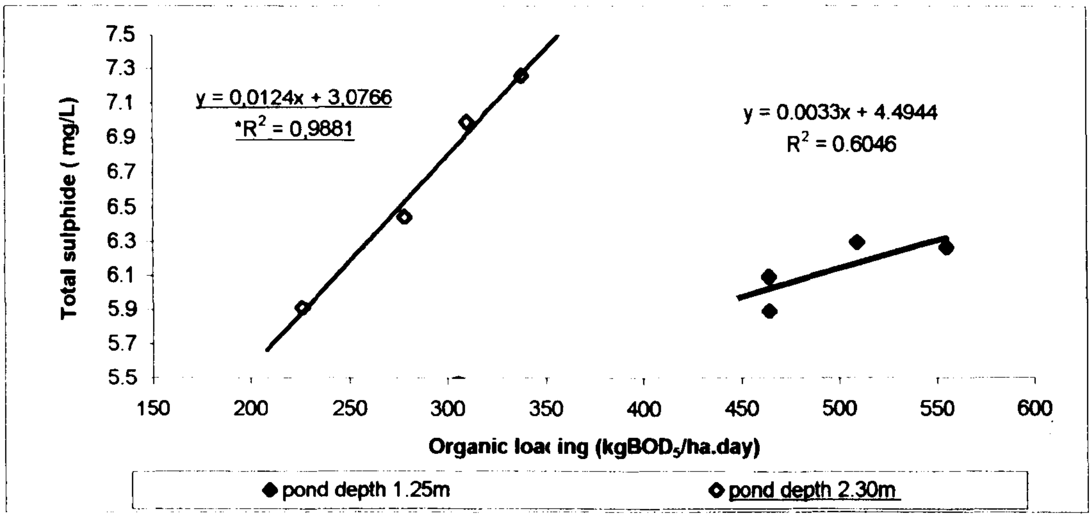


Figure 3.5. Total sulphide plotted against surface organic loading in the primary facultative ponds.

In contrast to NH_3 and by extrapolation of the curves presented in figure 3.6, H_2S was relatively higher in the deeper ponds than in the shallow ones. H_2S was calculated using the equation 2 (Hilton and Oleszkiewieg, 1988):

$$H_2S = \frac{TotalSulphide}{\left[1 + 1.02x10^{(pH-7)}\right]} \quad \text{(Equation 2)}$$

The increased H_2S values at high organic loadings were again a function of total sulphide concentration in combination with pH because in contrast to NH_3 the percentage of total sulphide as the volatile form (H_2S) increases with a decrease in pH.

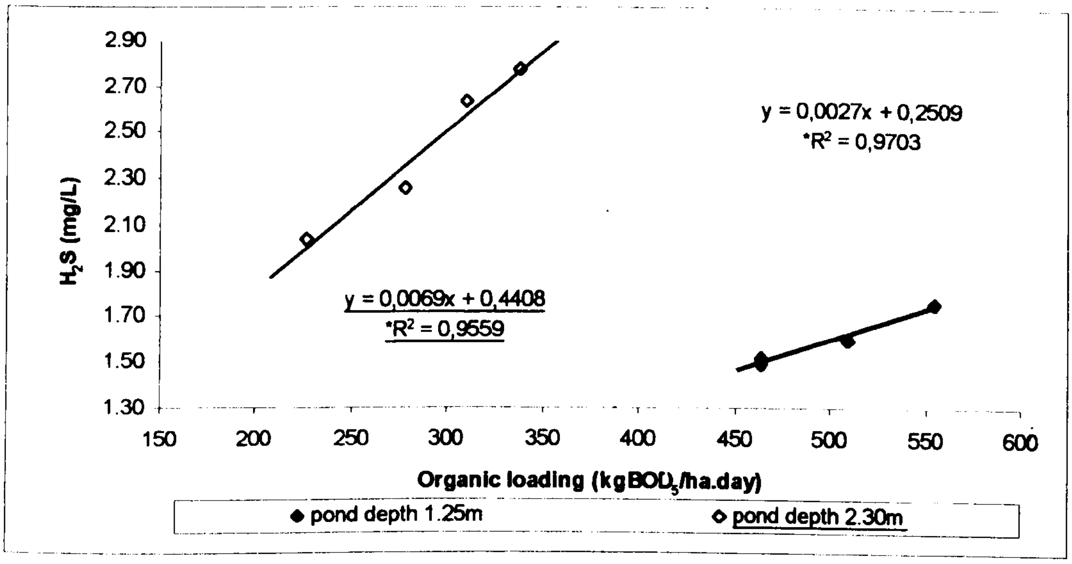


Figure 3.6. H_2S against surface organic loading in the primary facultative ponds.

3.3. The relationship between chlorophyll *a* and organic loading

Algae stratify in the water column of facultative ponds during the day in response to light intensity and, thus effluent samples taken at different times might have different chlorophyll *a* concentrations depending on the position of the algal band in the water column (Konig, 1984). Konig (1984) also showed in previous studies that samples collected at the sampling time of 08:00h in north-east Brazil had chlorophyll *a* values close to a daily computed mean derived from integrating samples taken throughout the day and night. Thus 08:00h samples could be considered relative to one another on a day to day basis.

This relationship of chlorophyll *a* to the depth of the pond was better understood by looking at the data for column samples (see details for column sampling in chapter 2.0) in which the average chlorophyll *a* concentration for the total pond depth was expressed per m^2 of pond surface as this eliminated the dilution effect of depth on chlorophyll *a* concentration. A negative regression was obtained for chlorophyll *a* per m^2 versus surface organic loading in the shallow and deep primary facultative ponds. The chlorophyll *a* expressed on an area basis was much lower in the deeper ponds than in the shallow ones for comparable surface organic loadings (figure 3.7).

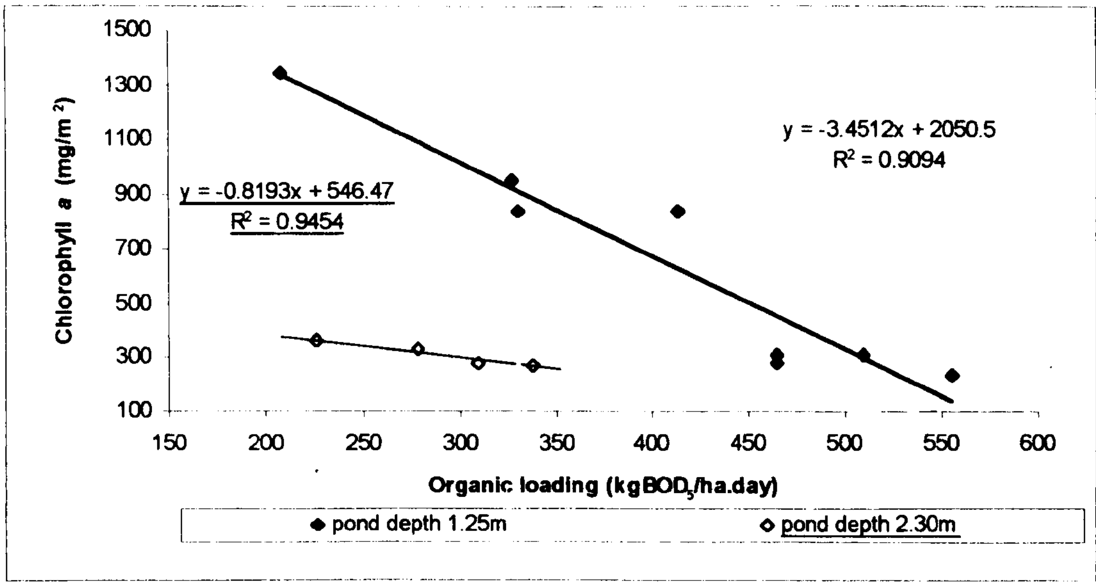


Figure 3.7. Chlorophyll *a* (mg/m^2) plotted against surface organic loading in the primary facultative ponds

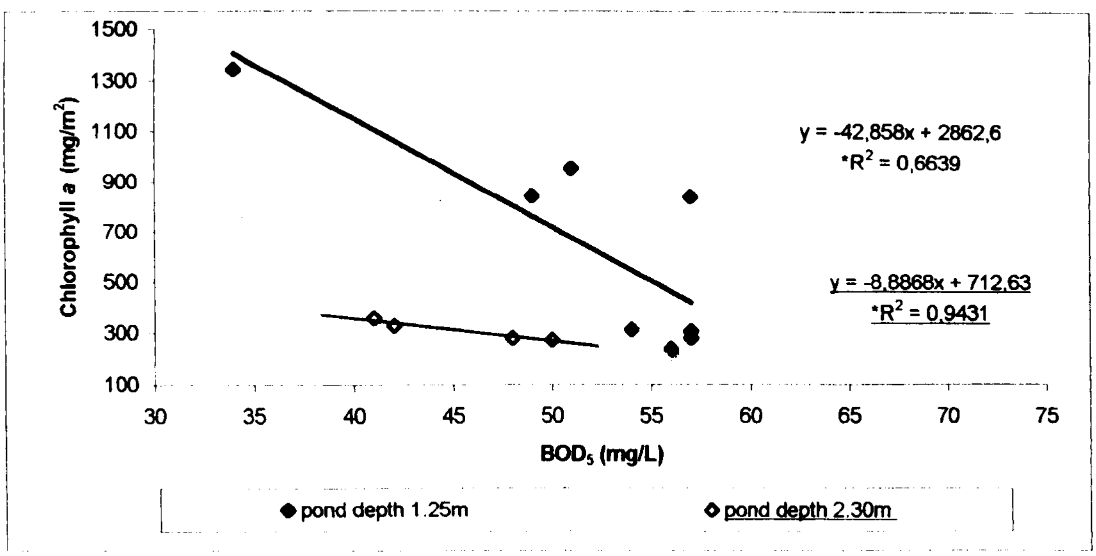


Figure 3.8. Chlorophyll *a* (mg/m^2) versus BOD_5 effluent in the primary facultative ponds

The relationship between pond algal biomass and effluent BOD_5 was a negative one but this was probably an indirect relationship as it had been shown that chlorophyll *a* (figure 3.8) decreased with increasing organic surface loading and effluent BOD_5 increased with increasing organic loading on the ponds.

When chlorophyll *a* concentration was compared with BOD_5 in the same effluent samples, a negative correlation was found and the relationship could be seen in figure 3.9 for both shallow and deep ponds. This figure demonstrated that the BOD_5 contribution by the algae to the total effluent BOD_5 increased as the effluent BOD_5 decreased. Put another way algae contributed more as a percentage of the effluent BOD_5 as the organic loading on the facultative ponds decreased. Therefore caution must be exercised when trying to determine the BOD_5 contribution by algae in different pond systems since the algal BOD_5 contribution is not constant.

The relationship between chlorophyll *a* and BOD_5 contribution might be a constant in pure algal cultures (König, 1984) but it was clear from this study that it may be difficult to separate the algal contribution to BOD_5 in pond samples as other non-algal solids will be retained on a filter. So with algal BOD_5 contributions one must accept the values obtained from pure algae cultures studies and even this can lead to inaccuracies because the relationship between BOD_5 and Chlorophyll *a* concentration will vary in the cells of the same algal species depending on the nutrients and light regimes under which they are growing.

In contrast to the linear relationship obtained between chlorophyll *a* and effluent BOD_5 no clear relationship exists between chlorophyll *a* (effluent or mg/m^2) and effluent COD in the primary facultative ponds in this study (figures 3.10 and 3.11).

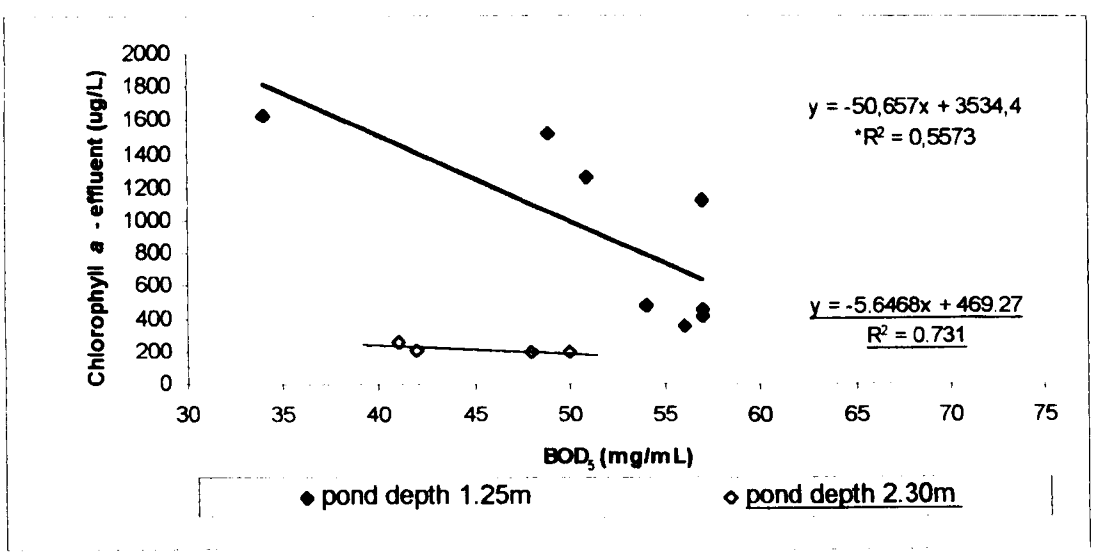


Figure 3.9. Chlorophyll *a* (effluent) versus BOD_5 effluent in the primary facultative ponds

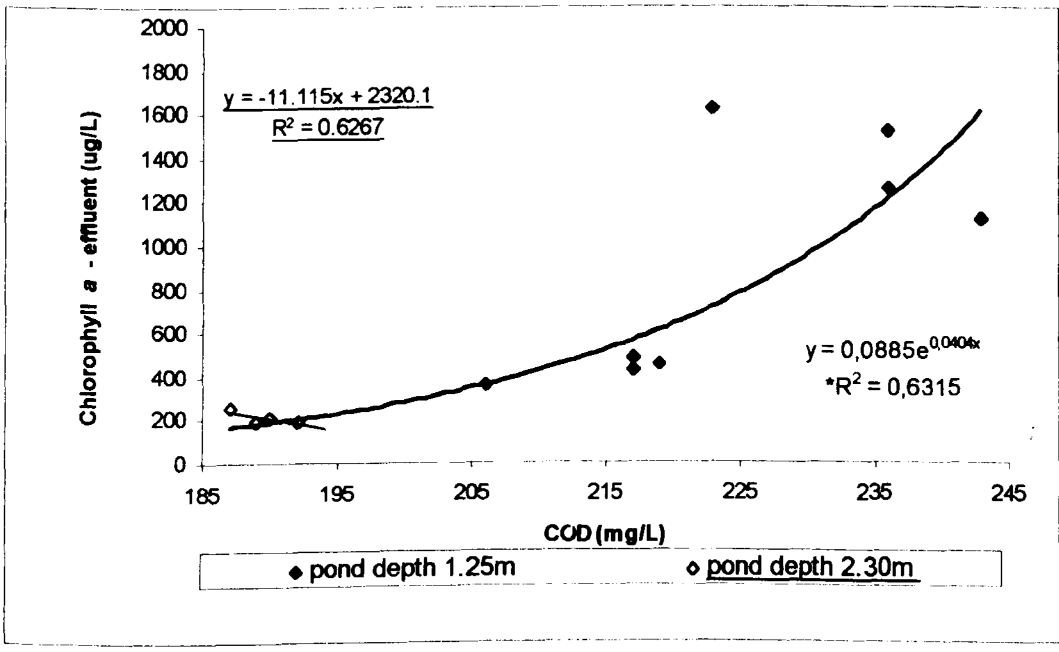


Figure 3.10. Chlorophyll *a* (effluent) plotted against COD in the primary facultative ponds.

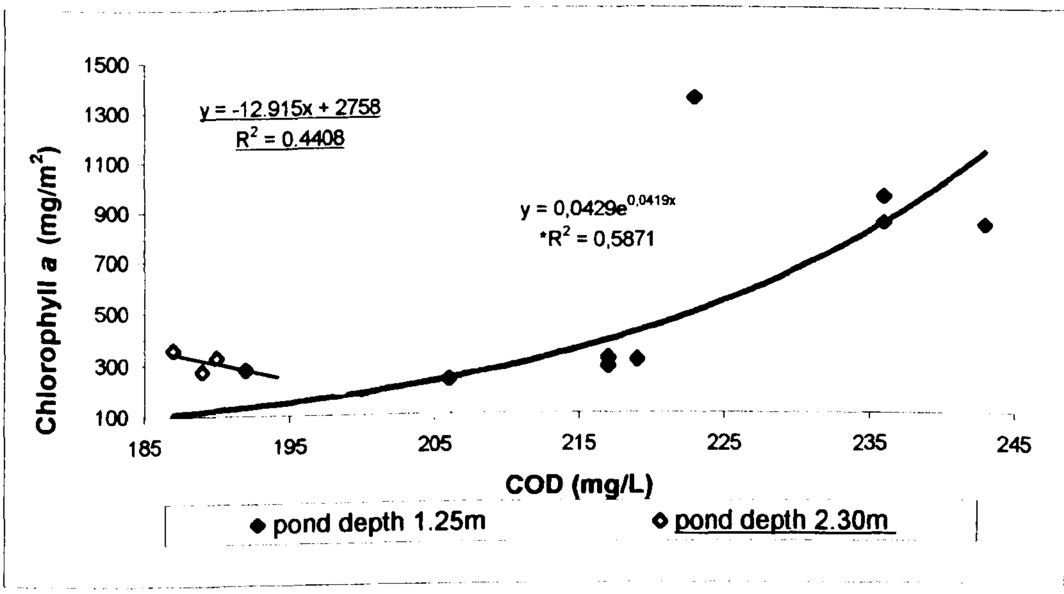


Figure 3.11. Chlorophyll *a* (mg/m^2) plotted against COD in the primary facultative ponds

3.4. The impact of ammonia and sulphide on chlorophyll *a* concentration

When chlorophyll *a* expressed as mg chlorophyll *a* per m^2 of pond surface was plotted against total ammonia (figures 3.12) a negative regression was obtained which largely mimics the curves obtained for surface organic loading versus chlorophyll *a* data. This might suggest that since total ammonia increases with increasing organic loading (figure 3.2) the apparent organic loading impact on algae may be due to the increase in total ammonia and thus only indirectly to BOD_5 loading.

However, this becomes less clear when the relationship between total ammonia and NH_3 is considered in the facultative ponds at different loadings (figure 3.13). NH_3 is considered to be more toxic to algae but there was no increase in NH_3 with increase in total ammonia. In fact, the contrary was found with the concentration of NH_3 increasing as total ammonia decreased. This relationship was probably because lower loaded facultative ponds have higher pHs (see figure 3.3). This was also supported by the previous findings that NH_3 decreased as organic loading increased (see figure 3.4).

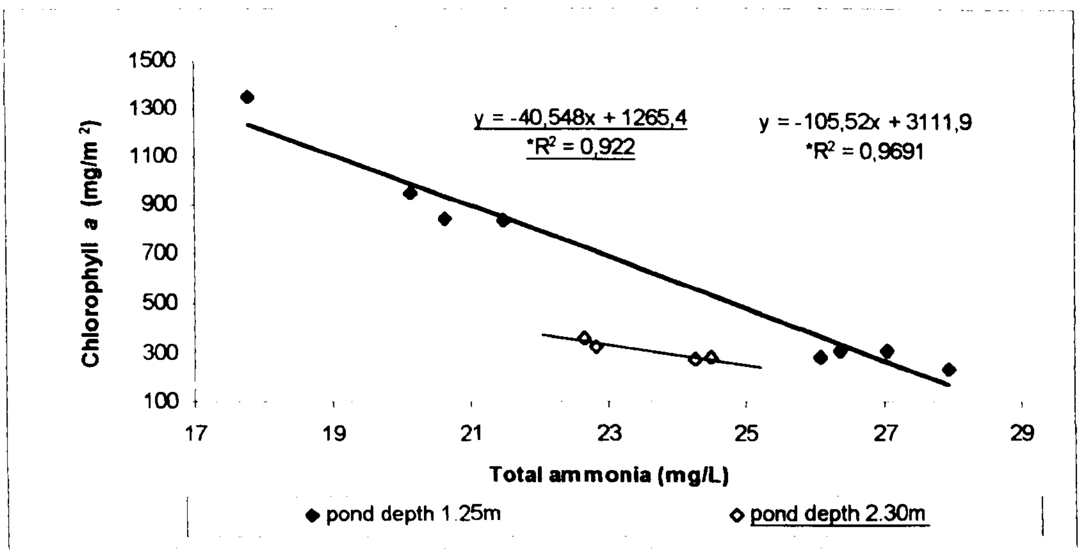


Figure 3.12. Chlorophyll *a* (mg/m^2) against total ammonia in the primary facultative ponds.

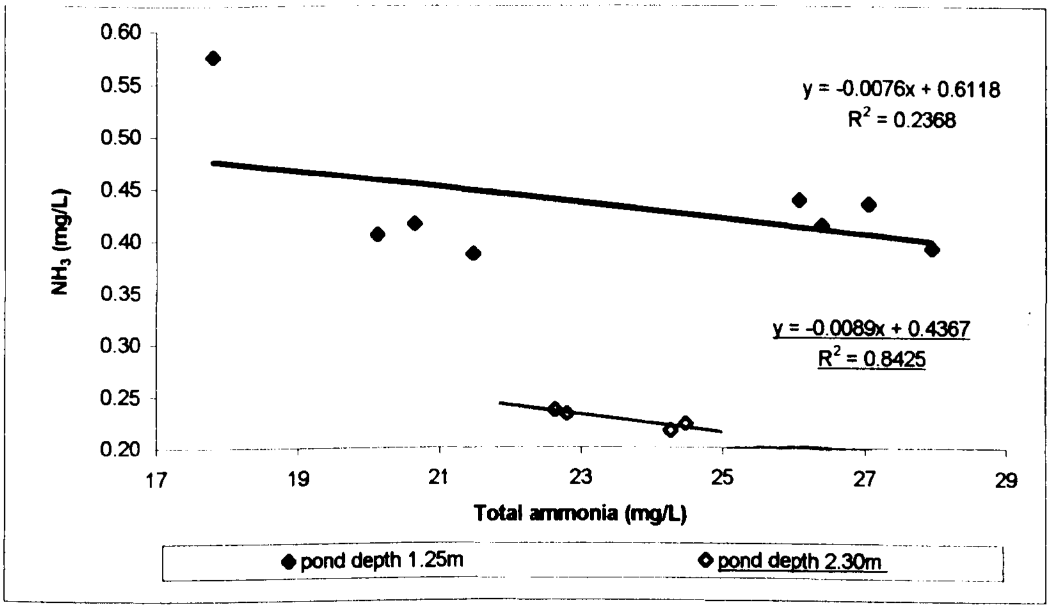


Figure 3.13. NH_3 plotted against total ammonia in the primary facultative ponds.

In the shallow facultative ponds there was a poor positive linear regression between chlorophyll *a* per m^2 (figure 3.14) and NH_3, although the figure shows that chlorophyll *a* appears to increase with increased free NH_3. This would suggest that the concentration of NH_3 (the toxic form of ammonia) never reached levels high enough to inhibit algal growth and thus algal biomass was not being controlled by ammonia concentrations in these facultative ponds whether shallow or deep.

Total sulphide and H_2S concentrations were seen to increase in both shallow and deep facultative ponds with increased organic loading (see figures 3.5 and 3.6 respectively). Thus the decrease in chlorophyll *a* concentration observed with increased total sulphide concentrations (fig 3.15), might be expected, although the inhibitory effect was more pronounced in the shallow facultative ponds than in the deeper ones.

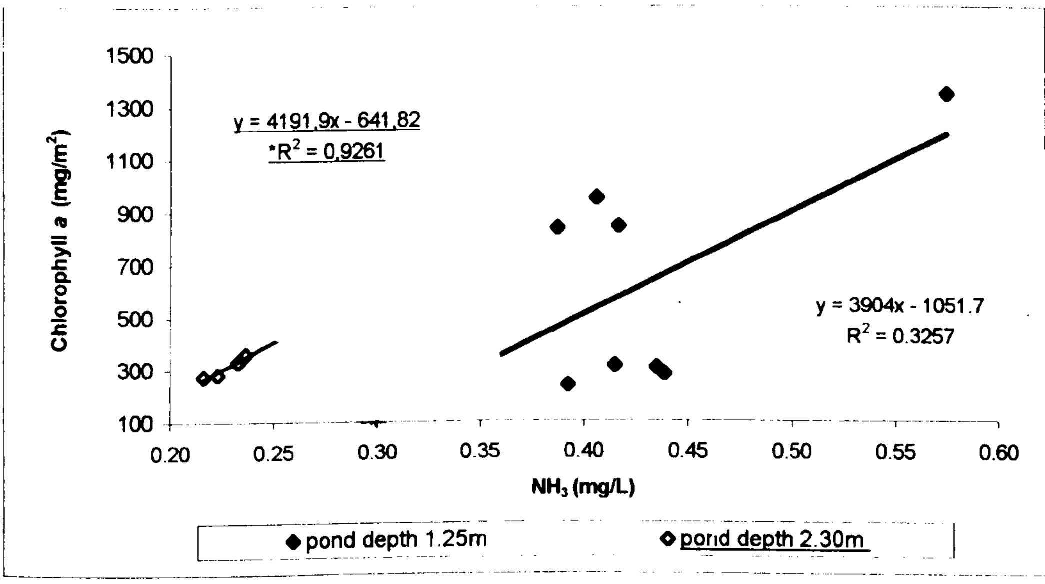


Figure 3.14. Chlorophyll *a* (mg/m^2) against NH_3 in the primary facultative ponds

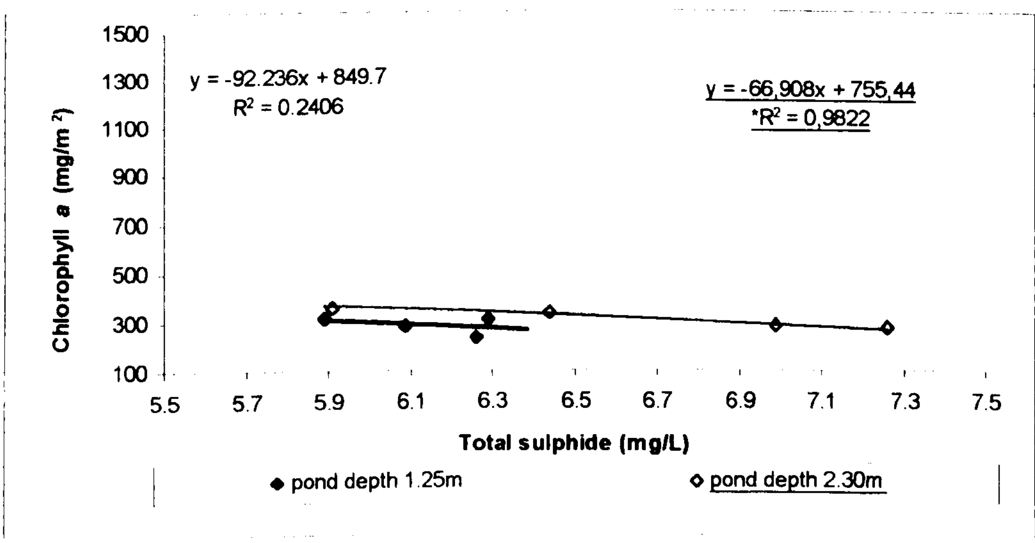


Figure 3.15. Chlorophyll *a* (mg/m^2) plotted against total sulphide in the primary facultative ponds

The data presented in figure 3.16 showed that chlorophyll *a* concentration also decreased as H_2S (the more toxic form of sulphide to algal photosynthesis) increased. The weakness in the data was the lack of results for total sulphide at the lower organic loadings for the shallow ponds (as referred to previously in section 3.2) as the sulphide values on the graph were already high and the algal population expressed as chlorophyll *a* had already decreased. Nevertheless data for total sulphide and H_2S versus chlorophyll *a* do show negative correlation in contrast to the ammonia data. Increased total sulphide concentration also gave an increase in H_2S (figure 3.17) and H_2S also increased in response to increase in organic loading (see figure 3.6). These data would suggest that sulphide might be the parameter controlling algal biomass in facultative ponds.

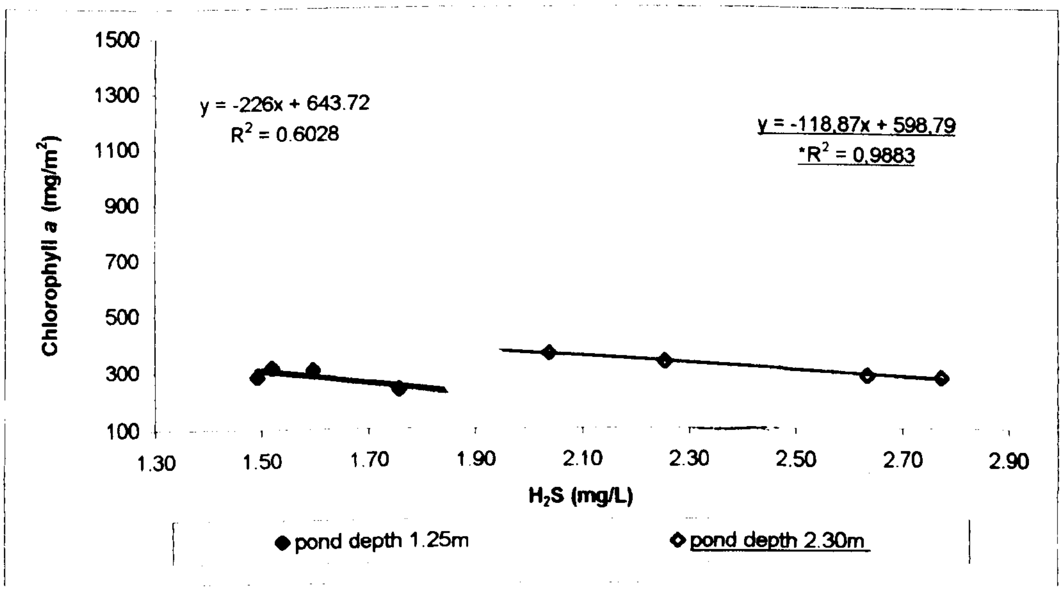


Figure 3.16. Chlorophyll *a* (mg/m^2) plotted against H_2S in the primary facultative ponds

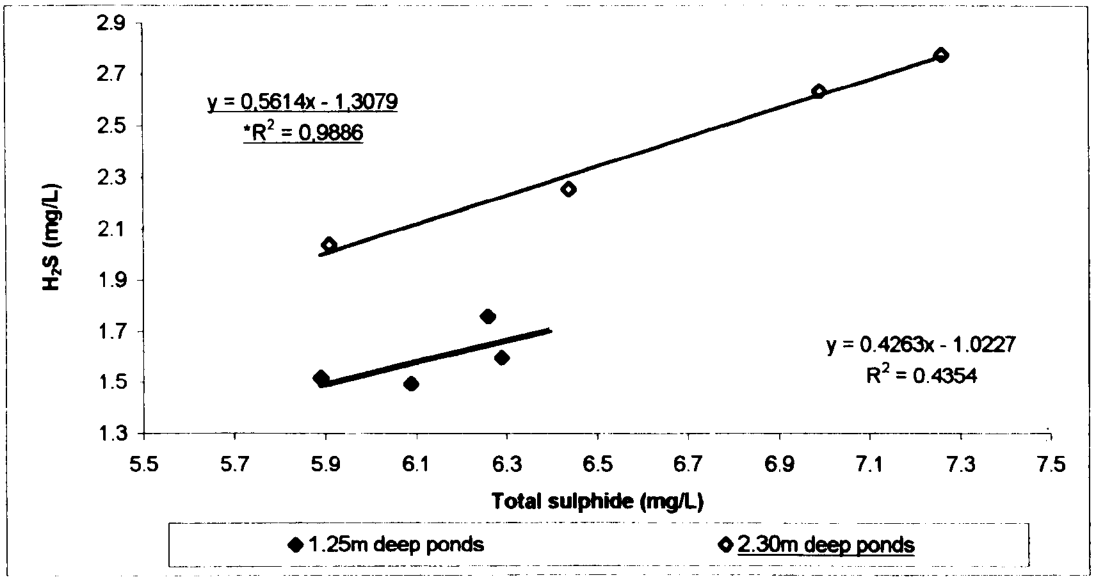


Figure 3.17. H_2S plotted against total sulphide in the primary facultative ponds

3.5. Faecal Coliform removal in the primary facultative ponds

The first order kinetics for faecal coliform removal (k_b) for the primary facultative ponds was calculated considering that these ponds were working closer to a completely mixed reactor than to a plug flow one. Using the Marais (1974) equation: $k_b = 2.6(1.19)^{T-20}$, it was possible to compare the theoretical values of k_b obtained from this equation using only the mean temperature value, since Marais found that k_b is highly temperature dependent; with k_b values obtained from real FC results. The Marais equation was mainly derived from studies on maturation ponds of approximately 1m deep, but in current design protocols this equation is extended to anaerobic and facultative ponds. The theoretical value of k_b, based on temperature using the Marais equation commonly used to design new WSP systems could be compared with k_b values derived from the actual FC results obtained from the primary facultative ponds using the Marais equation:

$$N_e = \frac{N_i}{1 + kb\ \theta} \quad \text{(Equation 3)}$$

where: N_e = number of FC per 100ml of effluent

N_i = number of FC per 100ml of influent

k_b = first order rate constant for FC removal, d^{-1}

θ = retention time, d

Which can be rearranged for k_b thus:

$$k_b = \frac{Ni - Ne}{Ne\theta} \quad \text{(Equation 4)}$$

Figure 3.18 shows the relationship between k_b and surface organic loading in shallow and deep facultative ponds. There seems to be a negative relation between k_b and the surface organic loading. In the deeper ponds, the k_b values did not vary very much but they seemed to increase with the loading, although a weak correlation coefficient was obtained (not significant at the level of 5%) when a regression curve was inserted. This graph also showed that the values of k_b were lower in the deeper ponds.

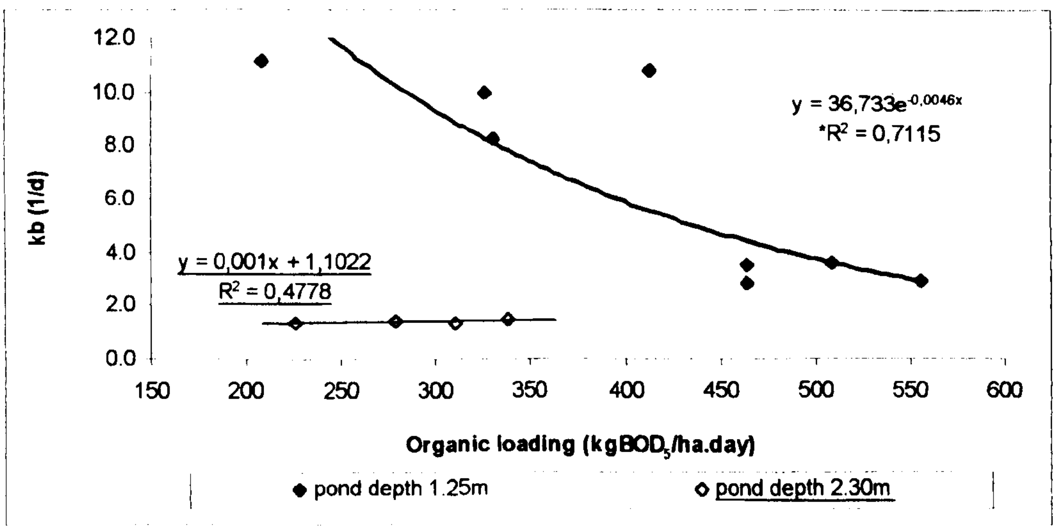


Figure 3.18. Faecal coliform removal (k_b) plotted against surface organic loading in the primary facultative ponds.

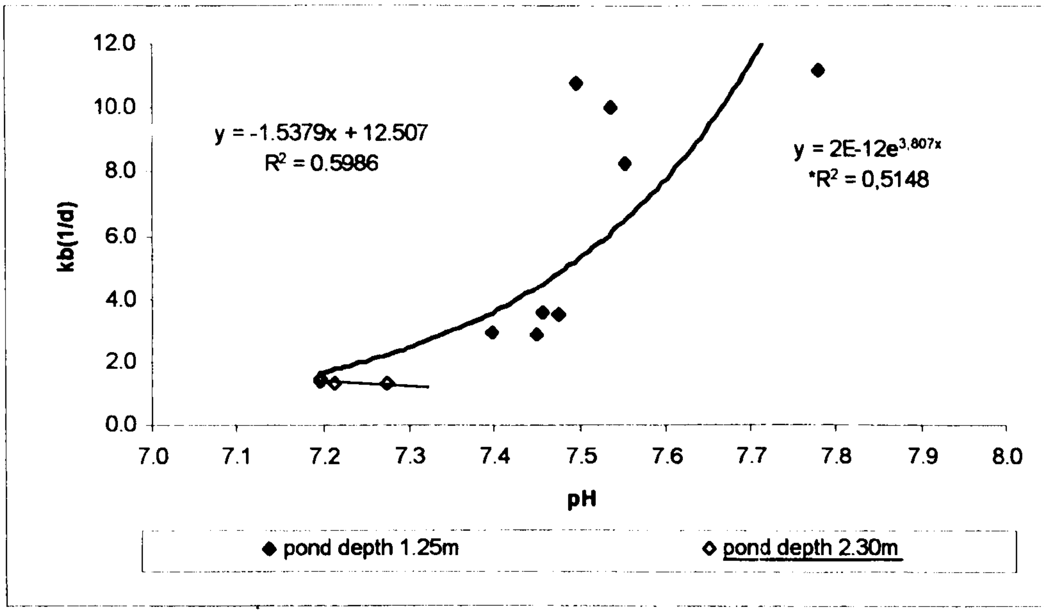


Figure 3.19. Faecal coliform removal (k_b) plotted against pH in the primary facultative ponds.

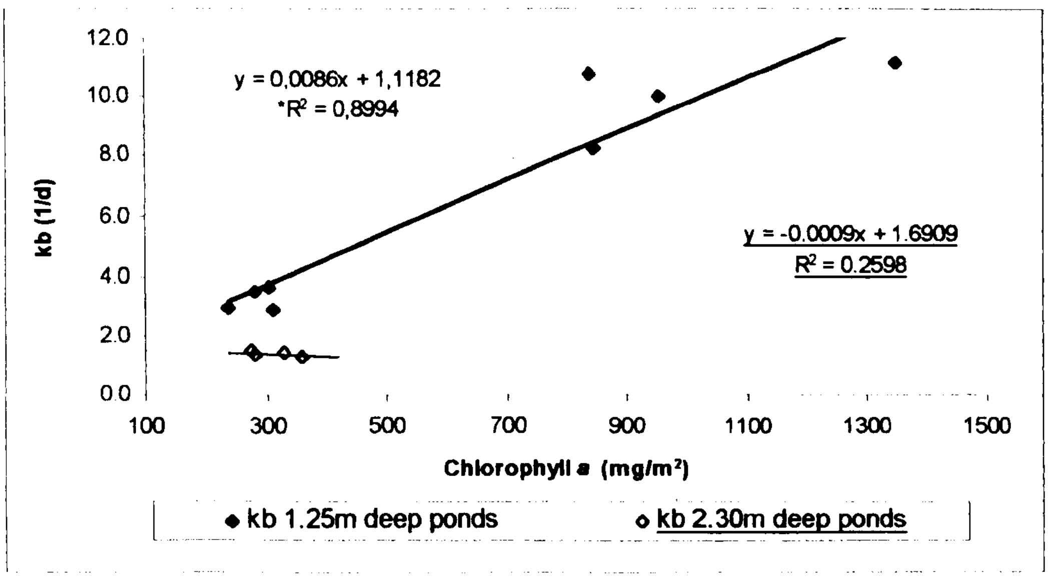


Figure 3.20. Faecal coliform removal (k_b) plotted against chlorophyll *a* in the primary facultative ponds.

When the relation between k_b and Chlorophyll *a* and pH was considered, it was noticed that in the shallow ponds k_b tended to increase with the increase in chlorophyll *a* and pH concentrations (Figures 3.19 and 3.20). This was not a surprise since it is known that the algae control other parameters like pH and dissolved oxygen through their photosynthetic activity. In the deeper ponds, k_b seemed unaffected by the algae as it did not change very much with variations in chlorophyll *a* and pH values. This might be due to the low concentrations of chlorophyll *a*, which did not change very much in these ponds.

When actual k_b values were compared with the theoretical one, it could be seen that in the shallow ponds when the surface organic loading ranged between 208 - 413 kgBOD_5/ha.day the actual k_b values were higher than the value 6.20 proposed by Marais for a temperature of 25°C. However when the surface organic loading range increased to 465-555 kgBOD_5/ha.day, all the k_b values were approximately 50 % lower than the value predicted by Marais. The k_b values found in the deeper ponds were all much lower than 6.20 ranging from 1.3 to 1.5.

Since in these primary facultative ponds k_b varied with organic loading and depth; temperature was not the only parameter affecting the k_b value.

3.6. Assessment of the efficiency of the primary facultative ponds

- Depth has no apparent impact on BOD removal in primary facultative ponds as deep (2.30m) and shallow (1.25m) gave comparable BOD_5 effluent quality for the same surface organic loading. Thus the increased retention time in the deeper ponds apparently does not enhance BOD_5 removal. Therefore the additional cost of excavation to construct deep facultative ponds would have no justification. However, where the profile of land would mean lower construction costs if the facultative ponds were deeper, this would apparently have no deleterious effect on performance.
- In systems comprising single ponds effluent quality will be directly proportional to surface organic loading. At 25°C, as in the study, a single lagoon of 1.25m depth and a surface organic loading of 225 kgBOD_5/ha.d gave an effluent quality of approximately 40 mg/L BOD_5 (total) which represents a removal efficiency of in excess of 80% which is at the very top end of BOD_5 removal efficiency recorded for primary facultative ponds.

• The linear regression line for these data would suggest that loadings as high as 500 $kgBOD_5/ha.d$ can be applied to primary facultative ponds at the temperature of 25 °C without a loss of efficiency. Current design procedures (Mara and Pearson, 1998) would recommend a maximum loading on primary facultative ponds of only 350 $kgBOD_5/ha.d$.

• In terms of microbiological quality of the effluent, deep facultative ponds (2.30m) exhibited much lower k_b than shallow ones (1.25m). Thus pathogen die-off is less in deep ponds than shallow ones even taking account of the increased hydraulic retention time given the same organic loading.

• At lower surface organic loading FC die-off (k_b) was much greater at lower loadings compared to high ones. However, this is less significant in terms of engineering design as primary facultative ponds are basically designed at the head of a series with the main role of BOD removal and FC removal in the main prerogative of the subsequent maturation ponds of the series.

3.7. Conclusions

• The increase in pond surface organic loading led to a decrease in BOD_5 effluent quality;

• Total ammonia and total sulphide concentrations were higher in the deeper ponds than in the shallow ones within the same range of surface organic loading, and they also increased with the increase in surface organic loading;

• H_2S increased with increasing surface organic loading but the opposite happened with NH_3;

• Chlorophyll *a* concentration was lower in the deeper ponds than in the shallow ones;

• Algae contributed more to the effluent BOD_5 as the surface organic loading on the ponds decreased;

• Sulphide seemed to be controlling algal biomass in these facultative ponds;

• No clear relationship could be seen between chlorophyll *a* (effluent or mg/m^2 of pond surface) and COD;

• FC k_b was inversely related to pond depth and organic loading.

Chapter 4. SYSTEM B – FIVE POND SERIES

In this part of the study, the interactions between various physico-chemical parameters in series of five waste stabilization ponds and their impact on process microbiology and thus effluent quality are considered.

The pond series comprised an anaerobic pond followed by a facultative pond and then three maturation ponds (see section 2.0 - Material and Methods for more details). The experiments were conducted sequentially using two different pond depths (1.00m and 2.20m). Since this study concentrated on the significance of algae (as chlorophyll *a*) and associated parameters in the treatment process, data for the raw sewage and the anaerobic pretreatment pond were not included (except when necessary). As the treatment process was a continuous one through the pond series, the data for various parameters have been treated as a continuum through the series for each depth used, unless otherwise stated.

Altogether, five experiments were conducted. Three experiments were conducted at three different organic loadings when the facultative and maturation ponds were 1.00m deep and subsequently two experiments at two different organic loadings when the depth of the ponds had been increased to 2.20m. The figures presented have combined the results of the different experiments for each depth. The values plotted for each pond are the mean of a minimum of 48 and 47 samples when the pond series were 1.00m deep and 2.20m deep, respectively.

In the previous chapter (chapter 3.0) the results were discussed for individually loaded primary facultative ponds. In this chapter, the data were analyzed for the performance of individual ponds in relation to one another in the series and for the performance of the whole series as a single treatment unit.

4.1. The effect of organic loading on effluent BOD_5 and COD

When effluent BOD_5 was plotted against surface organic loading (figure 4.1) a positive linear relationship (significant at the level of 5%) was obtained for both the shallow and deep pond systems, showing that the increase in surface organic loading, between 30 – 378 $kgBOD_5/ha.day$, led to a linear decrease in effluent quality in terms of organic matter. This pattern was similar to those found in the primary facultative ponds.

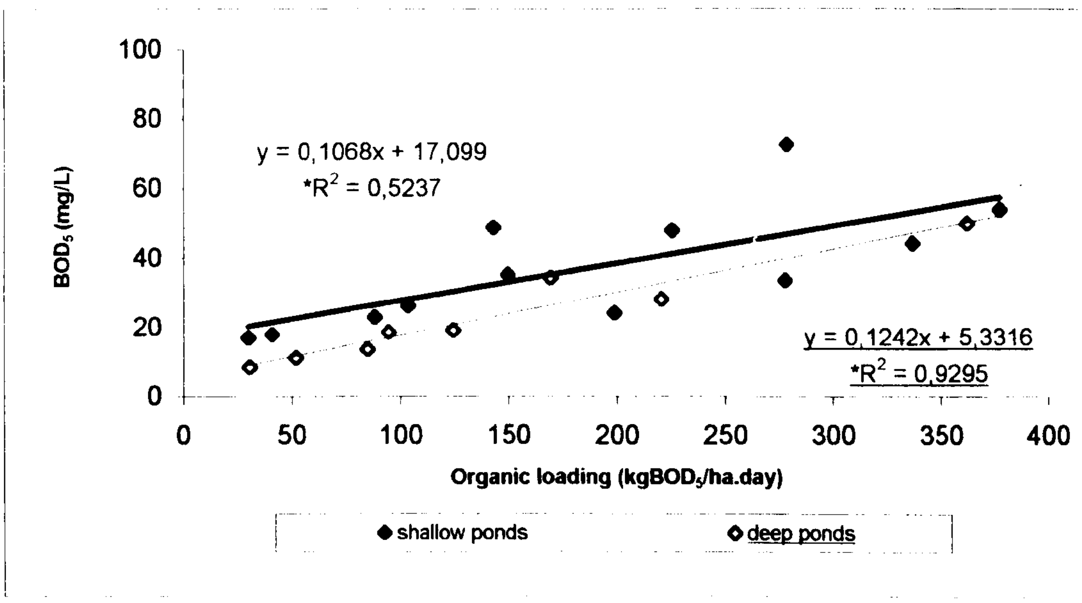


Figure 4.1. Effluent BOD_5 plotted against surface organic loading in the shallow (1.00m) and deep (2.20m) pond series.

* The regression is significant at the level of 5%
** The regression equation for the deeper ponds is underlined

In these experiments a good linear relationship was also obtained in the deeper ponds between COD and organic loading (figure 4.2) supporting the BOD_5 data in figure 4.1. However, this was not the case in the shallow system where the relationship between COD and surface organic loading was not significant.

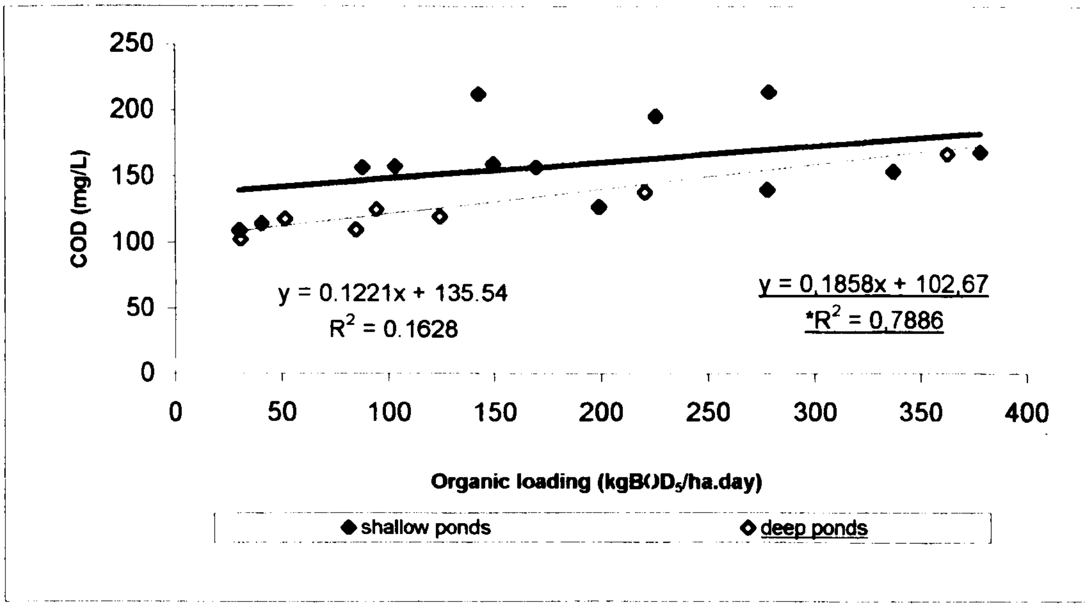


Figure 4.2. COD against the surface organic loading in the shallow (1.00m) and deep (2.20m) pond series.

4.2. BOD_5 removal in the series of ponds

According to figure 4.3, where the values of BOD_5 of the series of ponds (including the anaerobic pond) were plotted for two different depths against mean hydraulic retention time, it was noticed that to reach the effluent quality (BOD_5) requirements for wastewater discharge into rivers (25mg/L) imposed by the European community (CEC, 1991) a mean HRT of 19.5 days was necessary for the shallow series of ponds and 19.8 days for the deeper one. In a series of ponds most of the BOD removal occurs in the anaerobic and facultative ponds at the head of the series. The design of these ponds and the primary maturation pond are determined by BOD_5 loading (Mara and Pearson, 1998) and the deeper pond system (2.20m depth) gave a land saving of at least 32% over the land area requirements of the shallow system. Thus, in terms of organic matter removal the deep series of ponds would normally be more cost effective than the shallow ones, since they require less land area for the same treatment in the shallow series of ponds.

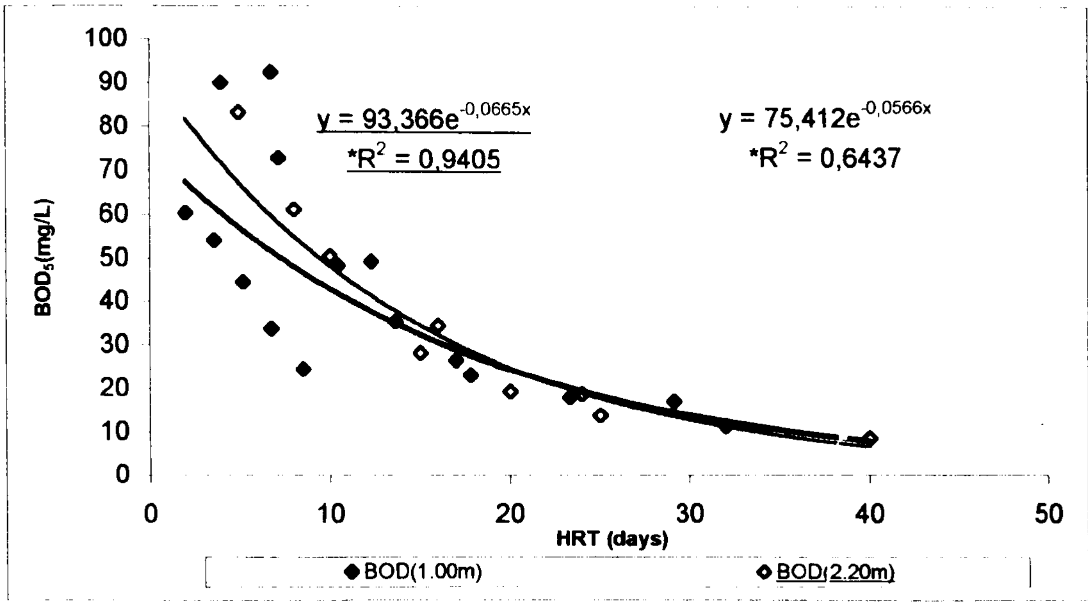


Figure 4.3. Effluent BOD_5 plotted against HRT in the shallow (1.00m) and deep (2.20m) pond series.

4.3. Ammonia, sulphide and pH in relation to surface organic loading.

As in the primary facultative ponds, there was a positive correlation between total ammonia and surface organic loading for both the deep and shallow ponds (figure 4.4).

Total ammonia concentration was also higher in the deep pond series compared to the shallow ones, mainly at the lower surface organic loadings, however the difference between the two curves was not pronounced, especially at higher surface organic loadings where they were very similar. In contrast, the free ammonia (NH_3) concentrations (calculated using equation 1 presented at section 3.2) were higher at the lower surface organic loadings (figure 4.5) due to the higher pH (figure 4.6). However, there was no clear difference in NH_3 concentration between the shallow and deep ponds.

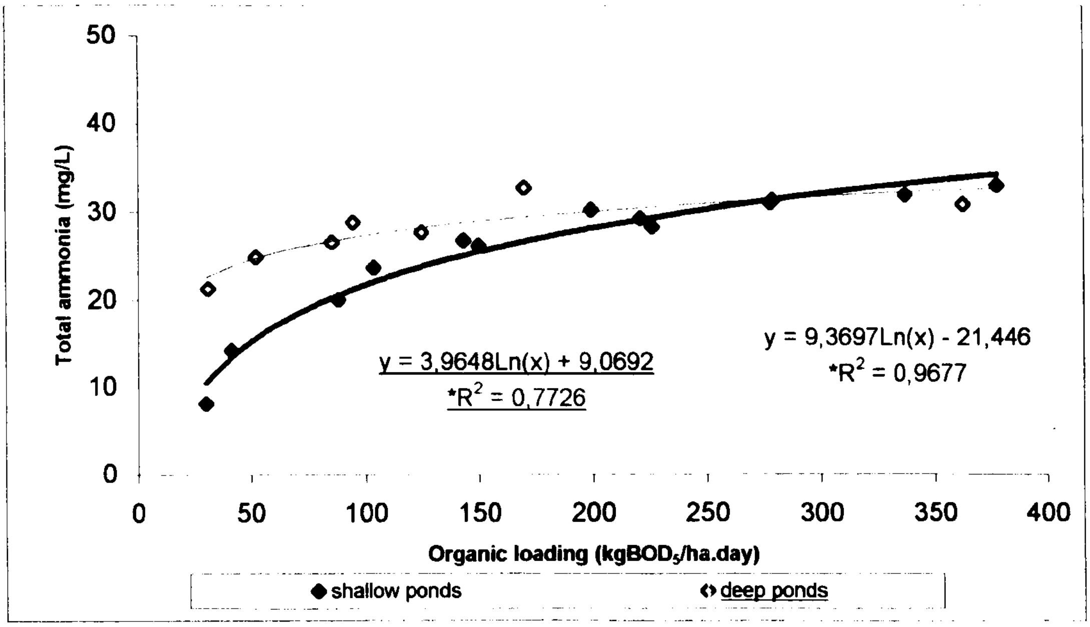


Figure 4.4.Total ammonia versus organic loading in the shallow (1.00m) and deep (2.20m) pond series.

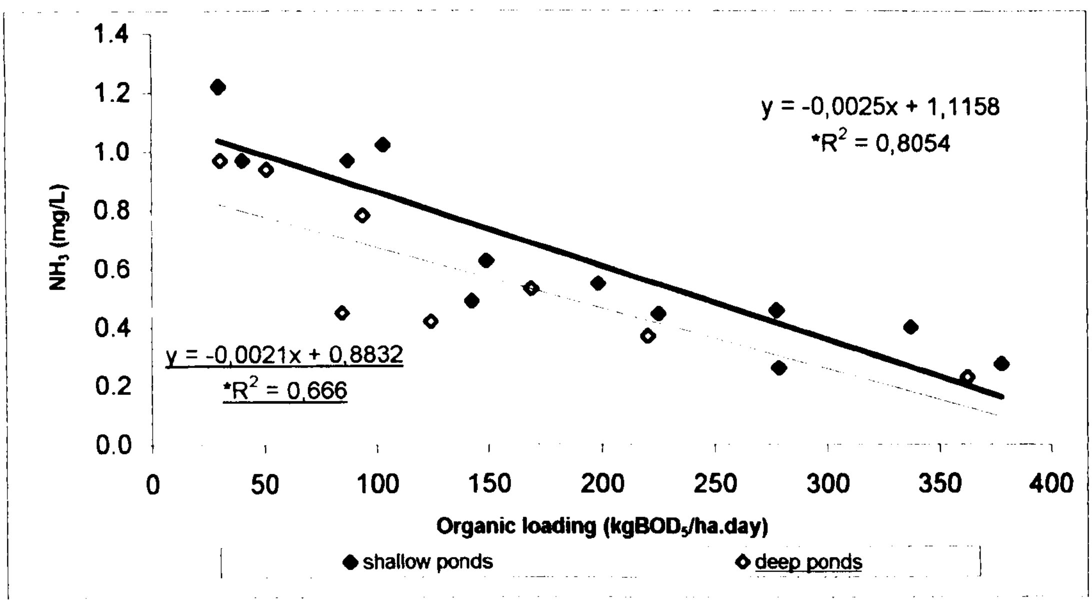


Figure 4.5. NH_3 concentration plotted against surface organic loading in the shallow (1.00m) and deep (2.20m) pond series.

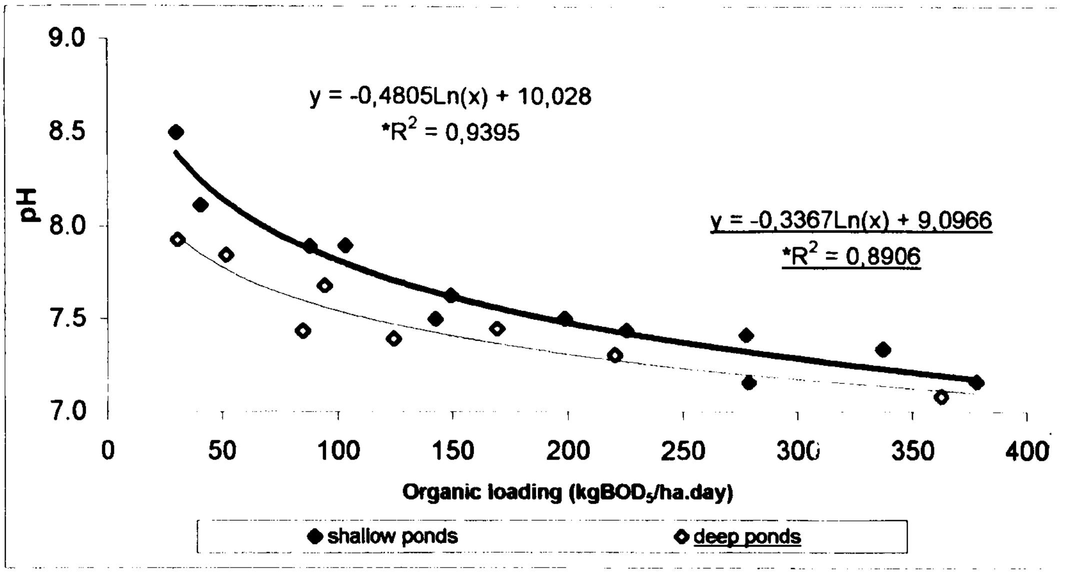


Figure 4.6. pH versus surface organic loading in the shallow (1.00m) and deep (2.20m) pond series.

The relationship between total sulphide and organic loading was a positive one (significant at 5%) for both the shallow and deep pond series (figure 4.7). H_2S also increased with increased organic loading (figure 4.8) since the increase in the surface organic loading buffers the system and prevents an increase in pH (figure 4.6). H_2S was calculated using equation 2 (presented at section 3.2). Here, again, the positive correlation between both total sulphide and H_2S against organic loading followed the same pattern observed in the primary facultative ponds, namely these parameters increase with the increase in surface organic loading. However, while in the primary facultative ponds the curves of H_2S and sulphide against organic loading for the two different depths were very distinct, in this series of ponds the differences between the two curves (for the two different depths) seemed to disappear with the increase in surface organic loading.

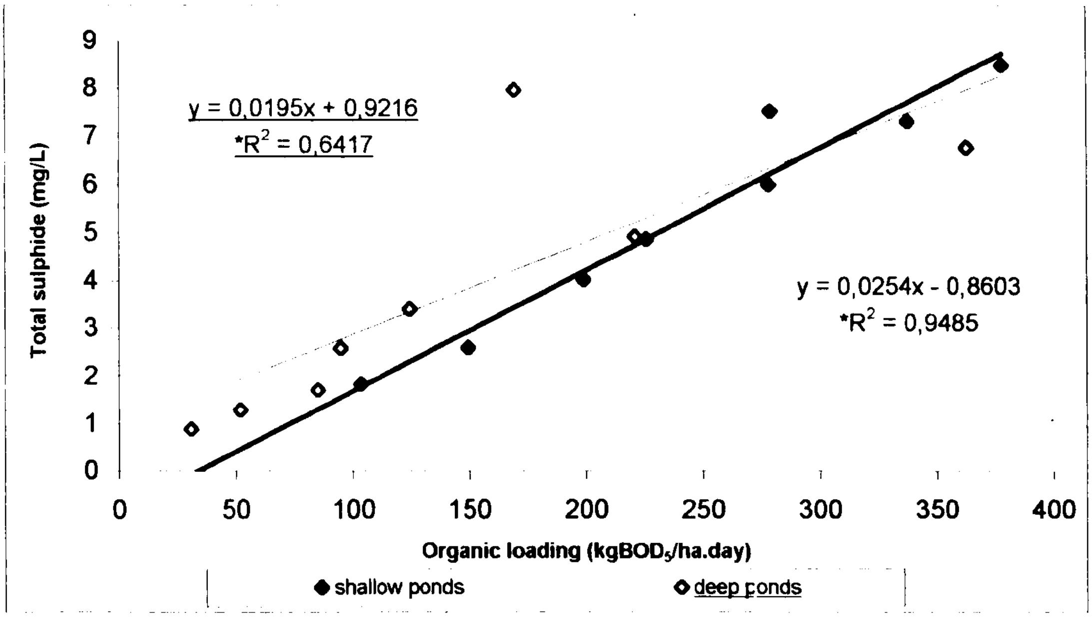


Figure 4.7. Total sulphide plotted versus surface organic loading in the shallow (1.00m) and deep (2.20m) pond series.

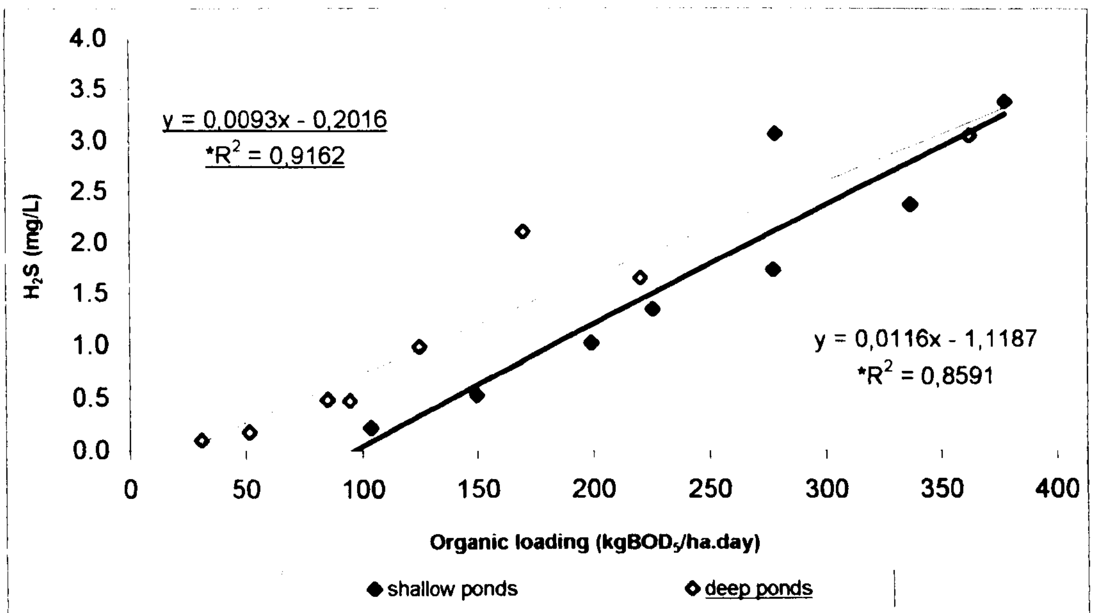


Figure 4.8. H_2S plotted against surface organic loading in the shallow (1.00m) and deep (2.20m) pond series.

4.4. The impact of surface organic loading on chlorophyll *a* concentration

The relationship between chlorophyll *a* and surface organic loading in both shallow and deep series of ponds was a negative linear one, repeating the same pattern observed for chlorophyll *a* against surface organic loading in the primary facultative ponds.

According to figure 4.9, chlorophyll *a* decreased faster in the shallow series of ponds than in the deep one, although there was no clear difference between the values of chlorophyll *a*/m^2 in the shallow series of ponds and in the deep one as was noticed in the primary facultative ponds (e.g. where the concentration of chlorophyll *a* was always higher in the shallow ponds than in the deep ones.)

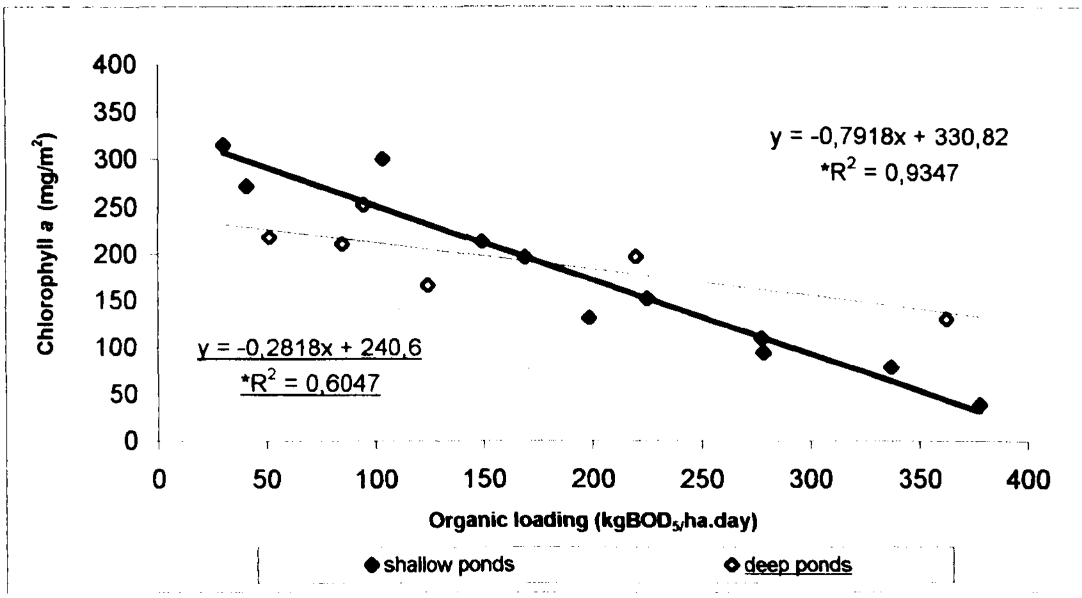


Figure 4.9. The effect of surface organic loading on chlorophyll *a* values in the shallow (1.00m) and deep (2.20m) pond series.

4.5. The effect of ammonia and sulphide on chlorophyll *a* concentration

The concentrations of ammonia and sulphide were plotted against chlorophyll *a* to determine the effect of these parameters on the algal biomass. A negative correlation existed between chlorophyll *a* and total ammonia in both series of ponds, although the curve for the deep series was not significant at the level of 5% (figure 4.10).

A negative correlation was also obtained between chlorophyll *a* and total sulphide (figure 4.11). However, when the more toxic forms of these parameters (i.e. NH_3 and H_2S) were plotted against chlorophyll *a*, only H_2S gave a significant negative relationship with chlorophyll *a* (figure 4.12) in both series of ponds. On the other hand, chlorophyll *a* tended to increase with the increase of NH_3 (figure 4.13). Thus these data suggest that sulphide (in the form of H_2S) might be the parameter controlling algal biomass in these series of ponds rather tnan NH_3. These results were similar to the ones for the primary facultative ponds.

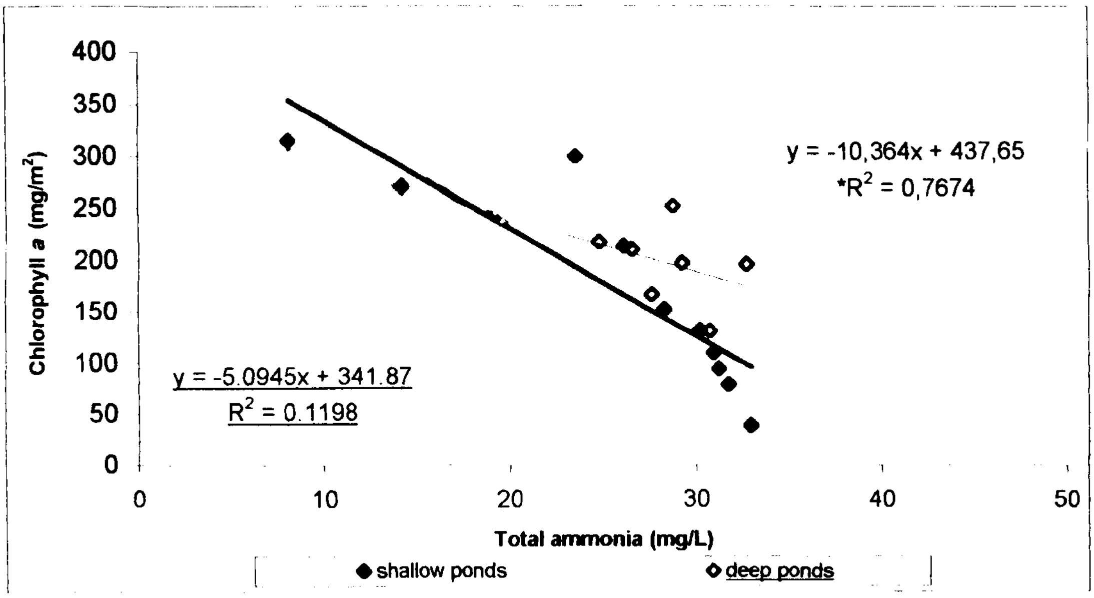


Figure 4.10. The effect of total ammonia on chlorophyll *a* concentration in the shallow (1.00m) and deep (2.20m) pond series.

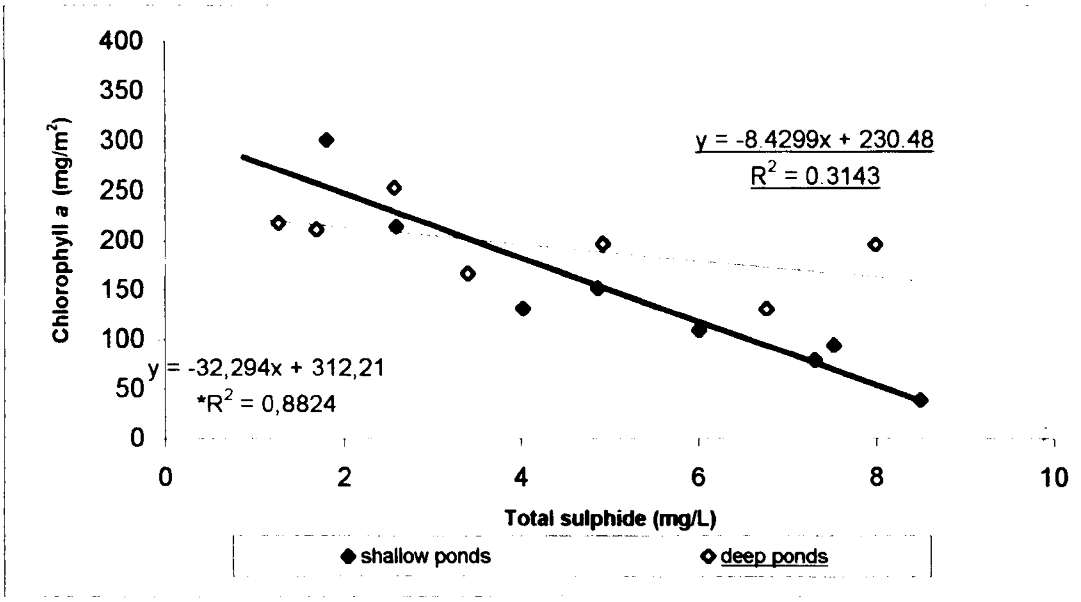


Figure 4.11. Chlorophyll *a* plotted against total sulphide concentration in the shallow (1.00m) and deep (2.20m) pond series.

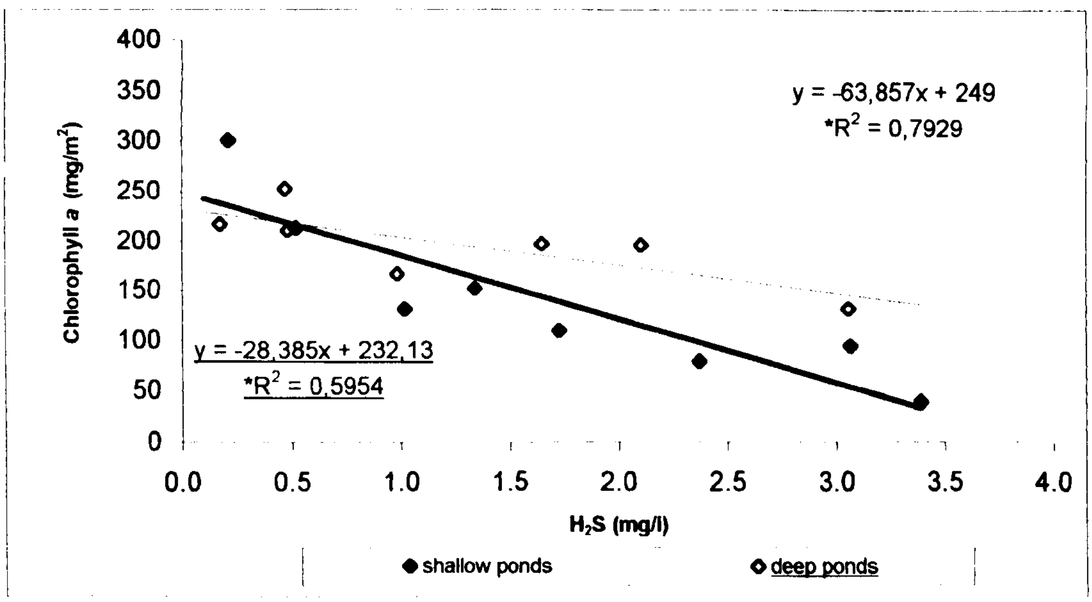


Figure 4.12. Chlorophyll *a* plotted against H_2S concentration in the shallow and deep pond series.

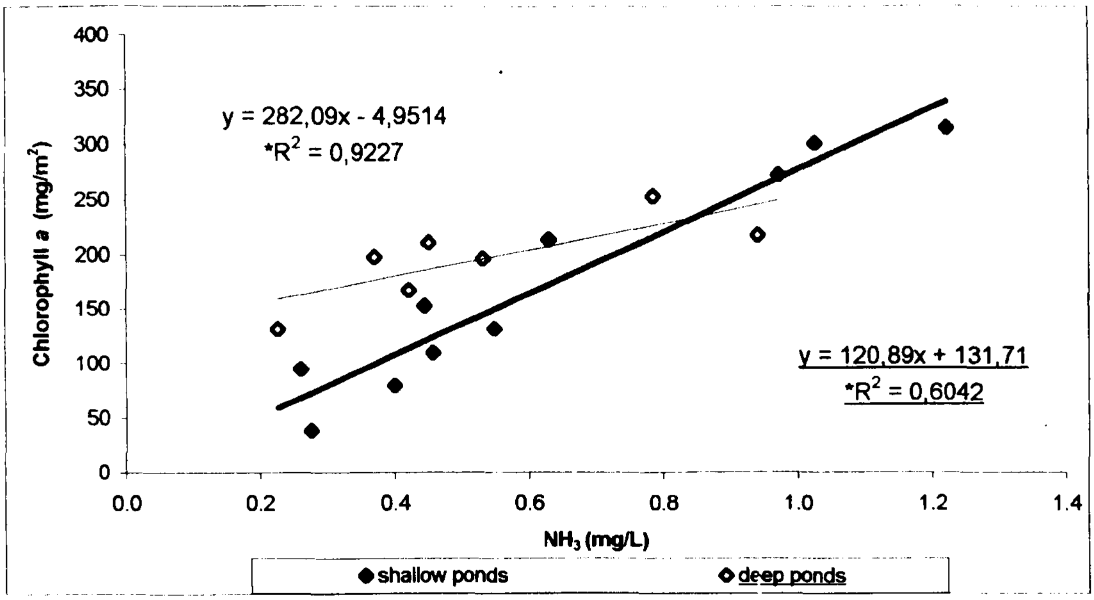


Figure 4.13. Chlorophyll *a* plotted against NH_3 concentration in the shallow and deep pond series.

4.6. Faecal coliform removal in the series of WSP

According to figure 4.14 (which includes the data for the anaerobic pond) FC decrease in concentration was faster in the shallow series of ponds than in the deeper one. For example, to reach the concentration of 10^3 cfu/100ml; recommended by WHO (1989) for unrestricted irrigation; a mean hydraulic retention time of 21.1 days was necessary in the shallow series of ponds and 32.1 days in the deep one.

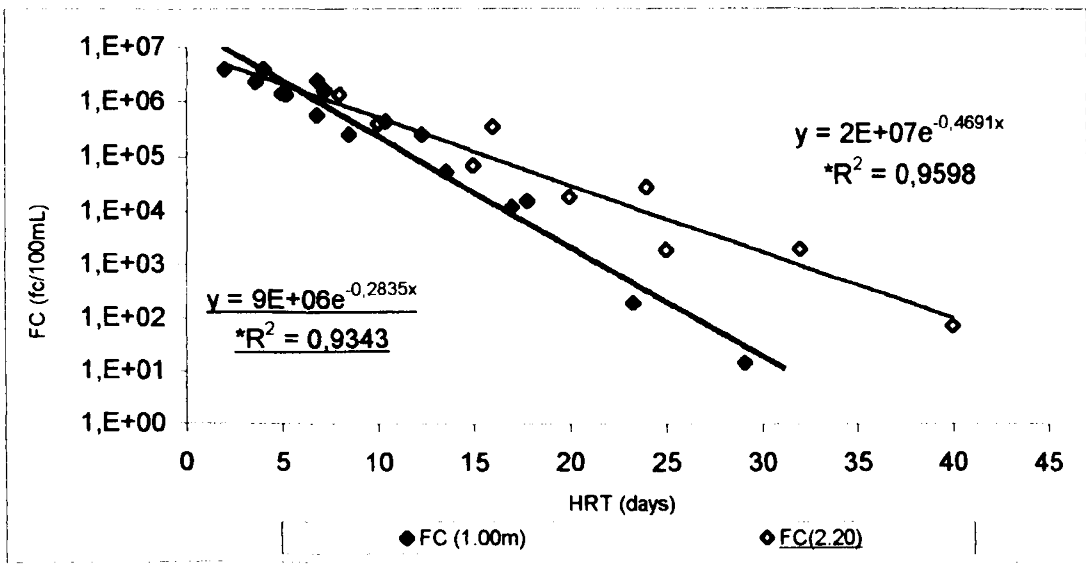


Figure 4.14. FC concentration plotted against (HRT) in shallow (1.00m deep) and deep pond series (2.20m).

Using the previous flow example of 10,000m^3/d and applying the equations obtained in figure 4.14 for the shallow pond series a volume of 211,000m^3 and an area of 21.1ha would be required to achieve an effluent quality of 10^3 cfu/100ml. Similarly, to reach the same effluent concentration in the deeper series, a volume of 321,000m^3 but an area of only 14.6ha for would be necessary. Thus the volume required by the deeper series to treat the same flow is 52% higher than the one required for the shallow series, but the area is smaller; i.e. 69% of the area necessary for the shallow series. Therefore, a rigorous financial analysis would be required to determine the best choice and this would probably depend on the price of land and construction costs (earth movement), which will vary from place to place. In a cost analysis of pond series of different depths, Athayde Jr. (1999) found that shallow ponds were less expensive than deep ones for the land costs found in the northeast of Brazil.

According to table 4.1, the k_b of the anaerobic ponds in all the series, irrespective of depth, was higher than the k_b for the secondary facultative ponds following them. Mills et al (1992), studying seven different waste stabilization pond systems in Kenya, found the same thing with the first pond in the series exhibiting higher k_b than that of the following ponds. They concluded that faecal coliform removal in these ponds was mainly due to sedimentation of these bacteria with the solids.

In general, the k_b value of the maturation ponds was higher than the ones for the preceding secondary facultative ponds in the series irrespective of depth. This would appear to be due to intense algal photosynthesis, which causes an increase in the pH and dissolved oxygen concentrations contributing to FC removal according to the general equation explaining the CO_2/ bicarbonate equilibrium in relation to photosynthesis. Equation 3:

$$2HCO_3 \Leftrightarrow CO_3^{2} + H_2O + CO_2 \qquad \text{(Equation 5)}$$

$$CO_3^{2} + H_2O \Leftrightarrow 2OH + CO_2 \qquad \text{(Equation 6)}$$

The k_b of the shallow and deep pond series were not much different except in experiment I (560 kg BOD_5/ha.d) which had the highest HRT of the shallow pond series.

The mean k_b of the series was lower than the value of 6.2 proposed by Marais (1974) from a temperature of 25°C in maturation ponds. However, Mills *et al.* (1992) also found k_b values lower than those calculated using the Marais (1974) equation, $k_b = 2.6\ (1.19)^{T-20}$, and then they derived another Arrhenius-like equation with the constants 0.712 and 1.166 instead of 2.6 and 1.19, respectively. The value computed from Mill *et al*'s equation is 1.53 $^{d-1}$ for a temperature of 25^{0}C. The results obtained with Mill *et al*'s equation were closer to the results found for the 1.00m 2.20m deep ponds series.

Table 4.1. k_b values in system B

Exp.	Pond	kb (1/d)*	Mean kb (1/d)*	HRT (days)	Cumulat. HRT (days)	Org.loading** ($kgBOD_5/ha.d$)	Depth (m)
I	A_1	02.22	3.18	6.8	06.8	560 (45)	1.25
	F_1	01.66		5.5	12.3	143	1.00
	M_1	02.74		5.5	17.8	88	1.00
	M_2	14.64		5.5	23.3	41	1.00
	M_3	02.09		5.8	29.1	30	1.00
II	A_1	03.23	0.94	2.0	02.0	1762 (139)	1.25
	F_1	00.46		1.6	03.6	378	1.00
	M_1	00.45		1.6	05.2	337	1.00
	M_2	00.86		1.6	06.8	278	1.00
	M_3	00.72		1.7	08.5	199	1.00
III	A_1	02.29	1.20	4.0	04.0	1071 (85)	1.25
	F_1	00.45		3.2	07.2	279	1.00
	M_1	00.87		3.2	10.4	226	1.00
	M_2	02.22		3.2	13.6	150	1.00
	M_3	01.00		3.4	17.0	104	1.00
IV	A_1	01.19	0.90	5.0	05.0	765 (35)	2.20
	F_1	00.52		5.0	10.0	363	2.20
	M_1	00.91		5.0	15.0	221	2.20
	M_2	00.58		5.0	20.0	125	2.20
	M_3	01.68		5.0	25.0	85	2.20
V	A_1	00.82	1.23	8.0	08.0	344 (16)	2.20
	F_1	00.39		8.0	16.0	170	2.20
	M_1	01.36		8.0	24.0	95	2.20
	M_2	01.65		8.0	32.0	52	2.20
	M_3	03.36		8.0	40.0	31	2.20

*kb calculated based on the equation 4 (section 3.5) for complete mixed flow. For a series of anaerobic, facultative and maturation ponds, equation 1 becomes:

$$N_e = \frac{N_i}{\left[(1 + k_b\theta_a)(1 + k_b\theta_f)(1 + k_b\theta_m)^n\right]}$$

where N_e and N_i refer to the number of FC per 100mL of the final effluent and raw wastewater respectively; the sub-scripts a, f and m refer to the anaerobic, facultative and maturation ponds; and n is the number of maturation ponds.

** Surface organic loading even for the anaerobic pond to enable a better comparison with the other ponds. The volumetric loading for the anaerobic pond, expressed in gBOD5/m3.day, is shown in the brackets.

4.7. Nutrient removal in this five pond series

As was explained in the Literature Review (section 1.4), the use of treated wastewater for irrigation presents two main advantages: the saving of clean water and the economizing on fertilizer. The latter being due to the presence of nutrients, such as nitrogen and phosphorus in the wastewater. However, high concentrations of nitrogen can be toxic to some crops. The value of total nitrogen is very important due to the changes in the nitrogen forms realized by bacteria. According to Ayers and Westcot (1985) a concentration of total nitrogen of less than 5mg/L will not detrimentally affect the crop growth. Nevertheless, ammonia is the nitrogen form most used to characterize sewage (Reis,1995), and it was ammonia that was analyzed in these series of experiments. In contrast to nitrogen, there is no mention of high concentrations of phosphorus detrimentally affecting plant growth.

The changes in concentration in total ammonia and orthophosphate, the form of phosphorus most rapidly assimilated by microrganisms and plants (Sawyer *et al.*, 1994), were analyzed in the series of five ponds at the two different depths of 1.00m and 2.20m at different organic loadings and retention times.

Figure 4.15. shows the relationship between total ammonia concentrations (expressed as arithmetic mean) and hydraulic retention time along the series of ponds, including the anaerobic pretreatment pond. The ponds are again treated as a continuum for each depth.

There was a tendency for total ammonia to decrease in concentration with the increase in the HRT along the series, irrespective of the series being either 1.00m or 2.20m deep. However total ammonia removal was highest in the shallow series, experiment I (560 $kgBOD_5/ha.d$), 84% removal in 29 days, although neither series reached the concentration of 5mg/L of ammonia stated by the Brazilian legislation (CONAMA, 1986) for discharge into receiving water bodies.

Figures 4.16 and 4.17 showed the relationship between total ammonia and pH and chlorophyll *a* in the series of ponds. It was seen that there was a decrease in total ammonia concentration with the increase in pH and chlorophyll *a* concentrations. This tends to confirm that the mechanisms of volatilization and assimilation by the algal biomass are the main ones responsible for total ammonia removal in these series, as suggested by Pano & Middlebrooks (1982), Ferrara & Avci (1982) and Arceivala (1986).

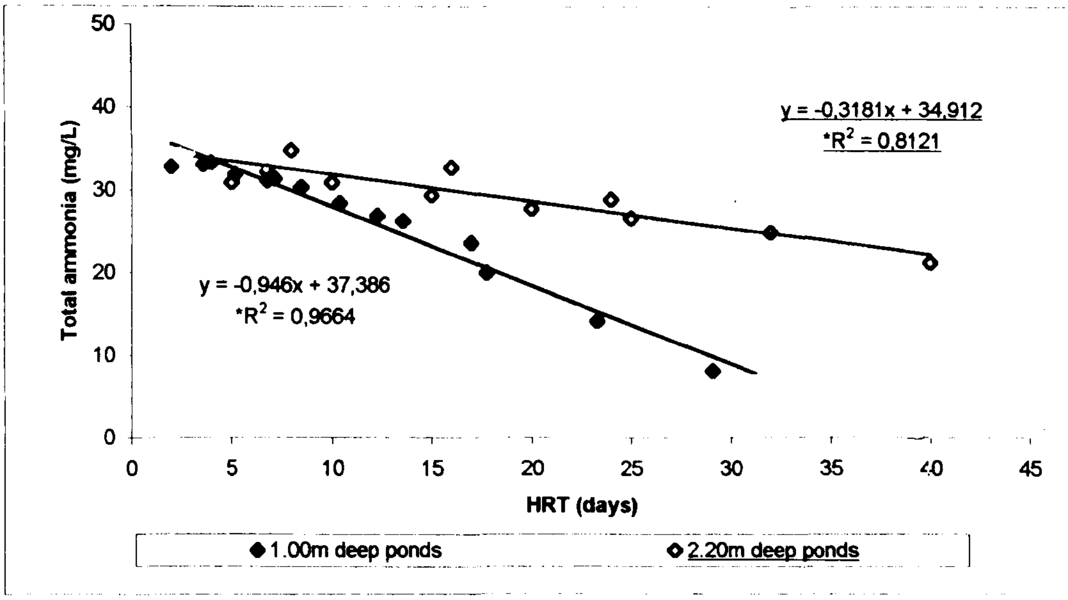


Figure 4.15. Total ammonia concentration against HRT at the series of ponds 1.00m and 2.20m deep.

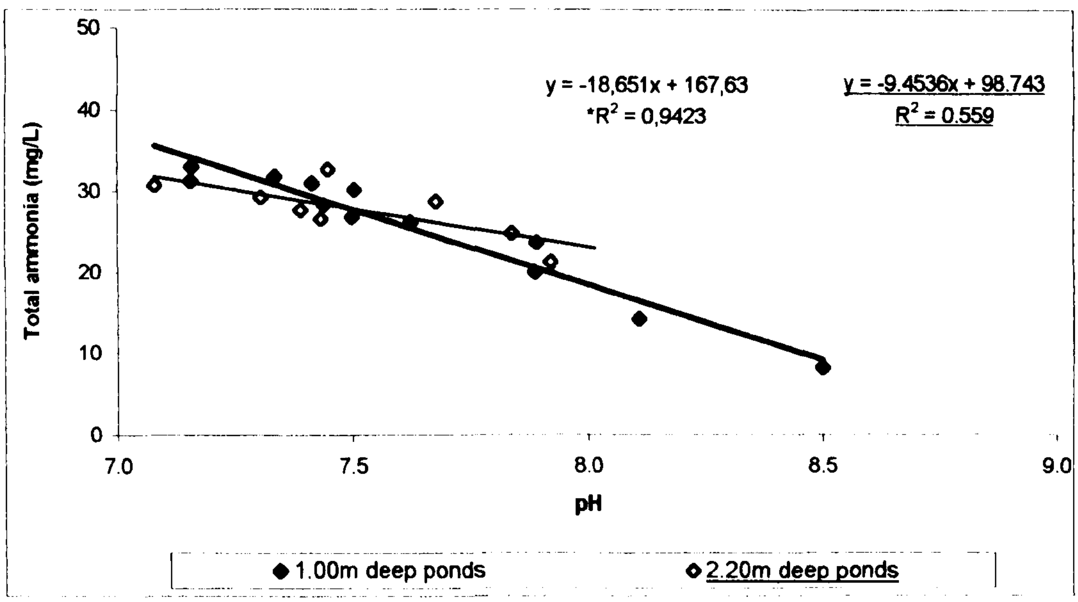


Figure 4.16. Total ammonia concentration plotted against pH in the 1.00m and 2.20m deep series of ponds.

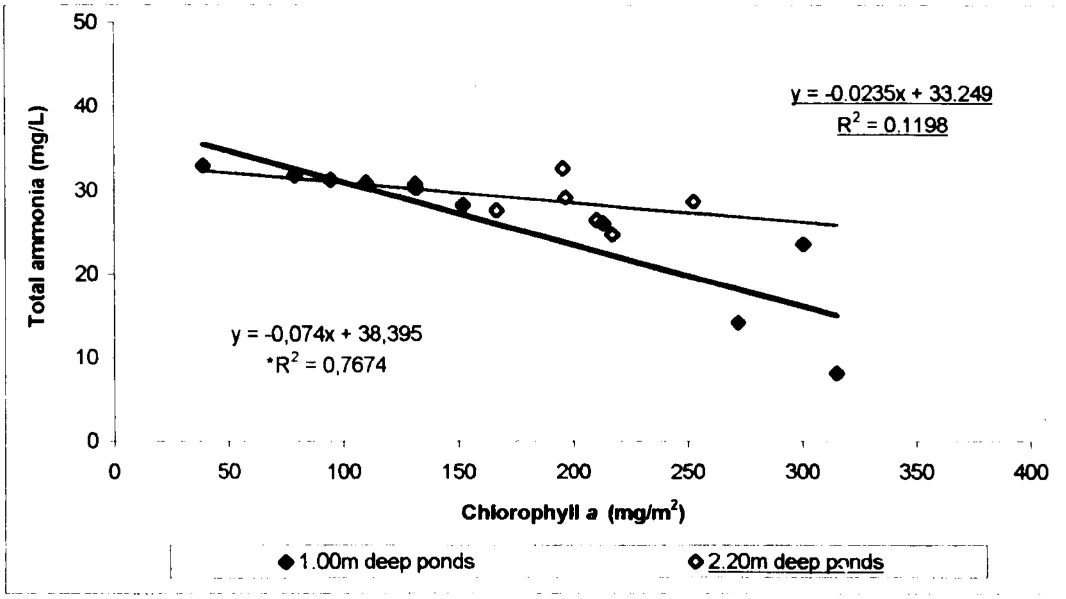


Figure 4.17. Total ammonia concentration plotted against chlorophyll *a* in the 1.00m and 2.20m deep series of ponds

It was seen that in the 1.00m deep series of ponds there was a difference in ammonia removal (see table 4.2). While in experiment I (560 kgBOD$_5$/ha.d), 84% of ammonia was removed, in experiment II (1762 kgBOD$_5$/ha.d) the removal was only 36%. This may be due to the shorter retention time in experiment II, since the concentration of ammonia in the raw sewage did not change very much. When the series of ponds became deeper, the same pattern was observed. In other words, experiment V (344 kgBOD$_5$/ha.d), which had the longest retention time, provided better ammonia removal than experiment IV (765 kgBOD$_5$/ha.d). Again, between these two pond series the concentration of ammonia in the raw sewage did not vary considerably. There was a slight difference between the concentrations of ammonia in the raw sewage when the pond series was shallow and when it became deeper, the same pattern was observed for orthophosphate.

Table 4.2. Ammonia removal in system B

Experiment	Depth (m)	Ammonia in the raw sewage (mg/L)	Ammonia in the anaerobic pond effluent (mg/L)	Ammonia in the final effluent (mg/L)	Removal efficiency (%)	Total hydraulic retention time (days)
I	1.00	49	32	8	84	29.1
II	1.00	47	33	30	36	8.5
III	1.00	49	33	24	51	17.0
IV	2.20	32	31	26	19	25.0
V	2.20	30	35	21	30	40.0

The figure 4.18 showed the orthophosphate concentration along time (HRT) in the shallow and deep series of ponds. In the 1.00m deep pond series was observed a general tendency of orthophosphate to decrease with the increase in the HRT. When the 1.00m pond series were loaded at 560 and 1071 kgBOD_5/ha.d, an orthophosphate removal of 39% and 8%, respectively were obtained; however when the 1.00m deep pond series was loaded at 1762 kgBOD_5/ha.d and at the loads (765 and 344 kgBOD_5/ha.d) applied to the 2.20m deep pond series, the concentrations of orthophosphate increased slightly along the series.

These results suggested that the concentrations of chlorophyll *a* and pH (which were below 9.5) were not high enough to perform a satisfactory orthophosphate removal. Even the efficiency in ammonia removal was not satisfactory, with only experiment I (560 kgBOD_5/ha.d) reaching 84%. Pearson (1996) states that in WSP systems ammonia removal can be as high as 95%.

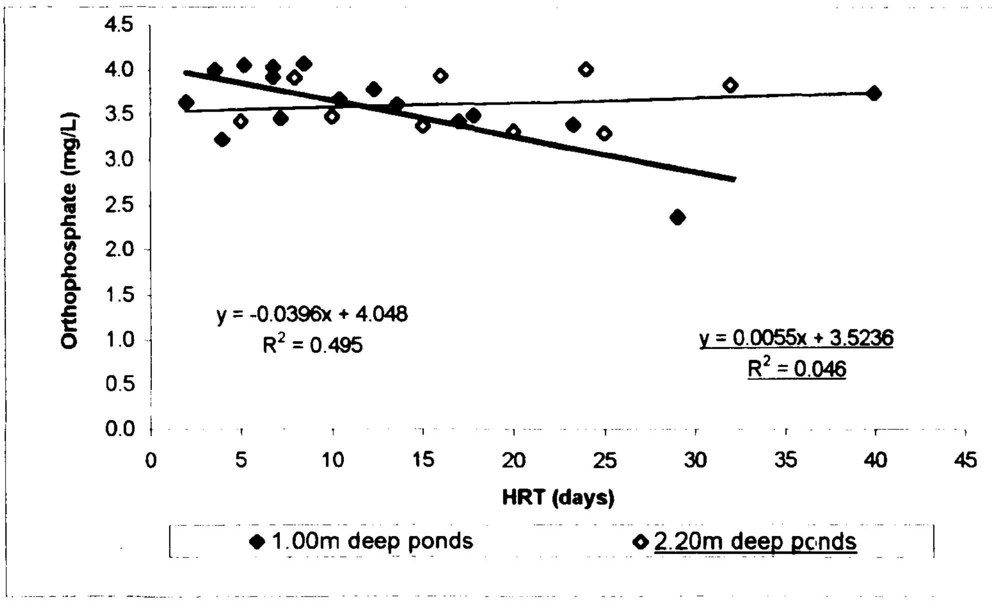


Figure 4.18. Orthophosphate concentrations against HRT at the series of ponds 1.00m and 2.20m deep.

4.8. Assessment of the efficiency of the five pond series

The data presented in this chapter establish that the shallow ponds are more efficient at both BOD_5 removal and faecal coliform removal than the deeper ones. However in terms of physical pond design the 2.20m deep pond series makes a significant saving in land area (>32%) over the shallow ponds when achieving the same effluent quality. In other words the additional HRT achieved per unit area with the deeper ponds more than compensates for the reduced efficiency. Exactly the same argument can be applied to faecal coliform removal. Thus the required BOD_5 removal and pathogen removal can be achieved with a lower land take in a 2.20m deep pond series compared to a 1.00m deep pond series. This means that the accepted practice of building facultative ponds to a pond liquid depth of between 1.5 and 2.0m and maturation ponds to a maximum depth of 1.5m represent unnecessary constraints on pond design and furthermore uses more land area than is necessary. The cost of land can be an expensive component of overall pond construction costs (Arthur 1983).

Unlike BOD_5 removal and FC removal the loss of efficiency in terms of nutrient removal in deeper ponds is not compensated for by the increased HRT. The removal of ammonium, which is the major form of nitrogen in ponds and phosphate requires shallow ponds and this is linked to the increased pH in the 1.00m compared to the 2.20m deep ponds.

Although deeper (2.00m deep) ponds provided a better engineering design solution for the key parameters of BOD_5 and FC removal, further research would be required to assess the impact of further increasing pond depths.

4.9. Conclusions

- The increase in surface organic loading between 30 –378 kgBOD_5/ha.d (loading on the facultative ponds) led to the decrease in BOD_5 effluent quality;
- The shallow ponds are more efficient at both BOD_5 removal and faecal coliform removal than the deeper ones. However in terms of physical pond design the 2.20m deep pond series makes a significant saving in land area (>32%) over the shallow ponds when achieving the same effluent quality;
- Total ammonia and total sulphide concentrations increased with increasing surface organic loading; and H_2S followed the same trend;
- The mean k_b of both series was lower than the value of 6.2 proposed by Marais (1974) to a temperature of 25°C;
- There was no clear difference between the values of chlorophyll *a* in the shallow and deep pond series;
- Sulphide (in the form of H_2S) controlled algal biomass in both series of ponds;
- There was a tendency for ammonia to decrease with the increase in HRT in both the deep and shallow pond series;
- Ammonia removal ranged from 36 to 84% in the shallow pond series and from 19 to 30% in the deep pond series;
- In no case was the final effluent concentration of < 5mg/L of ammonia obtained, which is a requirement for discharge in receiving water bodies stated by the Brazilian legislation (CONAMA, 1986);
- Orthophosphate removal ranged from 0 to 39% in the shallow pond series and in the deep pond series, a slight increase in orthophosphate concentration was observed.

Chapter 5. SYSTEM C: SHALLOW FIVE POND SERIES (INNOVATIVE SYSTEM)

In previous research conducted on this innovative system the impact of variations in pond geometry and depth on FC removal were considered. The results basically showed the apparent lack of impact of pond shape on pond performance and the advantage of individual shallow maturation ponds to enhance FC removal. Increased organic loading was also shown to increase the time required for FC removal (Pearson *et al*, 1996). Silva *et al* (1995) studied, nitrogen removal in this same system, and found that ammonia removal rates were highest in the maturation ponds and that there was a trade-off between depth and retention time in favor of the shallow ponds. In this chapter, the innovative system is considered as a series of five ponds (anaerobic pond, secondary facultative pond, primary maturation pond, secondary maturation pond and tertiary maturation pond) in which the depth of the secondary facultative pond varied between 1.00 – 2.00m and the depth of the maturation ponds between 0.39 – 1.00m. The mean depth of this series was 0.81m, excluding the anaerobic pond. In this way, this series of very shallow ponds would be compared, later, with the 5 pond series comprising ponds of 1.00m deep and when their depths had been increased to 2.20m (these series of ponds were the subject of the previous chapter). This very shallow pond series was studied at two different organic loadings namely (181 and 430 $gBOD_5/m^3.day$) on the anaerobic ponds giving surface organic loading of 250 and 770 $kgBOD_5/ha.day$ on the secondary facultative ponds. In all the figures presented in this chapter, the value plotted for each pond is the mean of a minimum of 45 and 23 samples under the lower loading regime and higher loading regime, respectively. The physical characteristics of the ponds did not change from one experiment to the other (for more details see chapter 2.0 – Materials and Methods).

5.1. FC removal in the five pond series of the innovative system

There was a clear positive statistical relationship between FC concentration and surface organic loading when the data for the secondary facultative ponds and maturations ponds for the two loading regimes were plotted together as a continuum (figure 5.1). The fitted curve showed that independent of which experiment was being observed, the concentration of FC dropped with the decrease in organic loading.

When the results for FC concentration were plotted against pH for both organic loading ranges, there was a strong negative exponential relationship (figure 5.2). The pH reached the highest levels (9.1) in the lower loading range (figure 5.3).

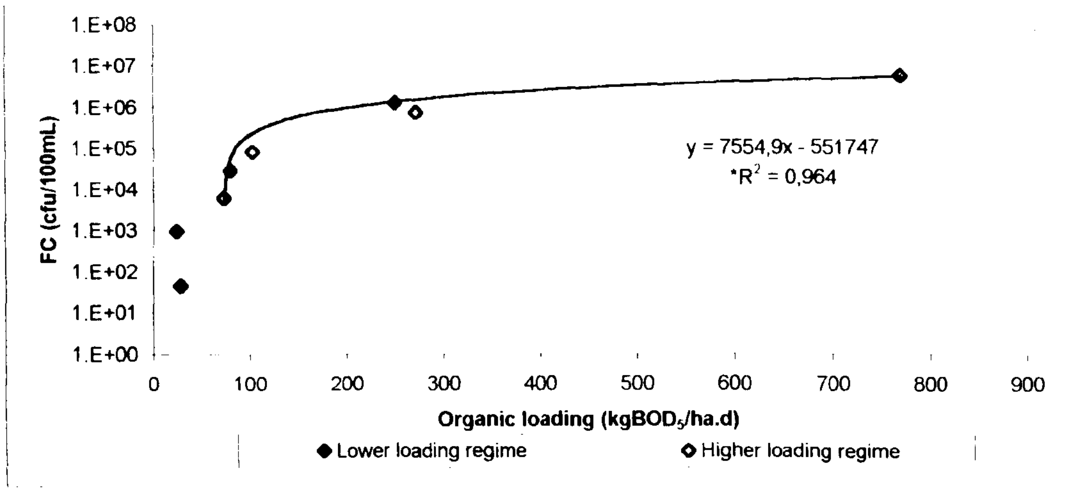


Figure 5.1. FC concentration plotted against surface organic loading for the secondary facultative pond and three maturation ponds of a five pond series (i.e. excluding the anaerobic pond) at the two organic loading regimes considered as a continuum.

*The regression is significant at the level of 5% in this figure and subsequent ones in this chapter unless otherwise stated.

Figure 5.4 shows the clear negative exponential relationship existing between FC concentration and HRT (mean hydraulic retention time). The FC data for the anaerobic pond was included and as before the results for the two different loads were plotted as a continuum.

There was no correlation between FC and chlorophyll *a* at both loading ranges suggesting that the algae *per se* did not contribute to FC removal, although they did it indirectly since they are responsible for the increase in pH and dissolved oxygen concentration. However, in this system, due to reasons that are not clear there was no significant correlation (at the level of 5%) between pH and chlorophyll *a* under the lower loading regime, although low loadings gave high pH at comparable chlorophyll a concentrations. However, there was strong correlation between pH and Chlorophyll *a* under the higher loading regime (figure 5.5).

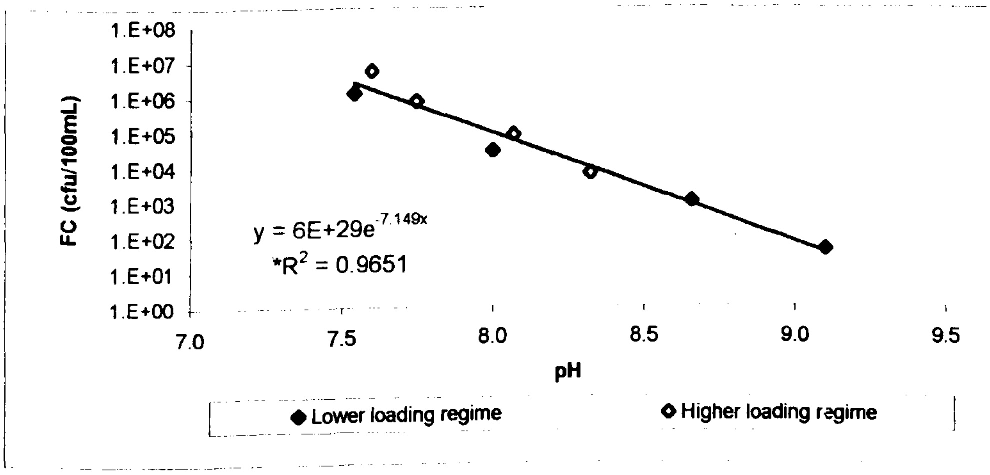


Figure 5.2. FC concentration plotted versus pH as a continuum in the innovative pond series under the two loading ranges (see legend to figure 5.1 for details)

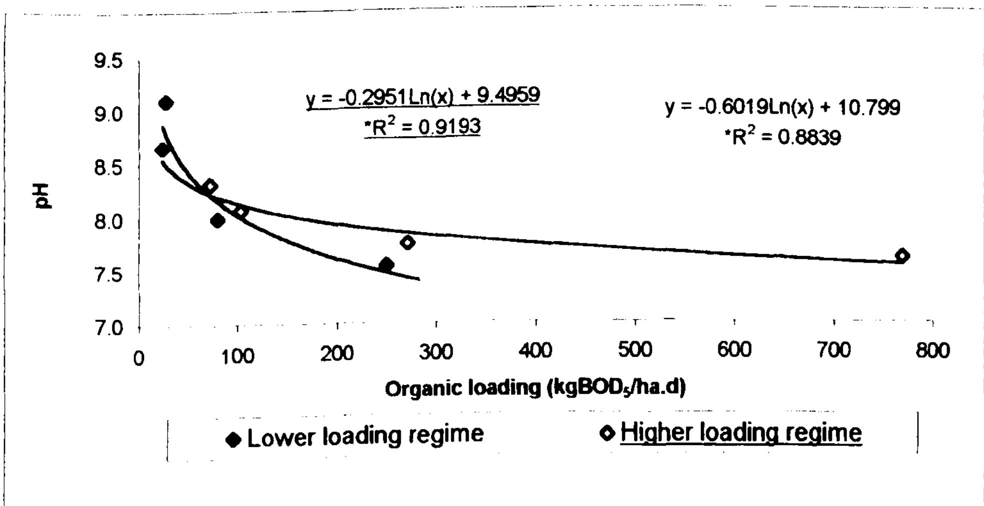


Figure 5.3. The effect of surface organic loading on pH in the five pond series of the innovative system.

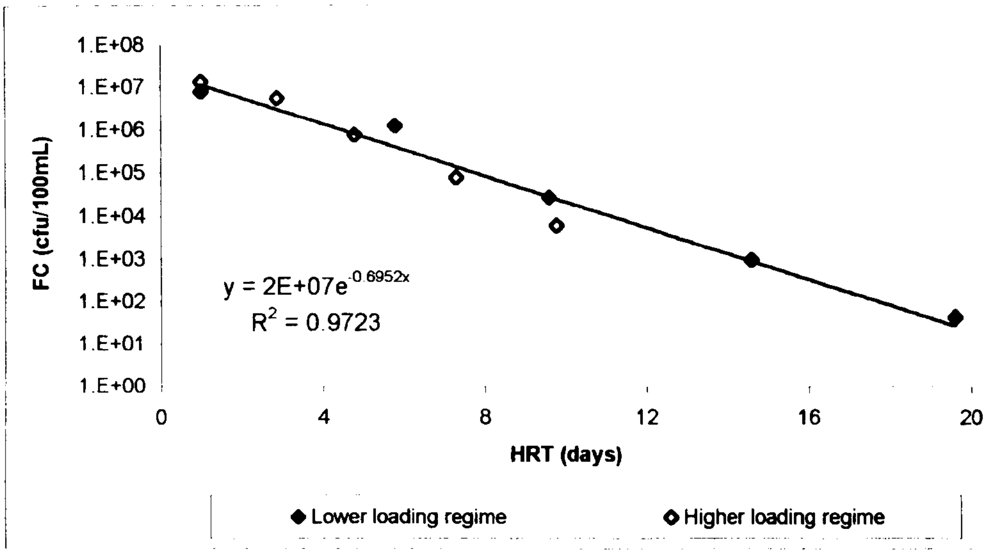


Figure 5.4. FC concentration plotted against mean hydraulic retention time (HRT) with the data for the lower and higher loadings plotted as a continuum.

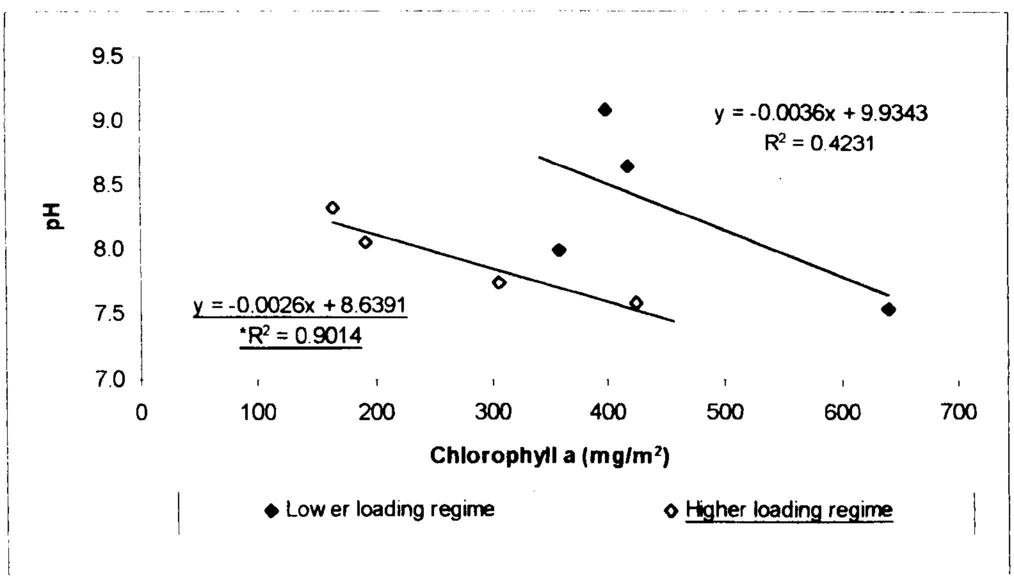


Figure 5.5. The relationship between pH and chlorophyll *a* under the two loading regimes of the innovative five pond series.

5.2. Algal biomass in the innovative system

Algal biomass was determined by the concentrations of chlorophyll *a* in this series of ponds. The chlorophyll *a* concentration was slightly higher in the lower loading regime, ranging from 358 – 639 mg/m^2, compared to the results for the higher loading regime (164 – 423 mg/m^2). However, there was no clear correlation (significant at 5%) between chlorophyll *a* and the surface organic loading (figure 5.6) when lower loading was applied to the pond series. In contrast, when the loading applied was much higher there was a positive relationship between chlorophyll *a* and the surface organic loading. This suggested that any relationship between the algal biomass and the surface organic loading was indirect and that other parameters were influencing chlorophyll *a* concentration.

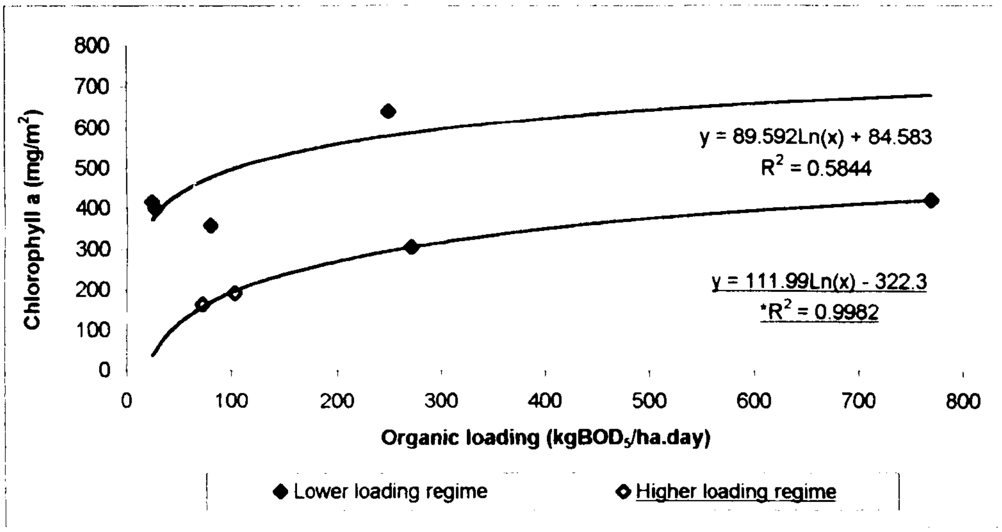


Figure 5.6. The effect of surface organic loading on the algal biomass in the innovative five pond series.

Chlorophyll *a* showed no clear relationship (significant at 5%) with COD at either loading regime (figure 5.7a and 5.7b). Similarly there was no clear relationship between chlorophyll *a* expressed as µg/L and effluent BOD (figure 5.8). However, when chlorophyll *a* was expressed as mg/m^2 of pond surface area a positive relationship could be found when chlorophyll *a* was plotted against BOD (figure 5.9).

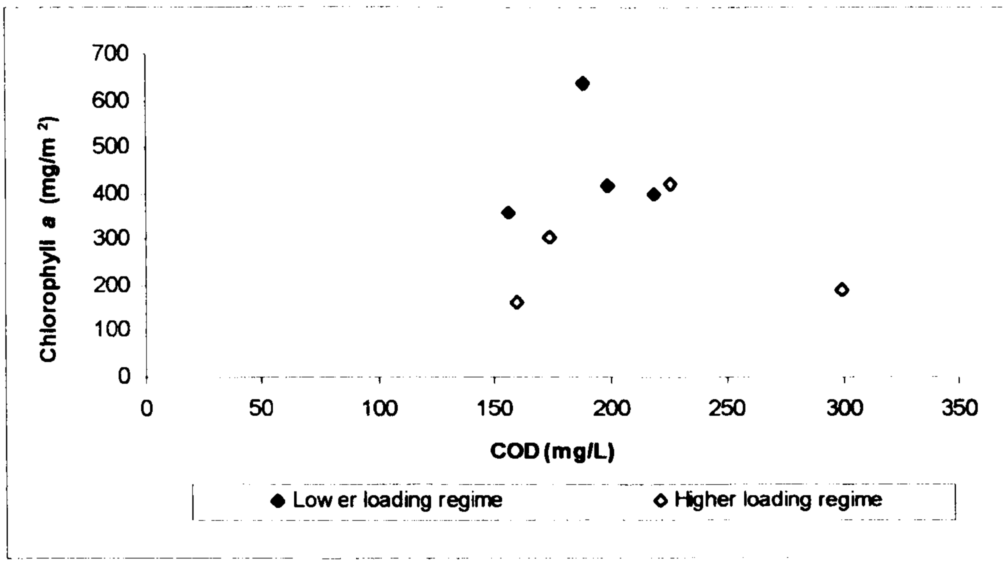


Figure 5.7a.Chlorophyll *a* plotted against COD in the innovative five pond series under both loading regimes.

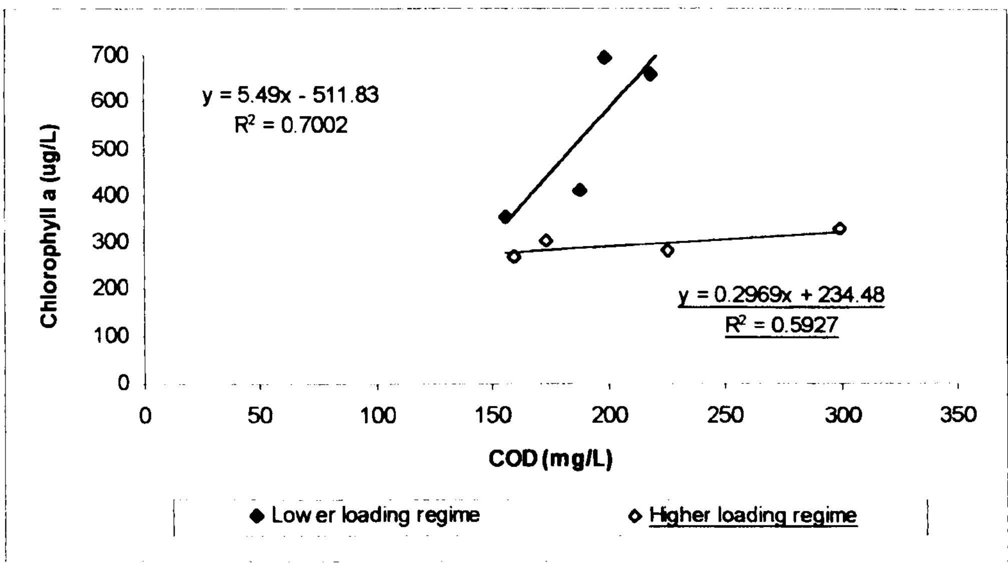


Figure 5.7b. Chlorophyll *a* (ug/L) plotted against COD in the innovative five pond series under both loading regimes.

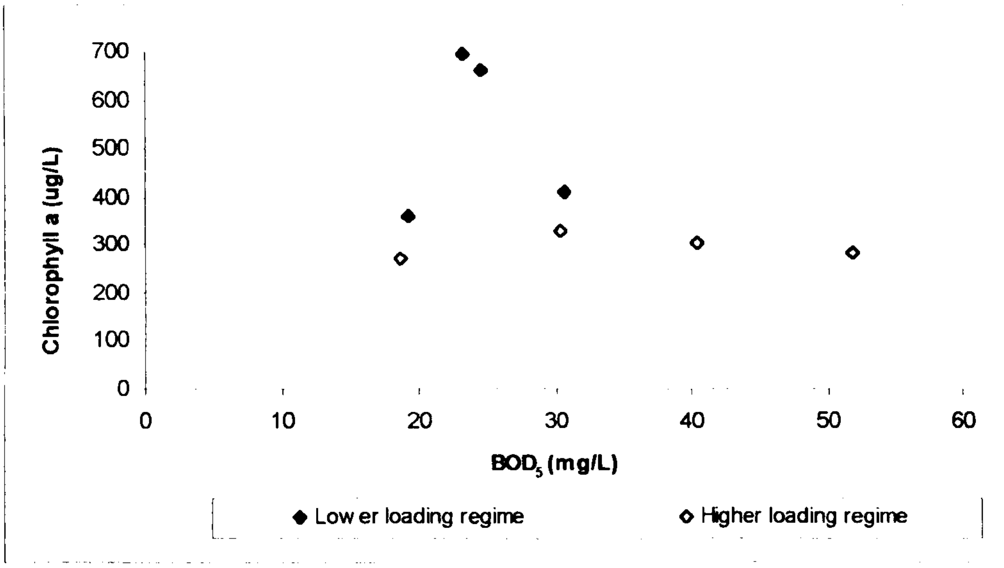


Figure 5.8. Chlorophyll a (ug/L) plotted against BOD_5 in the innovative five pond series under both loading regimes.

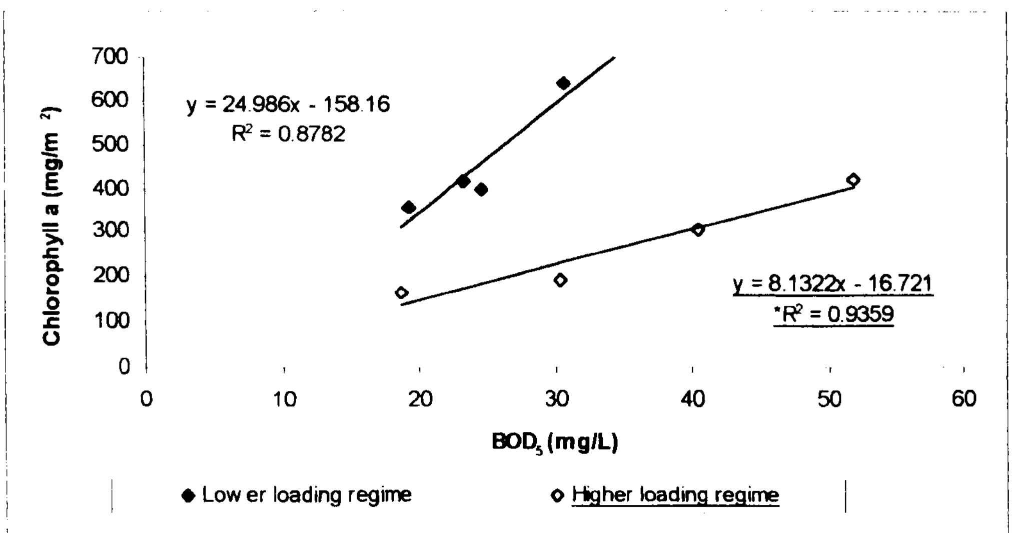


Figure 5.9. Chlorophyll a (mg/m^2) plotted against BOD_5 in the innovative five pond series under both loading regimes.

In order to see the effect of total ammonia, total sulphide and their unionized forms on algal biomass, chlorophyll *a* was plotted against these parameters. According to figure 5.10, there was a significant positive regression (at the of 5% level) between chlorophyll *a* and total ammonia at the higher loading. At the lower loading regime, there was a general tendency for chlorophyll *a* to increase with the increase in total ammonia concentration, but it was not statistically significant.

The figure 5.11 shows a positive significant (at the level of 5%) relationship between chlorophyll *a* and total sulphide under the two loading regimes. However, the regression for the lower loading regime was not considered, as the data were not well distributed. When H_2S was plotted against chlorophyll *a* (figure 5.12) the same positive relationship was observed as for total sulphide although the regression for the lower loading experiment was again not considered as the data were not well distributed. This means that chlorophyll *a* was not inhibited by the increase in H_2S concentrations as organic loading increased. A negative correlation was obtained only between chlorophyll *a* and NH_3 (figure 5.13) suggesting that in this shallow system NH_3 concentrations seemed to be controlling algal biomass, in the experiment with high organic loadings. At the lower loading regime, chlorophyll *a* also seemed to be inhibited by NH_3, but it was not statistically significant although the chlorophyll *a* values were higher for comparable NH_3 values. According to Pearson et al (1987) 13.2 mg/L of free NH_3 cause 50% inhibition to *Euglena*, the most sensitive algal genera to ammonia. Thus, as the values of NH_3 were not high in terms of potential toxicity to algae, perhaps some other factor could be also affecting algal biomass, for example grazing.

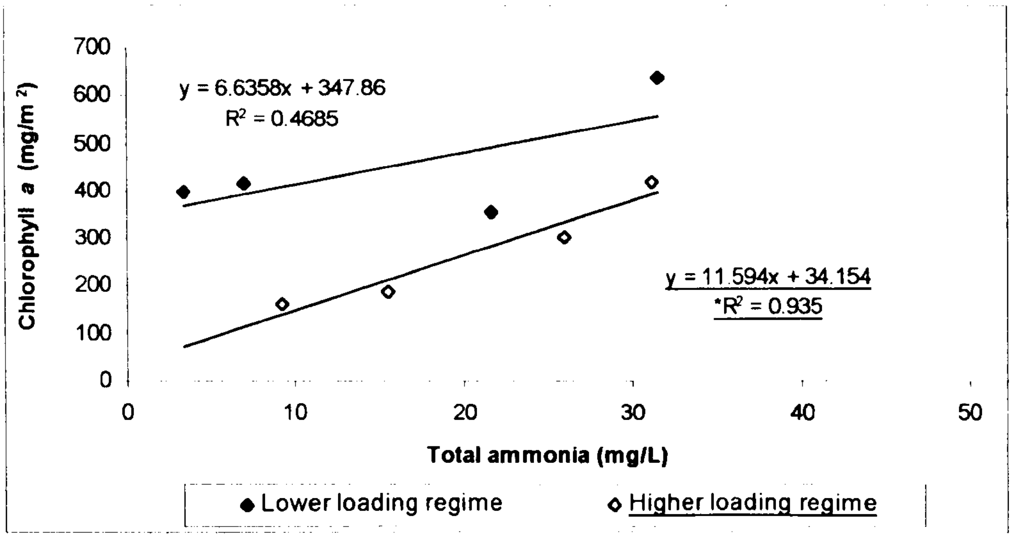


Figure 5.10. Chlorophyll *a* plotted versus total ammonia in the innovative five pond series

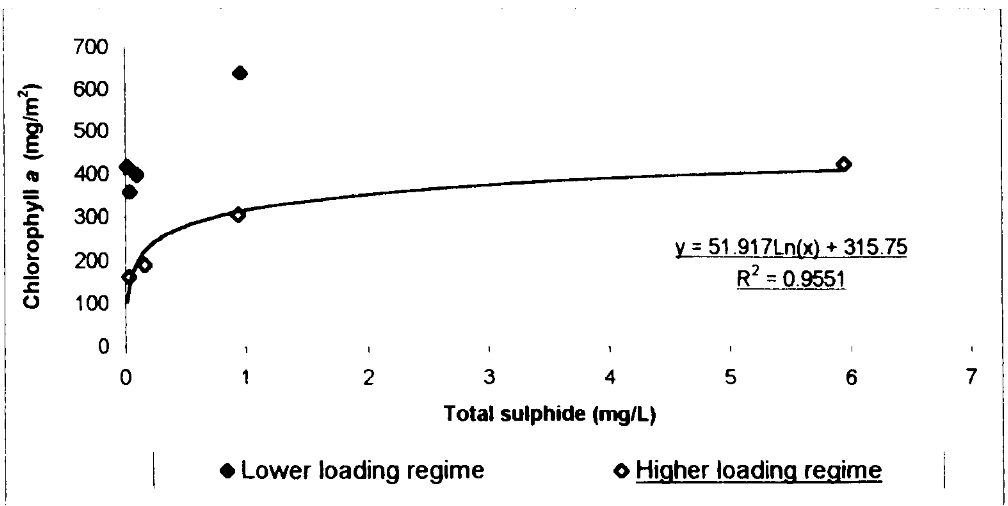


Figure 5.11. Chlorophyll *a* plotted against total sulphide in this shallow five pond series.

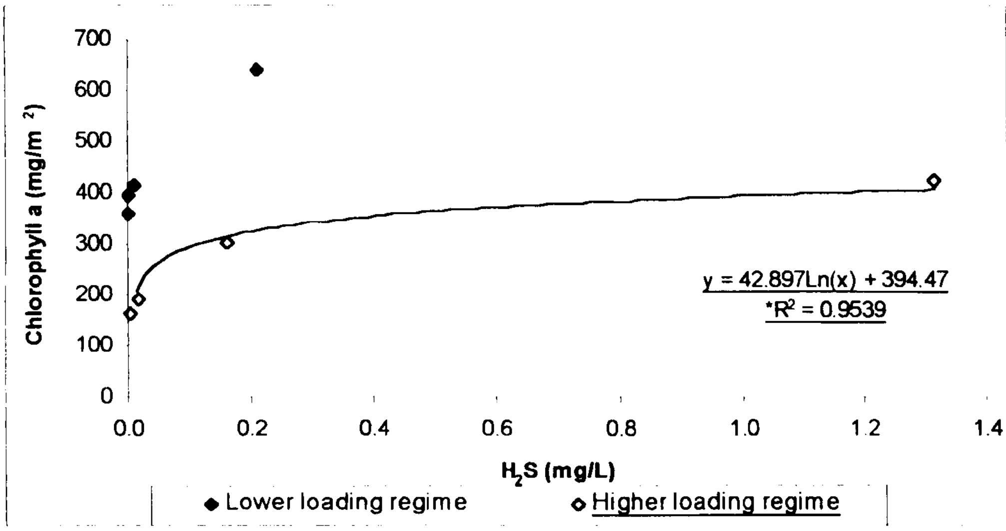


Figure 5.12. The relationship between chlorophyll *a* and H_2S in this shallow five pond series.

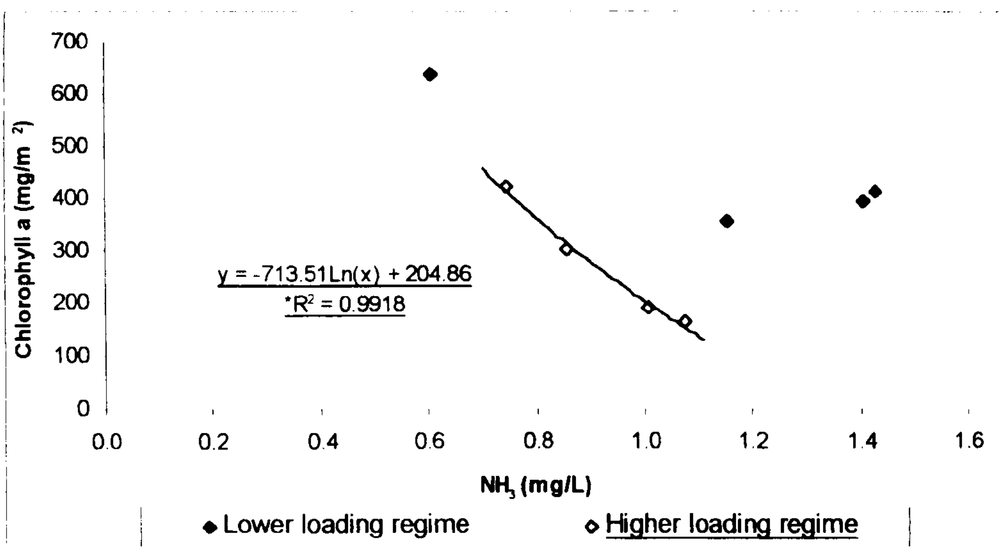


Figure 5.13. The relationship between chlorophyll *a* and NH_3 in this shallow five pond series

5.3. Nutrient removal

This shallow series of ponds was analyzed for nutrient removal efficiency. For the same reasons mentioned in the previous chapter: ammonia was the most significant form of soluble nitrogen as only very low concentrations of nitrite and nitrate (less than 1mg/L) were found. The study on nutrient removal in the pond series therefore concentrated on the behavior of ammonia and orthophosphate (form of phosphorus most rapidly assimilated by aquatic organisms).

Figure 5.14 shows the relationship between ammonia (arithmetic mean for each experiment) and hydraulic retention time along the series of ponds, including the anaerobic pretreatment pond.

There was tendency for ammonia to decrease with the increase in HRT under both lower and higher loading regimes. However, as table 5.1 shows ammonia removal efficiency (89.6%) was better at lower loadings than at the higher loading when removal dropped to 66.6%. Table 5.1 also shows that only at the lower loading regime did the final effluent (3mg/L) meet the 5mg/L requirement of the Brazilian legislation (CONAMA, 1986), for effluent discharge into receiving water bodies. This was probably due to the longer retention time with the lower loading regime, which enhanced volatilization conditions because of the higher pH (figure 5.3).

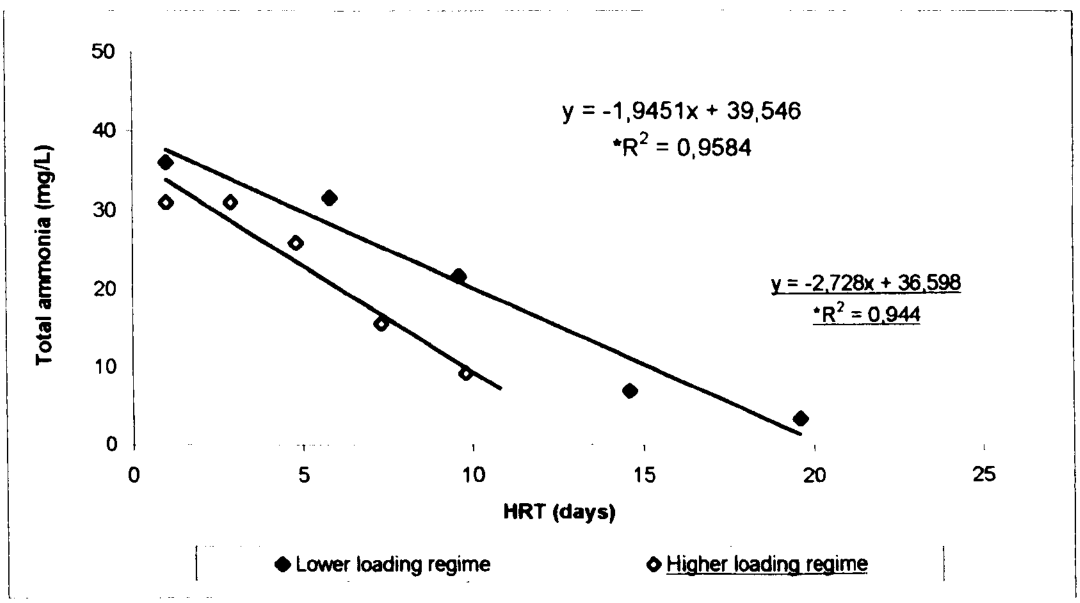


Figure 5.14. Total ammonia concentration plotted against HRT for both the lower and higher loading regimes in the innovative system including the anaerobic pond.

Table 5.1. Ammonia removal in system C

Loading on the secondary facultative pond ($kgBOD_5$/ha.d)	Ammonia in the raw sewage (mg/L)	Ammonia in the anaerobic pond (mg/L)	Ammonia in the final effluent (mg/L)	Removal efficiency (%)	Total hydraulic retention time in the series (days)
250	29	36	3	89,6	19,6
770	27	31	9	66,6	9,8

It was shown in table 5.1 that the concentration of ammonia increased from the raw sewage to the anaerobic pond. This was due to the bacteria activity in the decomposition of organic material in the sewage (Silva, 1982).

Figures 5.15 and 5.16 show the relationship between total ammonia and pH and chlorophyll *a* in the innovative pond series. It was seen the decrease in total ammonia concentration with the increase in pH, confirming the mechanism of volatilization as the main one responsible for total ammonia removal in these pond series. However, opposite to what happened in the 1.00m and 2.20m pond series, in the innovative system there was not a negative regression between total ammonia and chlorophyll *a* at both loading regimes.

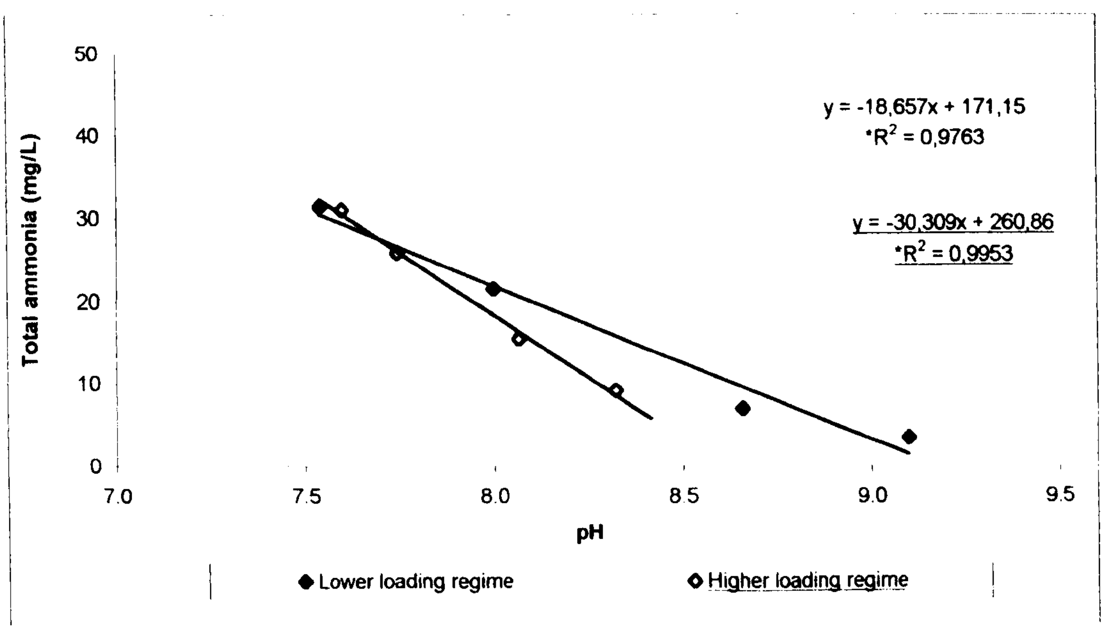


Figure 5.15. Total ammonia plotted against pH in the innovative pond series.

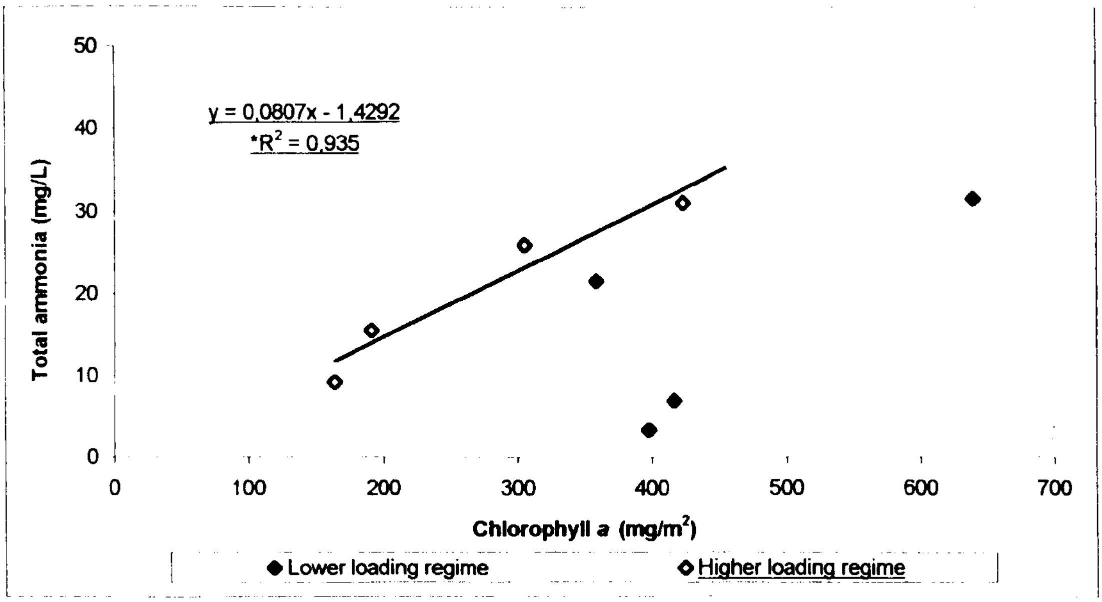


Figure 5.16. Total ammonia plotted against chlorophyll *a* in the innovative pond series.

Orthophosphate concentration decreased with the increase in HRT at both loading regimes (figure 5.17). Table 5.2 shows that, as was the case with ammonia, the best orthophosphate removal efficiency occurred at the lower loading regime probably as a consequence of increased precipitation at the higher pH (figure 5.18) since the algal biomass did not seem to have contributed to orthophosphate removal as is shown in figure 5.19. As with ammonia, orthophosphate concentration also increased in the anaerobic pond before decreasing through the series as a result of microbial mineralization particularly in the sludge layer which, due to neutral pH conditions, released orthophosphate into the water column (Silva *et al*, 1995).

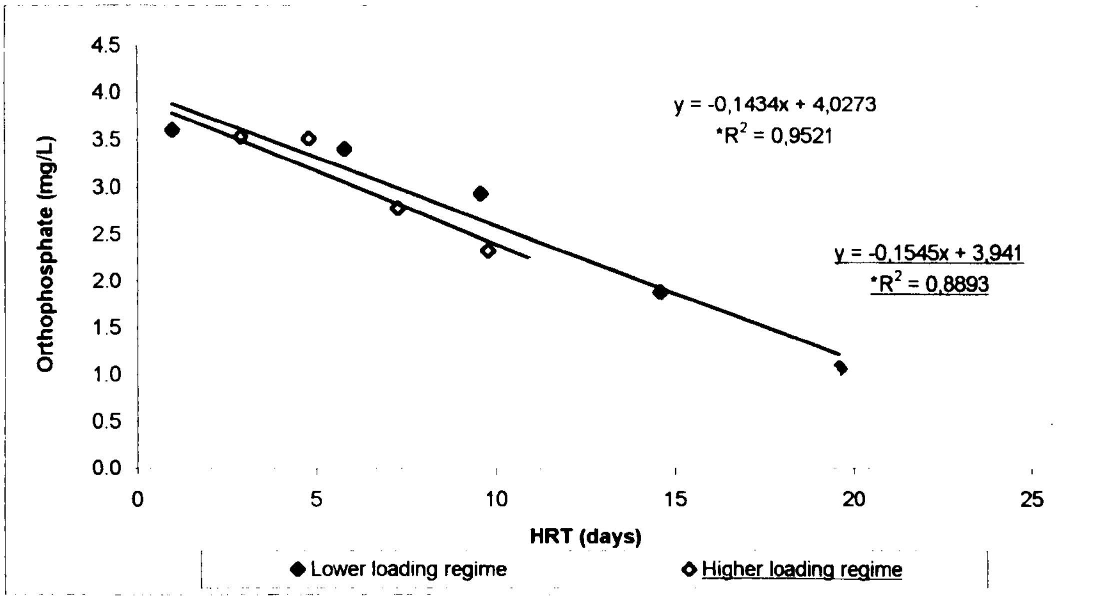


Figure 5.17. Orthophosphate concentration plotted versus HRT for both the lower and higher loading regimes in the innovative system including the anaerobic pond.

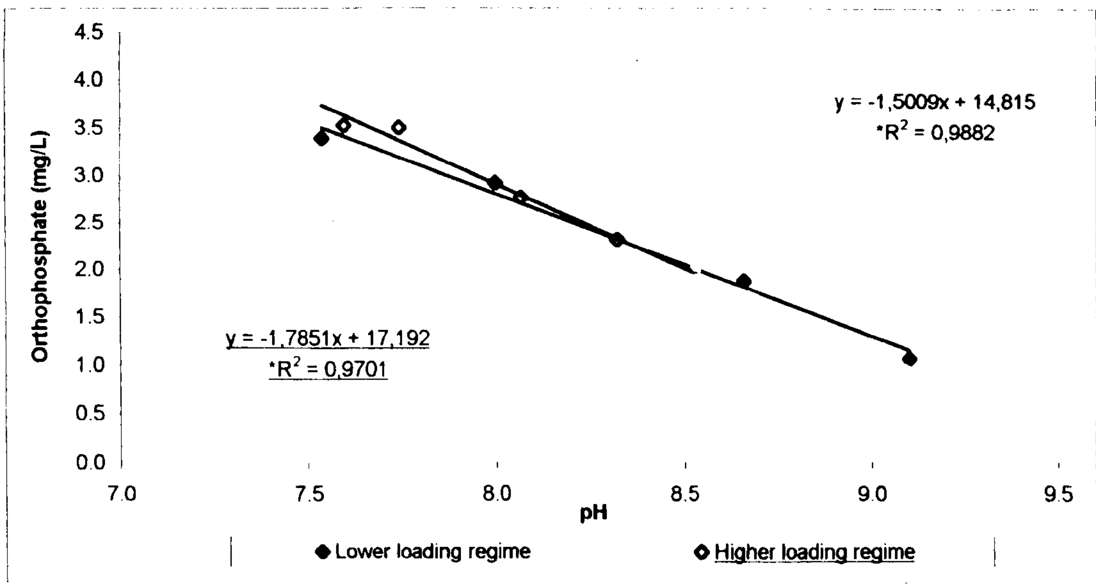


Figure 5.18. Orthophosphate concentration plotted versus pH for both the lower and higher loading regimes in the innovative system.

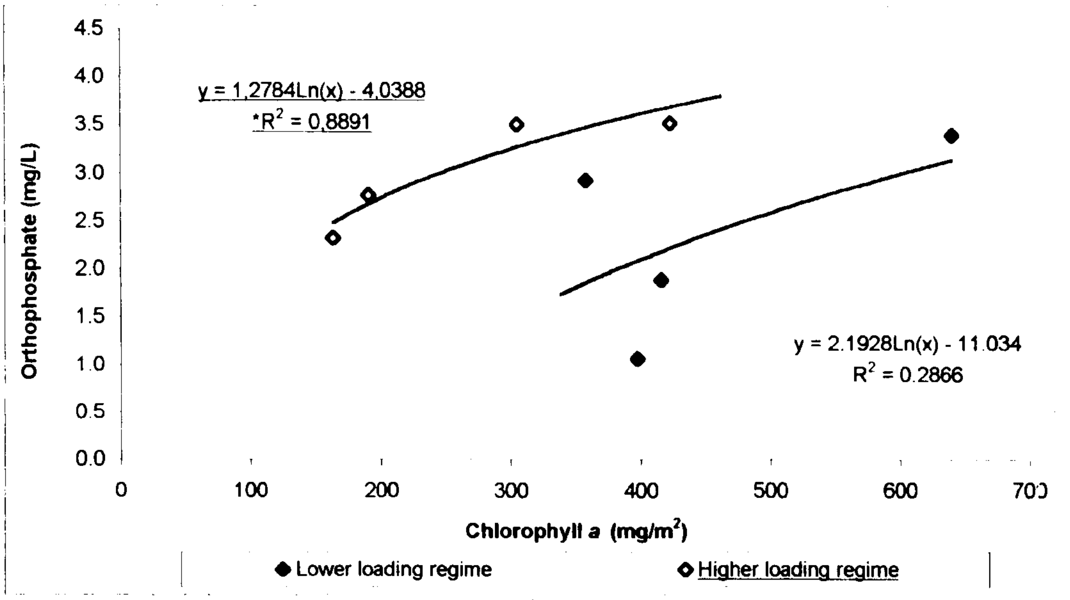


Figure 5.19. Orthophosphate concentration plotted versus chlorophyll *a* for both the lower and higher loading regimes in the innovative system.

Table 5.2. Orthophosphate removal in system C

Loading on the secondary facultative pond ($kgBOD_5$/ha.d)	Orthophosphate in the raw sewage (mg/L)	Orthophosphate in the anaerobic pond (mg/L)	Orthophosphate in the final effluent (mg/L)	Removal efficiency (%)	Total hydraulic retention time in the series (days)
250	2.2	3.6	1.1	50.0	19.6
770	2.6	3.6	2.3	11.5	9.8

5.4. Faecal coliform removal in three series of five ponds

The impact of depth on FC removal was compared in three pond series namely the shallow innovative system and the other two five pond series, one with ponds of 1.00m depth and the other with ponds of 2.20m depth, which were the subject of the previous chapter.

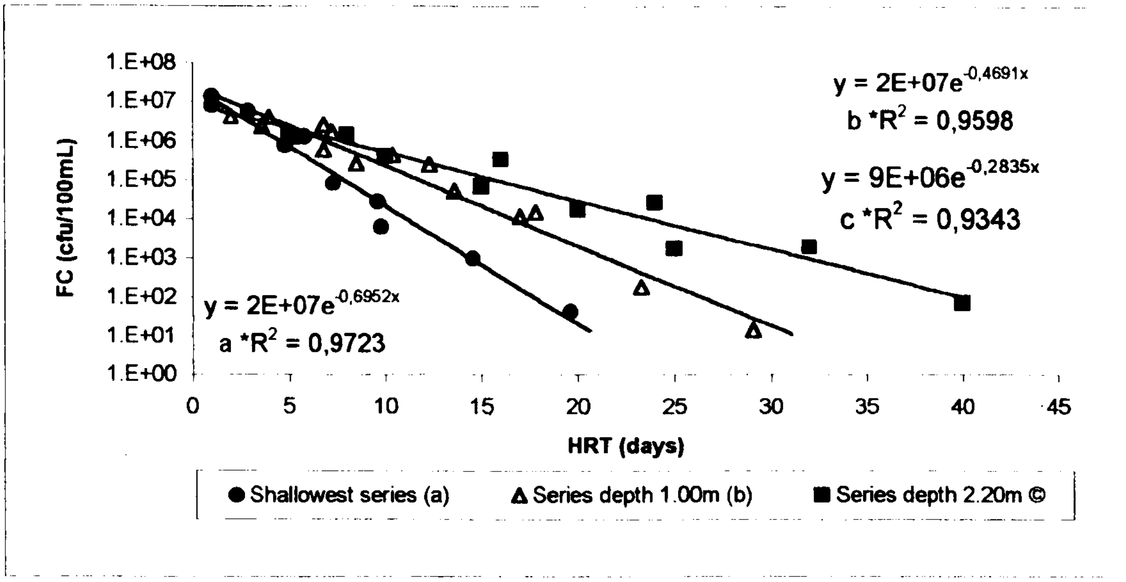


Figure 5.20. FC concentration plotted against hydraulic retention time (HRT) in the shallowest series (a), 1.00m deep series (b) and 2.20m deep series (c).

The results for figures 4.9 - and 5.4 showing the relationship between FC and HRT (including the data in the anaerobic pond) are combined in figure 5.20. In all three series FC concentration fell exponentially with time. The time necessary for the series to reach the concentration, for example, of 10^3 FC/100mL, recommended as a guideline by WHO (1989) for unrestricted irrigation varied with WSP depth. The innovative system with the shallowest maturation ponds was the fastest (14.2 days, to reach the above guideline concentration) whereas the deepest series required more than double the time (32.1 days) of the shallowest series. The 1.00m deep series achieved the desired concentration in 21.1 days.

Therefore, the results of these experiments demonstrated that depth had a clear effect on the efficiency of FC removal in pond series. According to Curtis (1990) depth is only an indirect factor on bacterial die-off rates, since the attenuation of solar radiation is cumulative with depth. As pointed out by this author, the decay rate of faecal bacteria in sewage treatment plants depends upon a number of factors, most of them high pH, high dissolved oxygen, increased temperature and photo-oxidation dependent on solar radiation, which in turn depend on the reactor depth.

Another factor acting in favor of the innovative system was its degree of sub-division, which reduces dead zones. The series from the innovative system comprised a total of 12 ponds. The sub-division of this series made the comparison with system B (five pond series) difficult.

In all three series, pH (figure 5.21) showed a significant negative correlation (at 5% level) with FC decay. However, while in the deepest series pH did not reach 8.0, in the tertiary maturation pond, M_{21} (see figure 2.2 in Materials and methods chapter) of the shallowest series; it reached 9.1.

In studying these series of ponds, it was observed that only in the 1.00m deep pond series there was a significant relationship between chlorophyll *a* and FC (figure 5.22), whilst in the other two no clear pattern could be determined. This might suggest that the effect of algae on FC removal was an indirect one through the impact of pH and dissolved oxygen concentrations (unfortunately dissolved oxygen data were not available), which would be affected by pond geometry. For example, if one accepts that surface light availability is a major factor controlling algal photosynthesis then deeper ponds will have relatively lager non-photosynthetic zones compared to shallow ones that will have a diluting effect on both pH and oxygen, thus reducing their levels. It would be interesting to know if their relationship would correlate more closely in artificial mixed ponds.

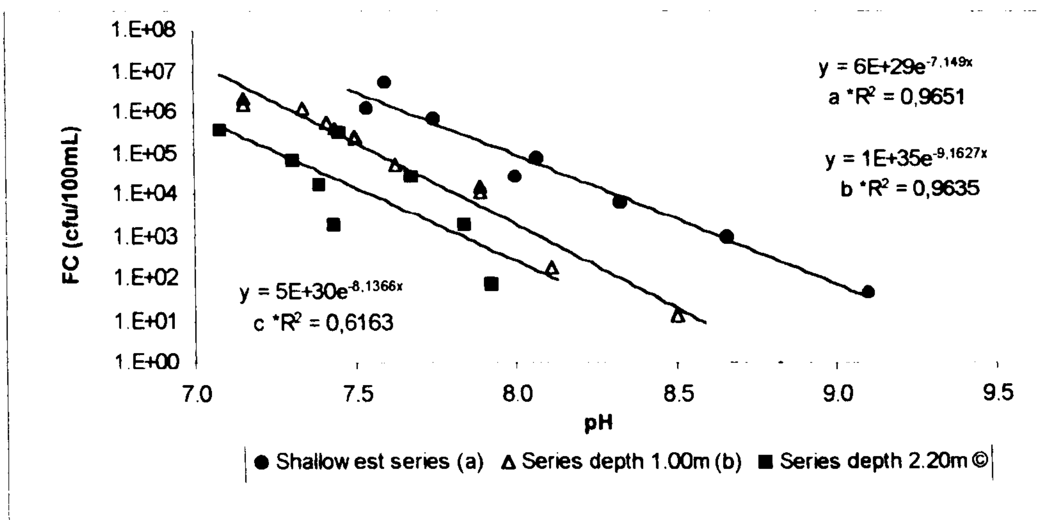


Figure 5.21. FC concentration plotted against pH in the shallowest series (a), 1.00m deep series (b) and 2.20m deep series (c).

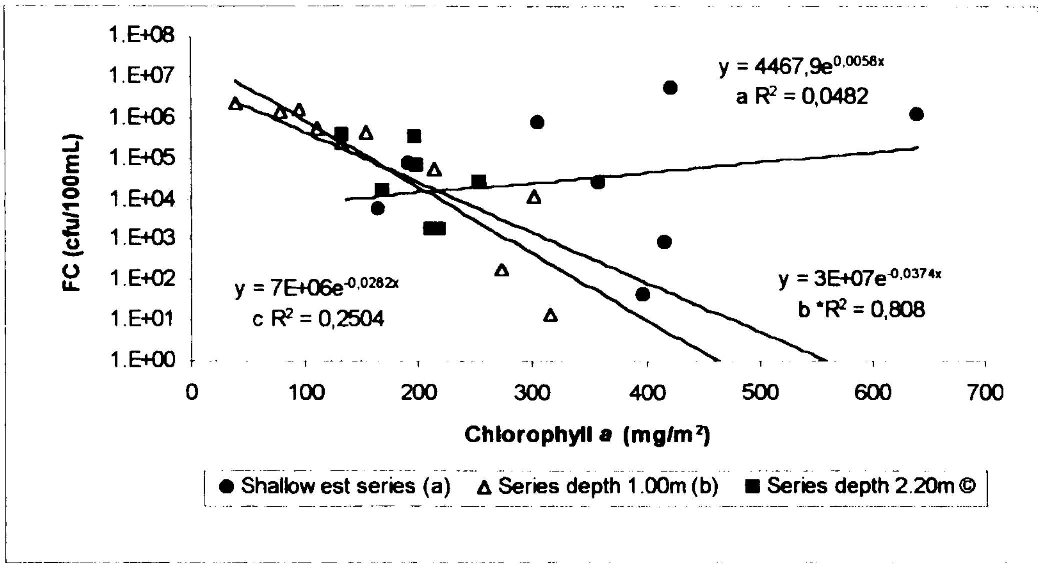


Figure 5.22. FC concentration plotted against chlorophyll *a* in the shallowest series (a), 1.00m deep series (b) and 2.20m deep series (c).

5.5. Algal biomass, expressed as chlorophyll *a*, in three series of five ponds

Algal biomass concentration (chlorophyll *a*) in this innovative system was compared with the two other series of ponds with depths of 1.00m and 2.20m studied in the previous chapter. As was mentioned before in chapter 2.0, in these series of ponds more than one level of organic loading was applied for the same depth and at both depths chlorophyll *a* dropped with the increased organic loading (figure 4.8). In contrast, in the innovative shallowest series with maturation ponds ranging from 1.00 down to 39cm the results for chlorophyll *a* concentration versus organic loading did not fit a continuum. Thus no clear pattern for chlorophyll *a* against surface organic loading at the lower loading regime could be seen (figure 5.6). However, at the higher loading (770 kgBOD$_5$/ha.day), chlorophyll *a* showed a significant (at the level of 5%) positive relationship with the organic loading, although the load applied was almost the double the maximum recommended (100 – 400 kgBOD$_5$/ha.day) for facultative ponds at a temperature of 25^{0}C (Mara & Pearson, 1998). Chlorophyll *a* concentration reached the highest values in the shallowest series (164 – 639 mg/m^2). In the other two series, chlorophyll *a* concentrations ranged between 39 – 315 mg/m^2 in the 1.00m deep pond series and 131 – 253 mg/m^2 in the 2.00m deep pond series.

In the pond series with depths of 1.00m and in 2.20m, the concentrations of H_2S seemed to be controlling chlorophyll *a* concentration (figure 4.9) whereas in the shallowest series, NH_3 seemed to be the controlling parameter (figure5.10). However, the concentration of NH_3 in this series was not high enough in terms of potential toxicity to algae, as explained before, and maybe this effect upon chlorophyll *a* concentration was a combination of other factors such as grazing.

5.6. Nutrient removal in three series of five ponds

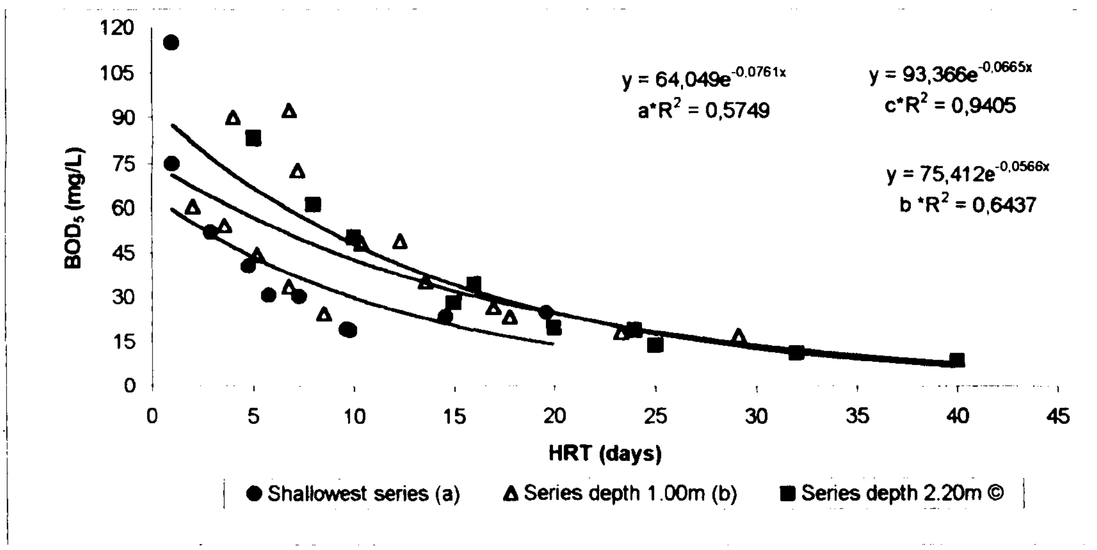
Comparing the results for nutrient removal in this innovative system with that obtained for the 1.00m and 2.20m deep pond series, it was observed that ammonia decrease with the increase in HRT in the three cases. According to the tables 4.2 and 5.1, the highest ammonia removal efficiency was found in the pond series with the shallowest maturation ponds (innovative system) under the lower loading regime with a total HRT of 19.6 days. This result was probably due to the shallow depth of the maturation ponds creating conditions for the ammonia volatilization (i.e. high pH). The 2.20m deep series of ponds, although operating with a longer HRT (i.e. 25 days), gave the lowest ammonia removal (19%) emphasizing the importance of depth and HRT. The degree of sub-division of the shallowest system also improved ammonia removal.

The best removal efficiency for orthophosphate (50%) was also found in the shallowest series of ponds at the lower organic loading regime. The high pH achieved in this series (9.1) suggests that orthophosphate removal was mainly by chemical precipitation (as hydroxyapatite). The 1.00m deep ponds series at the surface loading, in the anaerobic pond, of 1762 $kgBOD_5/ha.day$ ($\lambda v = 139 gBOD_5/m^3.day$) and the deeper pond series (2.20m) at the surface loading loadings of 765 $kgBOD_5/ha.day$ ($\lambda v = 35\ gBOD_5/m^3.day$) and 344 $kgBOD_5/ha.day$ ($\lambda v = 16\ gBOD_5/m^3.day$) there was a slight increase in orthophosphate concentration along the series of ponds.

5.7. BOD_5 removal in three series of five ponds

Figure 5.23 shows the regression between BOD_5 and HRT (including the anaerobic pond) in three pond series: shallowest series, 1.00m deep pond series and 2.20m deep pond series. The time necessary for these series reach the concentration, for example, of 25 mg/L recommended by the European Community (CEC,1991) for water discharge into rivers was 12.4; 19.5 and 19.8 days for the shallowest series, 1.00m deep pond series and 2.20m deep pond series respectively.

The shallowest series cannot be directly compared with the other two series, because besides depth there is another factor: the degree of sub-division of this series, which reduces dead zones. So, the results for BOD_5 removal in the 1,00m and 2.20m deep pond series suggest that deep ponds would normally be more appropriate for BOD_5 removal where land availability and costs are key factors.



5.23. Effluent BOD_5 plotted against HRT in the shallowest series (a), 1.00m deep series (b) and 2.20m deep series (c).

5.8. Assessment of efficiency of the five pond series

• In the case of FC removal, the trend of more efficient removal efficiency in shallow ponds was further accentuated in this innovative system with its shallow maturation ponds (1m – 0.39m depth). Since the geometry of the system is different to the five pond series described in Chapter. 4 a direct comparison cannot be made so as to determine whether the advantages shown by deeper ponds in terms of saving land area still holds when considering these ultra shallow ponds.

The data in figure 5.1 suggest that there is rapid die-off of FC at low organic loading and this correlates with high pH and good algal activity. Thus given that shallow maturation ponds had higher pH's than the deeper ponds there is an argument for making the last and cleanest pond in the series shallow i.e. 30-60cm to promote FC removal without significantly increasing land area since the previous ponds remain deeper.

• The shallow innovative system with maturation ponds ranging between 1-0.39m depth gave higher ammonia removal rates than the other two deeper five pond series. In all cases the lower the organic loading on the systems the higher the nitrogen (as ammonia) removal.

In the case of phosphorus, the same results were obtained i.e. shallow ponds gave better removal results and in fact in the 2.20m ponds no orthophosphate removal was observed.

These data show that shallow ponds, particularly shallow maturation ponds, will increase nutrient removal and this correlates with the higher pH's found there which increase ammonia volatilization and phosphorus precipitation. Therefore in terms of pond design these data would suggest perhaps a trade-off could be established in which the final one or two maturation ponds were very shallow i.e. 60cm in depth or less to ensure that good BOD and FC removals were accompanied by better nutrient removal efficiency.

5.9. Conclusions for chapter 5

• A positive relationship was found between FC concentration and organic loading. The decrease in load led to the decrease in FC concentration;

• No correlation was found between FC and chlorophyll *a* for both organic loading regimes in the innovative system;

• FC decay rate varied inversely with depth;

• The pH tended to increase along the innovative pond series under both loading regimes;

• The chlorophyll *a* concentration reached the highest values in the shallowest pond series (innovative system);

• There was no clear relationship between chlorophyll *a* and organic loading at the lowest loading regime in the innovative system, however at higher loading regime chlorophyll *a* increased with increasing organic loading. The reasons for this were not clear. In contrast in the 1.00m and 2.20m deep pond series, chlorophyll a concentration varied inversely with the superficial organic loading;

• H_2S seemed to be controlling chlorophyll a concentrations in the 1.00m and 2.20m deep pond series, but in the shallowest system NH_3 seemed to be the controlling parameter. Nevertheless NH_3 concentrations were not high enough, for instance > 13.2 mg/L, to cause 50% inhibition to the most sensitive algal genera, and maybe other factors were also affecting the algal biomass concentration;

• Algal biomass showed a positive correlation with BOD_5 only when it was expressed as mg/m^2 of chlorophyll *a*, but not when expressed as µg/l;

• There was not a significant relationship (at 5% level) between chlorophyll *a* and COD;

• Ammonia concentrations decreased with the increase of HRT at all depths of the ponds series;

• The highest ammonia removal efficiency (89.6% in 19.6 days) was found in the shallow system under the lower loading regime;

• Only the ammonia concentration in the final effluent (3mg/L) of the shallow system under the lower loading regime met Brazilian effluent standards (CONAMA,1986) of 5mg/L for effluent discharging into receiving water bodies;

• Orthophosphate concentration decreased with HRT along the pond series in the innovative pond series at both loading regimes;

•The best orthophosphate removal efficiency (50%) was observed in the shallowest pond series operated at the lower organic loading regime.

CHAPTER 6. ALGAL POPULATION IN THE INNOVATIVE POND SYSTEM

The nutrients (principally nitrogen and phosphorus) present in the WSP create an environment suitable for the development of algae. Variation in algal biomass and speciation in response to changes in physico-chemical and biological factors are essential features of an aquatic environment. The predominance of different algal species in response to changing ecological factors in WSP has been recognised for some time (Konig, 1984).

Algal growth and productivity in WSP are affected by many factors, such as solar radiation, temperature, organic loading and nutrient availability. According to Palmer (1969) organic pollution tends to influence the alga flora more than other factors in aquatic environments.

In the previous chapter, the interactions between such parameters as FC, chlorophyll *a*, sulphide and ammonia were considered in the shallow innovative five pond series. The physico-chemical data of this innovative series showed that parameters like ammonia and sulphide, which can be toxic to algae, were directly related to the surface organic loading.

As was described in the Materials and Methods chapter (section 2.0), this innovative system comprised other ponds, besides the ones considered in the five-pond series studied in the previous chapter. In fact this system comprised a total of 15 ponds, however 2 of them were not considered for this algal study since they were macrophyte and baffled ponds respectively. In the remaining 13 ponds of this pond system, distribution of algal genera in terms of diversity was investigated with a view to linking the presence of different algal genera to the degree of treatment achieved under the two organic loadings regimes of 250 and 770 $kgBOD_5/ha.d$ respectively applied to the secondary facultative ponds. The anaerobic ponds were not considered, as they do not generally contain algae.

Besides the measurement of chlorophyll *a* (discussed in the previous chapter) the phytoplankton biomass of the innovative shallow system was also investigated in terms of algal genera and number of cells. The samples for algal identification, counts and chlorophyll *a* were obtained at the same time as those for physico-chemical analyses (i.e. 08:00 to 9:00h.). The relative frequencies of different algal genera present in this system are determined from presence and absence data recorded for each pond during each different loading regime.

6.1. Algal diversity and biomass

The algal genera, and their classification into family, order and division for the two organic loading regimes of the innovative pond system, are presented at tables 6.2 and 6.3 respectively.

At the lower loading regime, the number of flagellate genera ranged from 6-8 and the non-flagellate ranged from 4 -11 depending on the pond (table 6.2). A total of 23 different genera was found in the system (table 6.1). At the higher loading regime, again taking the system as a whole, there was again a range of 6-8 genera of flagellate algal types and 7-14 non-flagellate genera (table 6.3). A total of 28 different genera was found in the system (table 6.1). Thus, it was observed a greater algal diversity at the higher loading principally amongst the non-flagellate species.

Six non-flagellate algal genera (*Gomphosphaeria, Anacystis, Zygnema, Fragilaria, Gomphonema and Nitzchia*) were found at the higher loading regime but not in the lower one. The genus *Gomphosphaeria* was found only in the highest loaded ponds (770 and 271kgBOD$_5$/ha.day), but with low frequencies. The genera Anacystis and Zygnema were present in all ponds of the system with surface organic loading ranging from 73 to 770kgBOD$_5$/ha.day. *Fragilaria* was found at the same loading range , but not at all ponds of the system. The genus *Nitzchia* was present only at the ponds loaded at 104 and 770kgBOD$_5$/ha.day whereas the algae *Gomphonema* was observed in the "cleanest" pond, the maturation pond loaded at 73 kgBOD$_5$/ha.day. Only one algal genus, *Spirogyra*, (with low frequency) was observed only at the lower loading regime and basically in the "cleanest" ponds i.e. the maturation ponds with low surface loading (25 – 71 kgBOD$_5$/ha.day).

When considering just the five secondary facultative ponds (F_{21}-F_{25}), they were equally loaded, in the first loading regime, they received 250 kgBOD$_5$/ha.d and in the second one 770 kgBOD$_5$/ha.d. These facultative ponds varied in depths from 1.00m to 2.00m. At the lower loading regime, the number of algal genera ranged between 10-14. No marked difference between the maximum number of flagellate and non-flagellate species could be seen, i.e. 7 in each case.

When these facultative ponds were loaded at 770 $kgBOD_5/ha.d$, the total number of algal genera increased ranging between 13 and 16. Here again, no great difference between the maximum number of flagellate and non-flagellate species could be seen (i.e. 7 and 9 respectively). However, at the higher loading regime, there was a slight increase in the total number of genera compared to the lower loading regime, despite the fact that the surface organic loading regime was almost double the maximum recommended, of 100 - 400 $kgBOD_5/ha.d$ for facultative ponds at a temperature of 25°C (Mara & Pearson, 1998). This high load therefore did not seem to be inhibitory to algal growth.

Table 6.1. Algal genera present at the innovative pond series under the lower and higher loading regime.

Algae	Lower loading regime	Higher loading regime
1.Cyanobacteria		
Gomphosphaeria	-	+
Anacystis	-	+
Oscillatoria	+	+
Arthrospira	+	+
Spirulina	+	+
2. Euglenophyta		+
Euglena	+	+
Phacus	+	+
3. Chlorophyta		+
Chlamydomonas	+	+
Chlorogonium	+	+
Pyrobotrys	+	+
Pandorina	+	+
Eudorina	+	+
Chlorella	+	+
Ankistrodesmus	+	+
Oocystis	+	+
Coelastrum	+	+
Scenedesmus	+	+
Micractinium	+	+
Dictyosphaerium	+	+
Closterium	+	+
Micrasterias	+	+
Zygnema	-	+
Spirogyra	+	-
4. Chrysophyta		
Cyclotella	+	+
Navicula	+	+
Fragilaria	-	+
Gomphonema	-	+
Nitzchia	-	+
5. Crytophyta		
Rhodomonas	+	+

The facultative ponds F_{21} - F_{24} and F_{25} have different geometries in terms of depth and length to breadth ratios, however this difference in geometry did not appear to have affected algal diversity. There was also no clear difference in the total number of algal genera between the facultative ponds of different geometries.

The secondary maturation ponds M_{16} to M_{19} also received equal organic loads albeit different ones at the different loading regimes, but their depths varied from 0.90 to 0.39m. At both loading regimes, there was again no marked difference in the maximum number of algal genera that would suggest any influence of depth, between the depth range of 0.90 to 0.39m, on algal diversity. There was predominance of non-flagellate algal genera over flagellate ones in these ponds confirming their maturation nature (Mara *et al*, 1992).

In relation to algal diversity, the maximum number of genera in the primary maturation pond M_{15} at the lower loading regime was similar to the secondary facultative ponds. However, there was difference in the algal types. The genera *Coelastrum* and *Micractinium* were found only in the primary maturation pond while *Cyclotella* and *Rhodomonas* in the secondary facultative ponds. At the higher loading regime, M_{15} behaved closer to the following secondary maturation ponds (M_{16-19}). Nevertheless, there was again differences in algal types; such as the genera *Gomphosphaeria* and *Cyclotella* were found only in the primary maturation pond (M_{15}) while *Spirulina, Chlorogonium, Closterium, Navicula, Fragilaria* and *Nitzchia* in the secondary maturation ponds.

The tables 6.2 and 6.3 show that the algal division, which predominated at both high and low loading regimes, was the Chlorophyta. According to Canter-Lund & Lund (1995) this algal division contains a larger number of genera and species than any other algal group and embraces a wide variety of structural forms. This algal division was considered by Palmer (1969) as the most pollution-tolerant algal group.

In the Chlorophyte division, the algal genera *Chlamydomonas* and *Pyrobotrys* showed the highest relative frequency in the ponds with the highest loadings under both loading regimes, but decreased in this frequency through the pond series. The algal genera *Chlorella* and *Scenedesmus* showed the opposite behaviour with their frequency increasing with the decrease in loading under the lower loading regime. At the higher loading regime, the same pattern was observed but was not as obvious. At the higher loading regime, *Scenedesmus* first appeared in M_{15}, the primary maturation pond, which received a loading comparable to the secondary facultative

Table 6.2 - Relative frequency of the algal genera (%) present in the innovative system – lower loading regime (surface organic loading in $kgBOD_5/ha.d$).

Algae/Ponds	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22
Organic loading	250	250	250	250	250	80	25	25	25	25	71	28	28
1- Cyanobacteria													
Chroococcales													
Chroococcaceae													
Gomphosphaeria **													
Anacystis **													
Oscillatoriales													
Oscillatoriaceae													
Oscillatoria **	83	78	67	33	50	87	76	80	89	87	93	85	57
Arthrospira **										2			
Spirulina **								2	2	4		2	
2- Euglenophyta													
Euglenales													
Euglenaceae													
Euglena *	98	100	96	93	93	100	100	100	91	98	98	98	100
Phacus *	46	24	24	44	44	61	28	13	20	15	39	2	2
3- Chlorophyta													
Volvocales													
Chlamydomonadaceae													
Chlamydomonas *	93	78	94	74	89	39	13	7	2	9	22	9	7
Chlorogonium *								2	2	4	4	4	4
Spondylomoraceae													
Pyrobotrys *	83	72	87	83	74	65	50	39	28	26	41	11	13
Volvocaceae													
Pandorina *	35	37	37	46	37	74	52	57	54	50	70	28	22
Eudorina *	11	15	9	13	13	63	61	59	74	72	54	35	39
Chlorococcales													
Oocystaceae													
Chlorella **	26	11	9	43	28	83	98	100	93	98	98	100	100
Ankistrodesmus **	7			2		46	54	65	61	65	54	67	67
Oocystis **	24	7	7	44	46	74	67	54	74	60	70	48	54
Scenedesmaceae													
Coelastrum **						4	2	13	7	13	7	13	11
Scenedesmus **	7	7	2	9	9	13	60	63	76	80	41	74	78
Micractiniaceae													
Micractinium **						4	7	9	9	9	9		2
Dictyosphaeriaceae													
Dictyosphaerium **				4		11	9	9	26	26	17	20	15
Zygnematales													
Desmidiaceae													
Closterium **											7		
Micrasterias **	4						4			2			4
Zygnemataceae													
Zygnema **													
Spirogyra **							4				7		
4- Chrysophyta													
Centrales													
Coscinodiscaceae													
Cyclotella **				2									
Pennales													
Naviculaceae													
Navicula **											2		
Fragilariaceae													
Fragilaria **													
Gomphonemaceae													
Gomphonema **													
Nitzschiaceae													
Nitzchia **													
5- Cryptophyta													
Cryptochrysidaceae													
Rhodomonas *		2		2			20	13	11	13	7	17	15
Non-flagellate genera	6	4	4	7	4	8	10	9	9	11	11	8	9
Flagellate genera	6	7	6	7	6	6	7	8	8	8	8	8	8
Total N° of genera	12	11	10	14	10	14	17	17	17	19	19	16	17
Cyanobacteria	1	1	1	1	1	1	1	2	2	3	1	2	1
Euglenophyta	2	2	2	2	2	2	2	2	2	2	2	2	2
Chlorophyta	9	7	7	9	7	11	13	12	12	13	14	11	13
Chrysophyta	0	0	0	1	0	0	0	0	0	0	1	0	0
Cryptophyta	0	1	0	1	0	0	1	1	1	1	1	1	1

* Flagellate algae

* * Non-flagellate algae

Table 6.3 - Relative frequency of algal genera (%) present in the innovative system –higher loading regime (surface organic loading in $kgBOD_5/ha.d$).

Algae/Ponds	F21	F22	F23	F24	F25	M15	M16	M17	M18	M19	M20	M21	M22
Organic loading	770	770	770	770	770	271	104	104	104	104	300	73	73
1- Cyanobacteria													
Chroococcales													
Chroococcaceae													
Gomphosphaeria **	4				4	9							
Anacystis **	30	26	22	30	30	17	13	22	13	30	18	4	26
Oscillatoriales													
Oscillatoriaceae													
Oscillatoria **	91	83	87	87	91	96	91	100	74	100	100	74	74
Arthrospira **	4	4				4			4	4		4	4
Spirulina **								9					
2- Euglenophyta													
Euglenales													
Euglenaceae													
Euglena *	100	100	100	100	100	100	100	96	100	100	100	96	91
Phacus *	22	23	52	30	30	100	87	96	65	87	86	57	57
3- Chlorophyta													
Volvocales													
Chlamydomonadaceae													
Chlamydomonas *	48	43	48	57	52	52	26	22	17	17	27	9	4
Chlorogonium *								4			5		
Spondylomoraceae													
Pyrobotrys *	83	100	96	91	96	87	43	61	43	30	82	17	13
Volvocaceae													
Pandorina *	52	48	48	26	43	70	30	35	44	43	45	30	13
Eudorina *	13		4	9	4	44	43	30	61	35	27	26	43
Chlorococcales													
Oocystaceae													
Chlorella **	39	39	39	48	61	48	61	57	61	52	50	57	39
Ankistrodesmus **	9			4		13	13	26	22	26	32	43	39
Oocystis **				9	17	17	17	13	13	9	27	4	9
Scenedesmaceae													
Coelastrum **	30	39	26	30	48	22	4	13	4		9		
Scenedesmus **						4	4	9	4	30	18	26	13
Micractiniaceae													
Micractinium **						4	4		13	13		4	13
Dictyosphaeriaceae													
Dictyosphaerium **						9	9	17	30	17	9	22	17
Zygnematales													
Desmidiaceae													
Closterium **							4				5		
Micrasterias **	4												
Zygnemataceae													
Zygnema **	26	26	17	9	22	48	52	57	35	48	50	17	34
Spirogyra **													
4- Chrysophyta													
Centrales													
Coscinodiscaceae													
Cyclotella **						4							
Pennales													
Naviculaceae													
Navicula **					4		17	17	9	9	14	13	4
Fragilariaceae													
Fragilaria **		4	9	4	4		9	17	13	4	14	9	4
Gomphonemaceae													
Gomphonema **												4	
Nitzschiaceae													
Nitzchia **			4						4	4			
5- Cryptophyta													
Cryptochrysidaceae													
Rhodomonas *	43	30	22	13	26	44	61	39	48	30	36	48	
Non-flagellate genera	9	7	7	8	9	13	13	12	14	13	12	13	12
Flagellate genera	7	6	7	7	7	7	7	8	7	7	8	7	6
Total N° of genera	16	13	14	15	16	20	20	20	21	20	20	20	18
Cyanobacteria	4	3	2	2	3	4	2	3	3	3	2	3	3
Euglenophyta	2	2	2	2	2	2	2	2	2	2	2	2	2
Chlorophyta	9	6	7	9	8	12	13	12	12	11	13	11	11
Chrysophyta	0	1	2	1	2	1	2	2	3	3	2	3	2
Cryptophyta	1	1	1	1	1	1	1	1	1	1	1	1	0

* Flagellate algae

* * Non-flagellate algae

ponds under the lower loading regime suggesting the sensitivity of this genus to higher organic loadings.

Palmer (1969), who studied the presence of algae in water polluted with organic wastes based on the results of 165 field workers, classified the chlorophytes *Chlamydomonas*, *Scenedesmus* and *Chlorella* among the top 8 pollution-tolerant genera. In this study, amongst the chlorophytes mentioned by Palmer only *Chlamydomonas* showed resistence to organic loading. According to Lund & Lund (1995) *Chlamydomonas* is the largest genus, with over 400 species. The species of this genus are encountered more frequently than any other members of the Volvocales and are to be found in a great variety of habitats (Prescott, 1980). *Chlorella* and *Scenedesmus* seemed not to be resistant to organic loading, which is in agreement with the results of Llorens *et al* (1993) when studying the primary productivity and production measurements and their relationship to the variations in nutrients (N and P), CO_2, pH, temperature, dissolved oxygen and solar radiation in a 8m deep sewage stabilization pond.

The algal frequency results obtained in this pond system suggested the flagellate algal genera *Pyrobotrys* as one of the most resistant to organic loading up to 770kgBOD5/ha.d.

The algal genus *Ankistrodesmus* also seemed to be affected by high organic loadings increasing in frequency with the decrease in loading, in accordance with de Noyelles (1967) who said that this algal genus and *Scenedesmus* are found in environments when the levels of organic loading were reduced from those favouring *Chlamydomonas*.

For reasons that are unclear, the genus *Zygnema*, not present under the lower loading was present in all the ponds under the higher loading.

The division Euglenophyta was represented by only two algal genera, *Euglena* and *Phacus*, which were present in all the ponds under both loading regimes. Lund & Lund (1995) stated that although Euglenophytes are common in water rich in organic matter, cells of *Phacus*, *Euglena* and *Trachelomonas* are commonest. Even those, which can photosynthesise, need organic nutrients, hence their abundance in places rich in rotting animal or planting matter. It is often difficult or impossible to grow Euglenophytes in the laboratory in the absence of bacteria, which, presumably, produce the nutrients they need in the process of decomposing organic matter.

The abundance of the Euglenophyta in sewage ponds can be related to high average concentrations of oxidizable organic matter and high concentrations of free carbon dioxide (Munowar, 1970; Konig, 1984). Alternatively, Provasoli (1958), Provasoli and Pintner (1960) and Konig (1984) have suggested that the distribution of *Euglena*, which utilises ammonia as its sole source of inorganic nitrogen may be more dependent upon high levels of ammonia in the sewage than it is on carbon sources.

In Palmer's list (1969) of the 60 most pollution-tolerant algal genera in order of decreasing emphasis based on 165 authorities, *Euglena* heads the list with 172 points. In the algal study of the innovative system, this resistance of *Euglena* to high organic loadings was confirmed, as it was found even at the surface organic loading of 770kgBOD$_5$/ha.day with maximum ammonia concentration of 31 mg/L. Thus *Euglena* presented the highest frequencies in the ponds of highest loadings and highest ammonia concentration, and in fact, seemed to be ubiquitous showing a high frequency (more than 50%) in all the ponds under both loading regimes.

The genus *Phacus* was also present in all the ponds under both loading regimes. Nevertheless, its frequency ranged from 2 to 61 at the lower loading regime and from 22 to 100 in the higher one. However, at both loading regimes this genus reached the highest frequencies at a similar loading range, i.e. between 71-250 kgBOD$_5$/ha.day at the lower loading regime and 73-271 kgBOD$_5$/ha.day at the higher loading regime.

The algal division Cyanobacteria was represented by only three algal genera: *Oscillatoria*, *Arthrospira* and *Spirulina* in the lower organic loading regime. In the higher loading regime, besides these three genera, two cyanobacterial genera appeared: *Gomphosphaeria* and *Anacystis*. However, the genus *Oscillatoria* was the only one to be present in all ponds under both loading regimes confirming the ubiquitous nature of this genus and its resistance to high organic loading as stated by Palmer in whose list it occupies the second position below Euglena. Lund & Lund (1995) stated that *Oscillatoria* is an extremely common genera, though its planktonic species are absent in many oligotrophic waters.

The algal divisions Chrysophyta and Cryptophyta were not well represented in the pond system (especially under the lower loading regime). The division Cryptophyta was represented by only one genera namely *Rhodomonas*. At the lower loading regime, this genus reached the highest frequencies at the loading range 25-28 kgBOD$_5$/ha.day, and at the higher loading regime between 73-300 kgBOD$_5$/ha.day.

The frequency data gave only a general indication of the importance of various algal genera occurring in the innovative pond system. A few algal genera were only found very rarely which made it difficult to relate their presence to the environmental conditions prevailing in the ponds, e.g. *Arthrospira, Spirulina, Closterium, Spirogyra, Cyclotella, Navicula, Gomphonema , Nitzchia* (tables 6.2 and 6.3) . In order to see the impact of organic loading on the algal biomass in terms of total algal numbers (biomass) the algal genera *Euglena, Oscillatoria, Scenedesmus, Chlamydomonas, Chlorella, Pyrobotrys, and Ankistrodesmus*, that were present at both loading regimes and represented the most common genera, were plotted against organic loading. The results for algal counts were transformed into $\log_{10}$ [(cell + 1)/mL]. Just as the pond series at different loadings were plotted as a continuum for physico-chemical data, the same approach was used for algae.

The figures 6.1 and 6.2 show that the algal biomass [(log cells +1)/mL] of *Euglena* and *Oscillatoria* did not change with the organic loading under either loading regime, confirming the frequency data that these algal genera were ubiquitous in the ponds at different organic loadings. No significant statistical relationship between the loading and algal/cyanobacterial biomass could be found.

The algal genera *Chlamydomonas* and *Pyrobobotrys* increased in cells numbers with the increase in organic loading under both loading regimes, confirming their resistance to high organic loadings (figures 6.3 and 6.4) suggested by the frequency data.

According to figures 6.5 and 6.6, where algal biomass was plotted against organic loading combining the result for both loading regimes, the algal genera *Scenedesmus*, and *Ankistrodesmus* showed a tendency to decrease in numbers with increasing organic loading. In the case of Chlorella, the situation was less clear with cell numbers tending to decrease slightly with increased loading although the results were not statistically significant.

The results of algal diversity and counts showed that different algae had different tolerance to organic loading. With the decrease of organic loading, algae that adapted better to conditions of low organic matter concentration replaced the algae more tolerant to organic pollution. Nevertheless, it was also seem that there were algae, which could survive at conditions of high and low organic loadings.

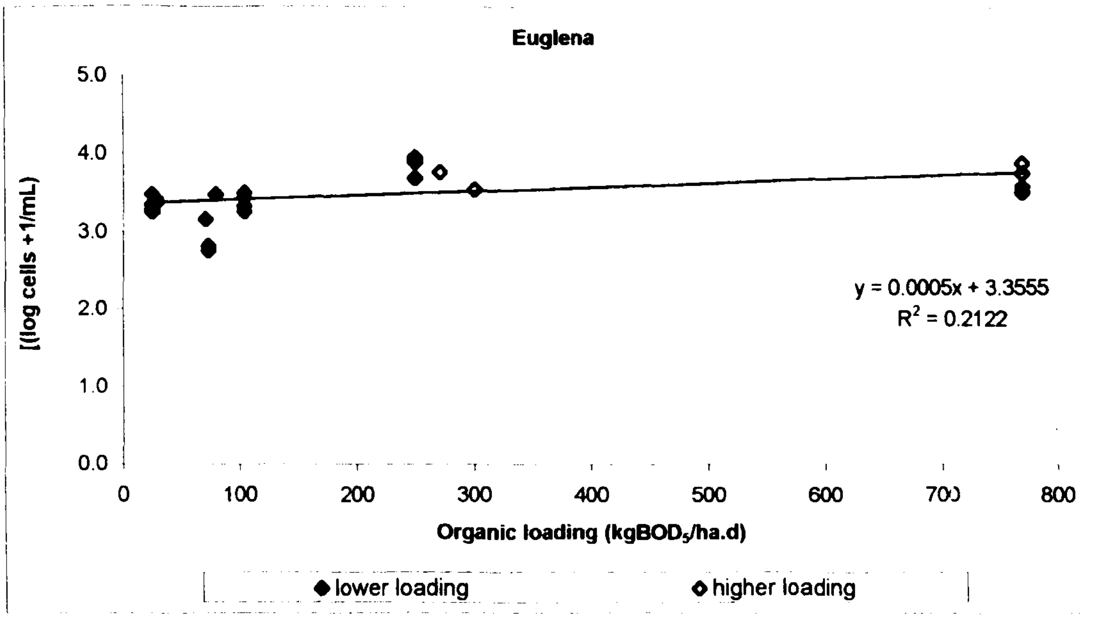


Figure 6.1. *Euglena* biomass plotted against surface organic loading under both lower (250 kgBOD_5/ha.d) and higher (770 kgBOD_5/ha.d) loading regimes applied to the five pond series of the innovative pond system.

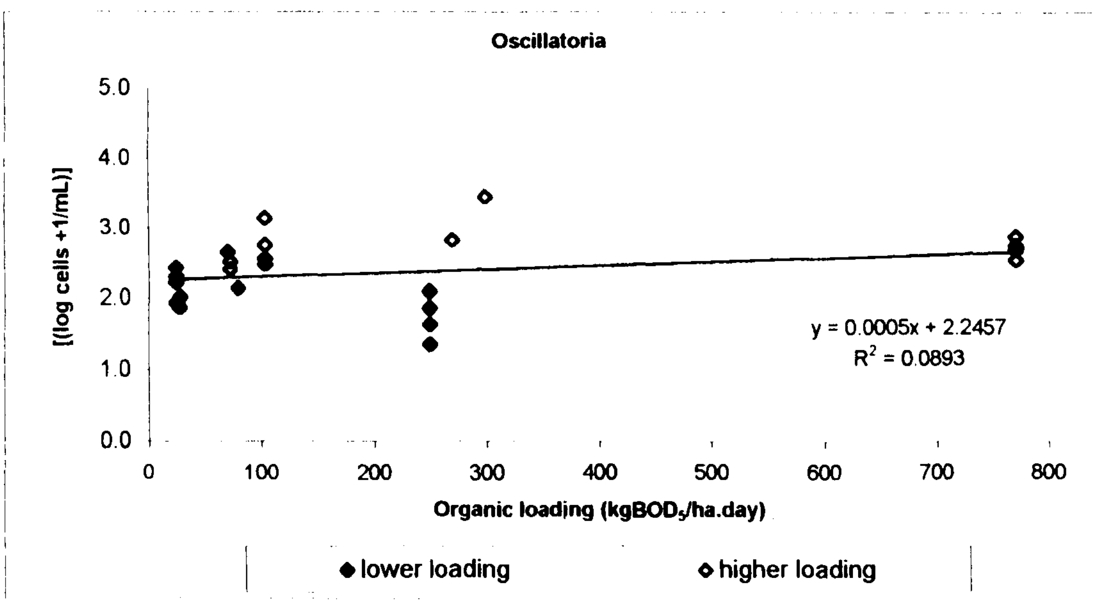


Figure 6.2. *Oscillatoria* biomass plotted against surface organic loading under both lower and higher loading regimes applied to the five pond series of the innovative pond system.

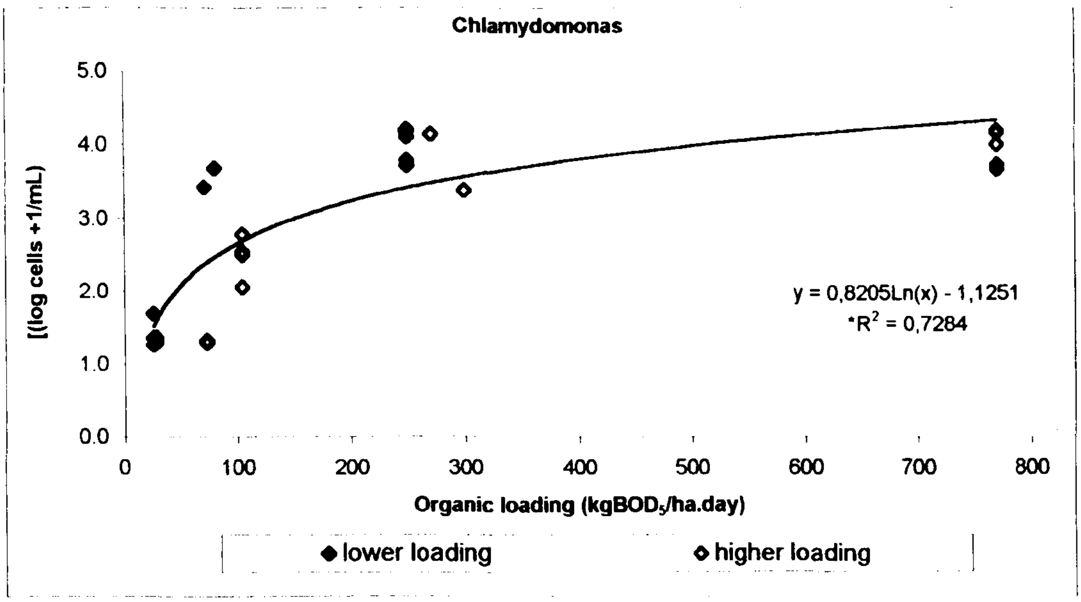


Figure 6.3.Chlamydomonas biomass plotted against surface organic loading under both lower and higher loading regimes applied to the five pond series of the innovative pond system.

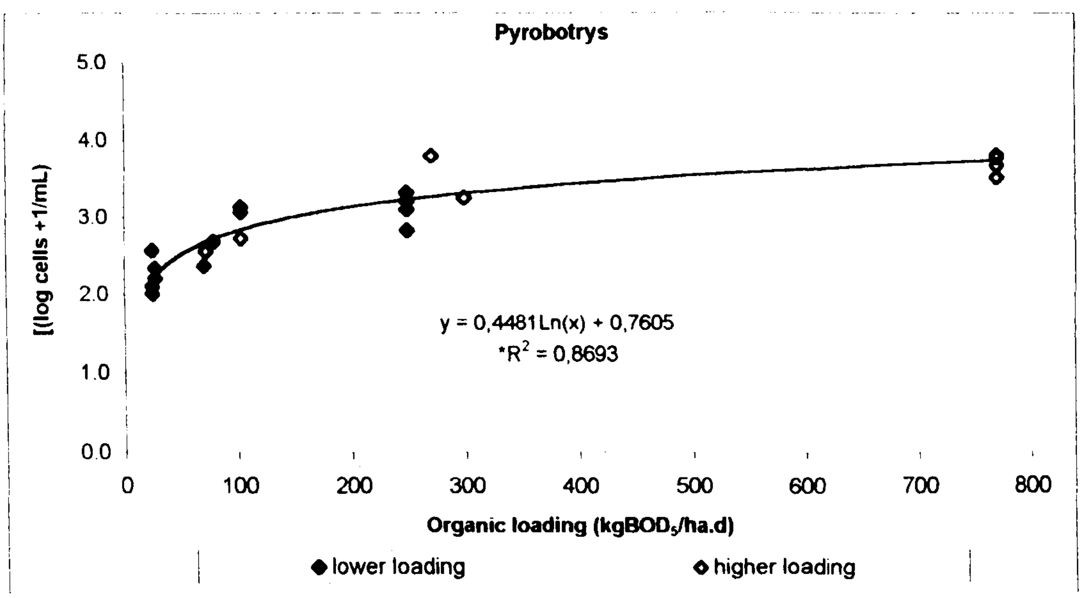


Figure 6.4. *Pyrobotrys* biomass plotted against surface organic loading under both lower and higher loading regimes applied to the five pond series of the innovative pond system.

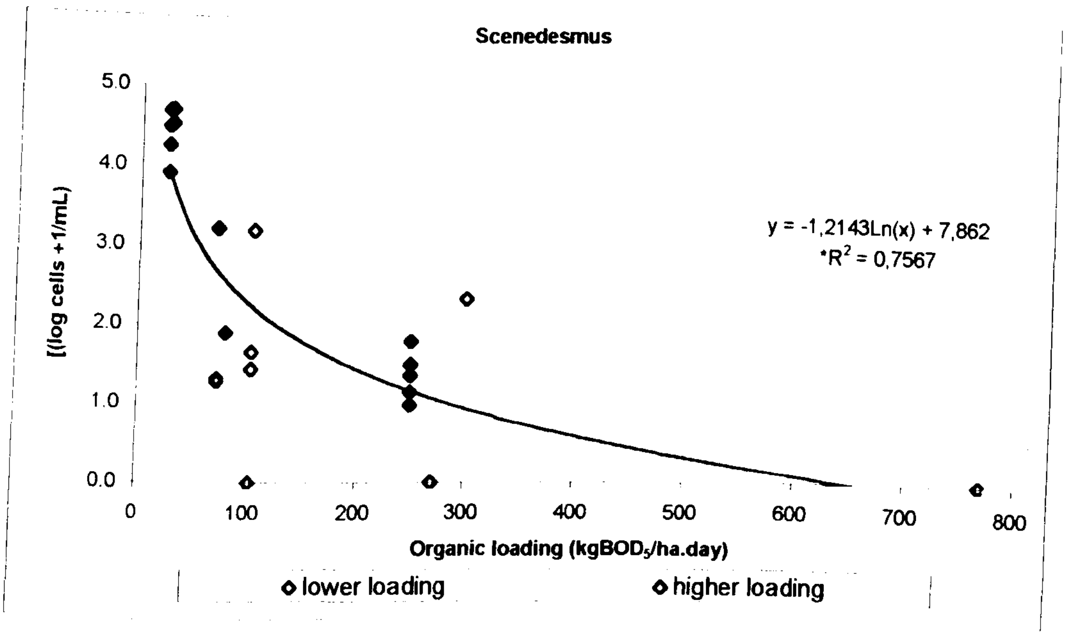


Figure 6.5. *Scenedesmus* biomass plotted against surface organic loading under both lower and higher loading regimes applied to the five pond series of the innovative pond system.

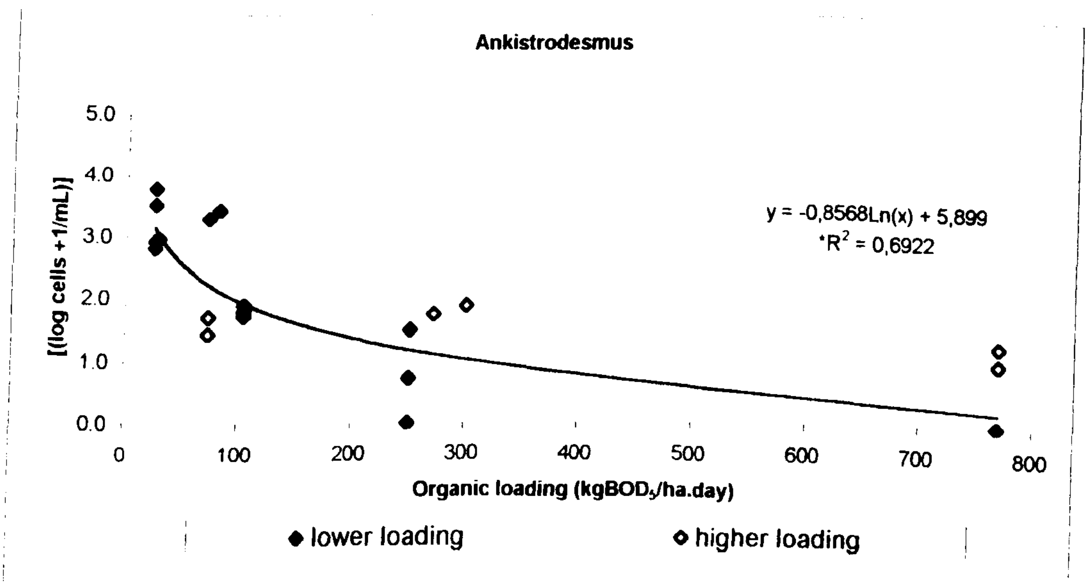


Figure 6.6. *Ankistrodesmus* biomass plotted against surface organic loading under both lower and higher loading regimes applied to the five pond series of the innovative pond system.

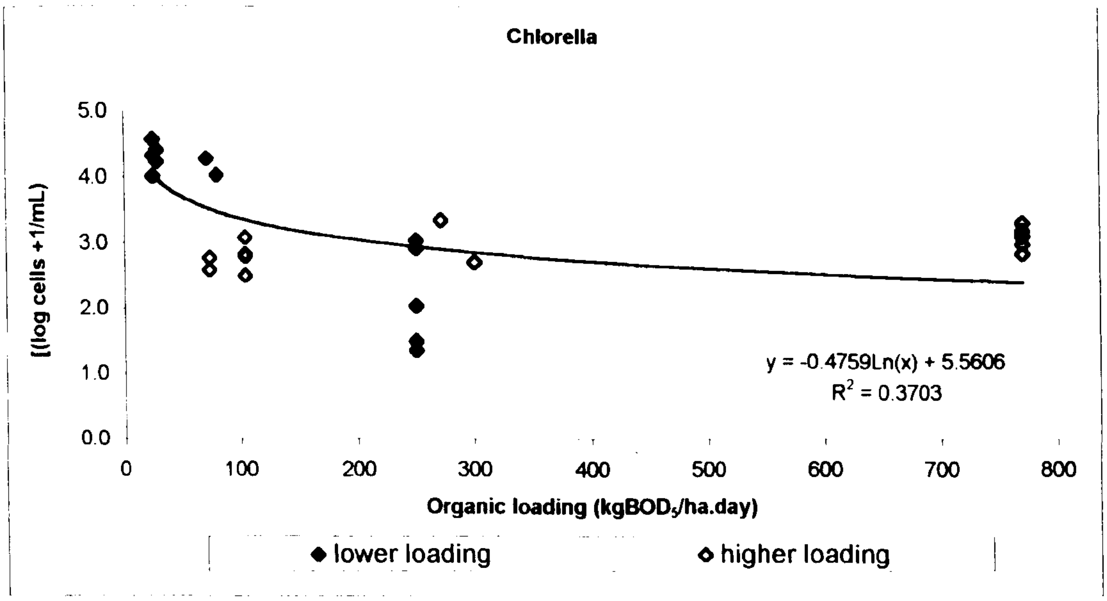


Figure 6.7. *Chlorella* biomass plotted against surface organic loading under both lower and higher loading regimes applied to the five pond series of the innovative pond system.

Figures 6.1 to 6.7 show the biomass concentration for seven algal genera in relation to the variations in surface organic loading. Besides these seven genera, figures 6.8 (*a* and *b*) and 6.9 (*a* and *b*) show, in a general way, the biomass (expressed as arithmetic mean) of other algae and their relationship with pond type in the innovative five pond series. The figure for each loading regime was split in two (*a* and *b*). In the first one, all the algae are shown; in the second one (b) the genera with higher cell numbers are not included so that the scale can be altered to show more clearly the variation in algae present at lower concentrations.

In figure 6.8 a *Chlorella* more clearly exhibited its preference for lower organic loading than was apparent in figure 6.7; presenting the highest biomass in the tertiary maturation pond, M_{21} loaded at 28 $kgBOD_5$/ha.d; and in figure 6.9a where a higher loading was applied to the ponds, it was also present but at lower concentrations.

Scenedesmus was present in greater numbers in the secondary and tertiary maturation ponds at the lower loading regime (figure 6.8a). However, it was virtually absent at the higher loading regime (figure 6.9b).

Ankistrodesmus, in figures 6.8 and 6.9, again showed its sensitivity to high organic loadings being found predominantly in the maturation ponds at the lower loading regime and virtually absent at the higher loading regime.

At both loading regimes, the genus *Oscillatoria* was present at all ponds although presenting higher number at the higher loading regime.

The algae *Chlamydomonas, Pyrobotrys* and *Euglena* showed higher cell numbers at the secondary facultative ponds and primary maturation ponds at both loading regimes.

The genus *Phacus*, present at both loading regimes, reached its highest biomass at the primary and secondary maturation ponds under the higher loading regime.

The decrease in loading along the series led to the appearance of other genera like *Eudorina* and *Dictyosphaerium* in the maturation ponds when the system was submitted to the lower loading regime. When the highest loading was applied, algae like *Pandorina, Rhodomonas, Fragilaria* and *Navicula* were found in the maturation ponds.

The figures 6.8 and 6.9 shows, under both loading regimes, the algal succession in the five pond series of the innovative system as a result of the treatment achieved at each stage. At the ponds higher loaded the genera more tolerant to high organic loading predominate, in the following stage with the decrease of organic loading (especially at the maturation ponds M_{16} – M_{18} and M_{21}) algae more sensitive to organic loading substitute the previous ones.

Figure 6.8a. Algal biomass concentration along the five pond series of the innovative system at the lower loading regime, 250 kgBOD$_5$/ha.d on the secondary facultative pond (all the algae included).The organic loading is expressed in kgBOD$_5$/ha.d.

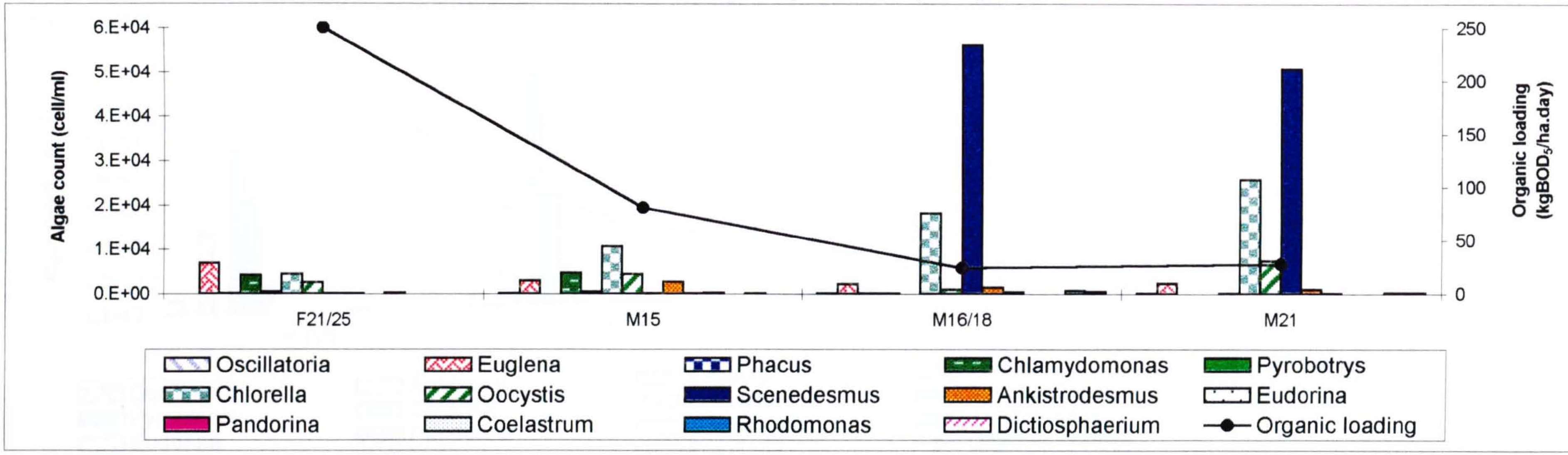
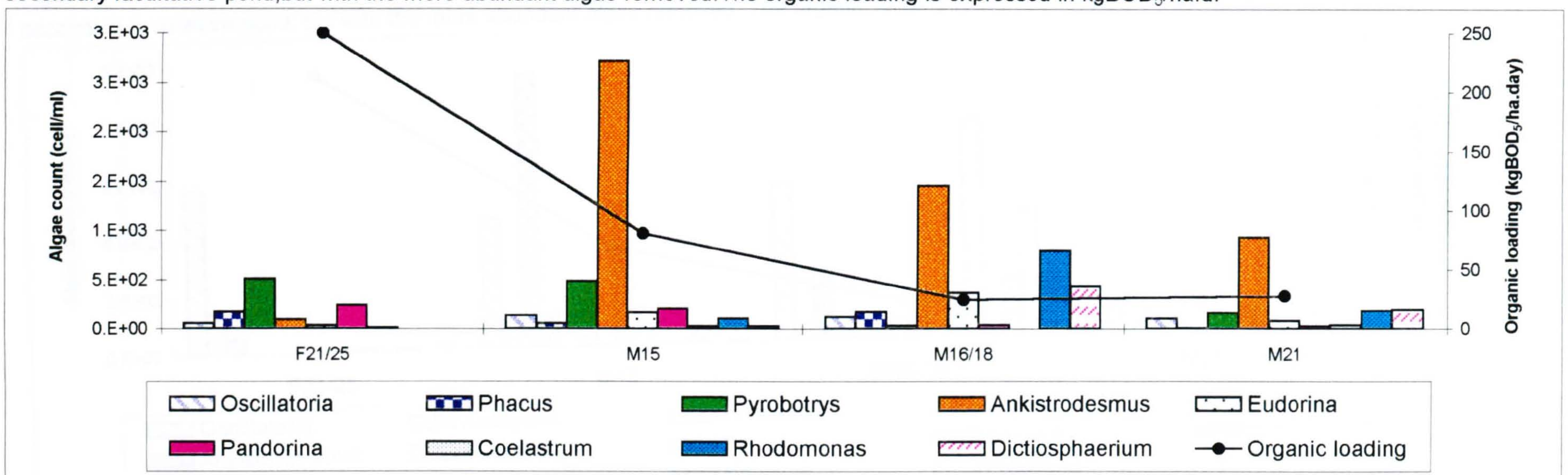


Figure 6.8b. Algal biomass concentration along the five pond series of the innovative system at the lower loading regime, 250 kgBOD$_5$/ha.d on the secondary facultative pond,but with the more abundant algae removed.The organic loading is expressed in kgBOD$_5$/ha.d.



$F_{21/25}$ (250 kgBOD$_5$/ha.day) - M_{15} (80 kgBOD$_5$/ha.day) - $M_{16/18}$ (25 kgBOD$_5$/ha.day) - M_{21} (28 kgBOD$_5$/ha.day)

Figure 6.9a. Algal biomass concentration along the five pond series of the innovative system at the higher loading regime, 770 kgBOD_5/ha.d on the secondary facultative pond (all the algae included). The organic loading is expressed in kgBOD_5/ha.day.

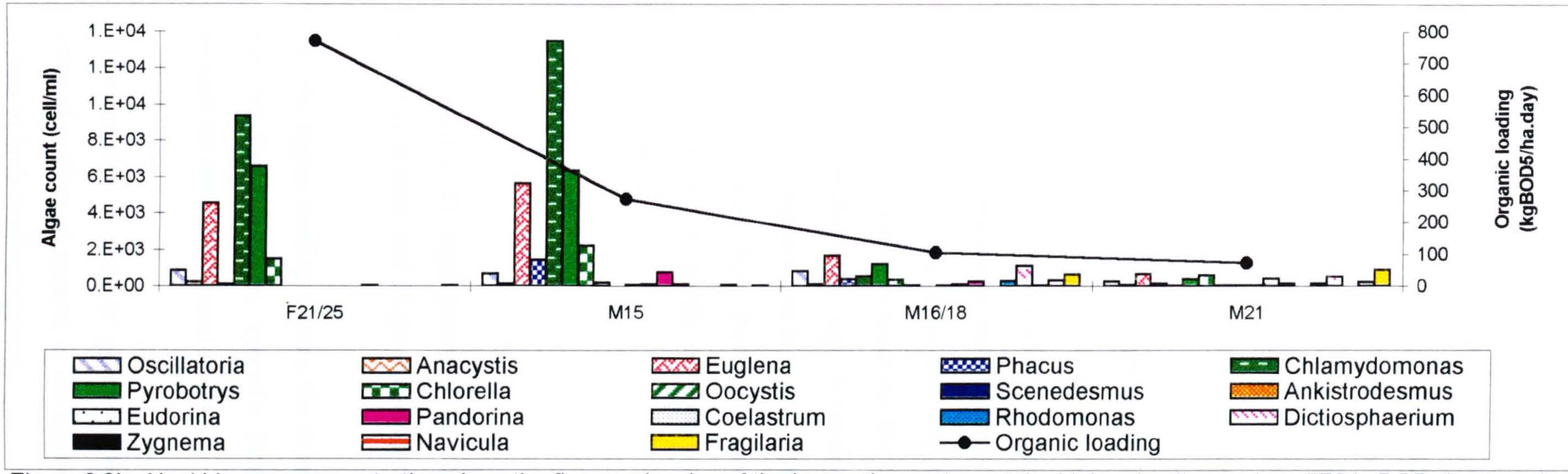
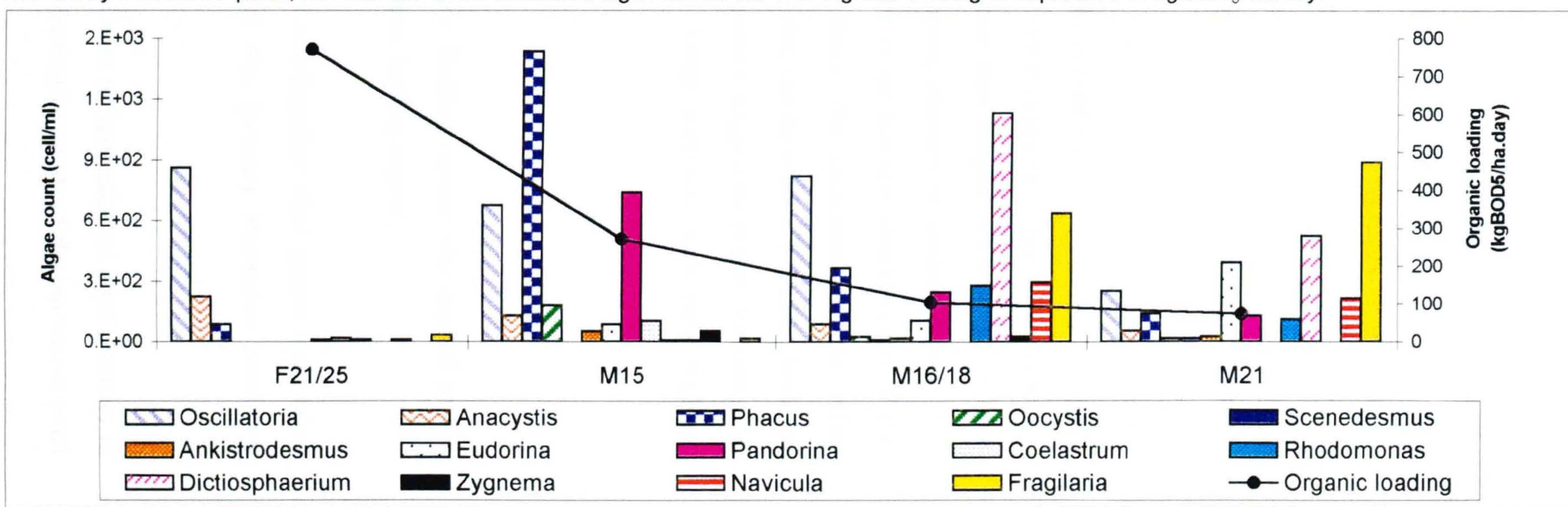


Figure 6.9b. Algal biomass concentration along the five pond series of the innovative system at the higher loading regime, 770 kgBOD_5/ha.d on the secondary facultative pond, but with the more abundant algae removed. The organic loading is expressed in kgBOD_5/ha.day.



$F_{21/25}$ (770 kgBOD_5/ha.day) - M_{15} (271 kgBOD_5/ha.day) - $M_{16/18}$ (104 kgBOD_5/ha.day) - M_{21}(73 kgBOD_5/ha.day)

6.2. Assessment of efficiency

In this chapter, it was explored the role of algal diversity in enabling us to predict pond performance. The advantage of being able to link the dominance of algal genera to treatment efficiency and changes in treatment efficiency is that this would provide a more rapid and less expensive assessment than the necessity of monitoring a series of physico-chemical parameters. Additionally, changes in algal diversity might provide an early warning of loss in pond performance and thus loss in effluent quality. So, that corrective measures could be taken before environmental damages occurs.

In this study, the frequency data could not be used as a tool to determine treatment efficiency, although there were algal genera only present in either low loaded regime (i.e. *Spirogyra*) or higher loaded regime (i.e. *Gomphosphaeria, Anacystis, Zygnema, Fragilaria, Gomphonema and Nitzchia*). However their frequencies were low to be a useful measure. Where algae can help determining changes in loading regime in a particular pond system is studying the changes in the biomass concentration of the genera *Chlamydomonas* and *Scenedesmus*. Since one increases and the other decreases with the increase in organic loading, they can be used as a double check. In this study, unlike others, there was no clear pattern suggesting higher frequency of flagellate genera in ponds of high loading. These contrasts with early findings (Pearson,1987).

6.3. Conclusions

• There were 23 algal genera when the lower loading regime was applied to the innovative system, and 28 genera under the higher loading regime;

• At the higher loading regime the number of non-flagellate algal genera was higher than the flagellate ones, specially from pond M_{15} (primary maturation pond) on through the maturation pond series;

• The variation in pond or geometry did not seem to have affected algal diversity in terms of number of genera, in the secondary facultative ponds of the innovative pond system at both loading regimes;

• In the maturation ponds M_{16}-M_{19}, equally loaded, the depth range of 0.39-0.90 did not seem to have affected algal diversity under either loading regime. There was predominance of non-flagellate algal genera in these ponds confirming their maturation state;

• The algal division Chlorophyta predominated in the pond system irrespective of the loading regime;

• The division Euglenophyta was represented by two genera *Euglena* and *Phacus*, which were present in all the ponds of the series under both loading regimes;

• The Cyanobacteria were represented at the lower loading regime by the three genera: *Oscillatoria*, *Arthrospira*, and *Spirulina*. At the higher loading, two additional cyanobacterial genera appeared namely *Gomphosphaeria* and *Anacystis*;

• The algal divisions Crysophyta and Cryptophyta were not represented by many genera, especially under the lower loading regime. At the low loading regime, the division Crysophyta was represented by *Cyclotella* and *Navicula*, which showed low frequencies and the division Cryptophyta by the genus *Rhodomonas*. At the higher loading regime, three additional genera belonging to the division Chrysophyta were found: *Fragilaria*, *Gomphonema* and *Nitzchia*.

• The algal genera *Euglena*, *Chlamydomonas* and *Pyrobotrys* showed resistance to high organic loadings. The highest biomass (in terms of cell numbers) of these algae was found at the secondary facultative ponds and primary maturation ponds at both loading regimes;

• *Scenedesmus* and *Ankistrodesmus* were sensitive to high organic loadings and presented higher biomass in the maturation ponds at the lower loading regime. At the higher loading regime, they were virtually absent;

• The genus *Chlorella* exhibited its preference for lower organic loading showing the highest biomass under the lower loading regime in the tertiary maturation pond, M_{21}, loaded at 28 $kgBOD_5$/ha.day. *Chlorella* was also present at the higher loading regime, but with lower biomass;

• The decrease in loading along the series led to the appearance of other genera at both loading regimes. At the lower loading regime *Eudorina* and, *Dictyosphaerium* were found in the maturation ponds. At the higher loading regime, also in the maturation ponds, the genera *Pandorina, Rhodomonas, Fragilaria,* and *Navicula* were observed.

7. WASTEWATER STORAGE AND TREATMENT RESERVOIRS – WSTR

The effluent from sewage treatment plants is approximately constant all the year round, however the water demand for agricultural purposes increases mainly in the dry season. To avoid the wastage of this effluent, the use of wastewater storage and treatment reservoirs (WSTR) to both treat and store wastewater for use when necessary has shown good results. Although the use of WSTR is already common practise in some countries, studies regarding their performance, as a sewage treatment unit are still relatively scarce, most of them being reported from Israel, for example Juanico and Shelef, 1991, 1994).

The removal of faecal coliform from the three WSTR described in the Material and Methods chapter was the basis of another PhD thesis (Athayde Jr, 1999). This mentioned work showed that FC decay during the filling phase of batch-fed WSTR was very reduced or nil, and thus, for the removal of these bacteria, these reactors should be filled as quickly as possible, subject to the maximum organic loading that would not cause odour production. The highest loading employed in this study was 667 $KgBOD_5/ha.d$, corresponding to a filling time of 15 days and no odour emissions were noticed. In the resting phase, FC decay rate decreased exponentially with time. The natural death of FC with time was the most significant contribution to the k_b value.

In this chapter, the relationship between algal biomass (expressed as chlorophyll *a*) and related parameters such as sulphide and ammonia in three 6.50 m deep batch-fed WSTR operating under different loading regimes will be considered. In a total, eight different experiments were performed to observe WSTR performance. From the eight experiments, in three of them the WSTR were fed with raw wastewater (RW) and in the other five with anaerobic pond effluent (APE). The APE and the RW were not studied in this work but the basic physico-chemical characteristics of the raw sewage and anaerobic pond effluent are presented in table 7.1 as taken from Athayde, Jr. (1999). The physical and operational characteristics of the eight experiments performed on the WSTR were described in the Materials and Methods chapter (Chapter 2).

Table 7.1. Characterisation of the RW and APE from the city of Campina Grande.

		FC (cfu/100mL)	BOD_5 (mg/L)	COD (mg/L)	TS (mg/L)	SS (mg/L)
RW	95% conf. LL	1.84E7	118	321	1113	169
	mean	2.47E7	134	374	1174	191
	95% conf. UL	3.31E7	152	426	1235	215
	sample size	52	45	42	46	47
APE	95% conf. LL	5.82E6	83	224	1009	57
	mean	8.10E6	98	263	1051	67
	95% conf. UL	1.13E7	116	302	1093	78
	sample size	28	27	29	27	27

Table 7.2 shows the surface organic loadings applied to the reservoirs in the different experiments.

Table 7.2. Surface organic loading (λs) applied to the WSTRs.

WSTR	Experiment	λs ($kgBOD_5$/ha.d.)
I*	I	178
	II	376
	III	117
II*	I	124
	II	667
III**	I	111
	II	265
	III	292

* Filled with APE

** Filled with RW

7.1. Daily variation of parameters in the WSTR

The studies on diurnal variations were carried out on one WSTR at one loading (667 $kgBOD_5/ha.d$) because of limitation on resources.

The pH, dissolved oxygen (DO), temperature and Chlorophyll *a* are dependent on the light incidence and penetration through the water column. However, there was no significant relationship between chlorophyll *a* concentrations and pH and DO (figures 7.1 and 7.2). In order to see the behaviour of each of these parameters in daily cycles, 24-hours profiles were performed. Figures 7.3 to 7.10 presented examples of typical 24-hour profiles of temperature, pH, DO, and chlorophyll *a* respectively. A weighted mean was calculated for these parameters concentrations in the water column at each sampling time.

The maximum temperature recorded during the 24-hour experiments was 30 °C at the 5cm level at 12:00h and 16:00h, and the minimum temperature recorded at any depth was 24 °C after 18:00h at different depths. The mean temperature for the water column measured at 8:00h when the WSTR were still completely mixed (see below) never differed more than 1°C from the mean temperature computed from measurements at various depths for the whole day, and thus can be considered representative for the whole day.

During day hours the thermocline developed at different positions, but always in the top 200cm. During the night, the water column was homogeneous (no temperature gradient) or the upper layers were cooler. These cooler upper layers forced them towards the bottom of the reservoirs, due to its higher specific weight, so causing mixing (turnover) of the reservoir contents. The time of the turnover was between 22:00h and 24:00h and stayed until 6:00h.

The maximum pH found in the 24-hour daily profiles was 8.6 at the 25cm level at 16h; and the minimum pH, 7.0, was found at 600cm at 20h. The maximum difference between the mean pH value for the 8:00h water column and the mean pH for the whole profile was less than 0.5, indicating that the mean pH for the water column at 8h was representative for the whole day.

The DO concentration reached 20mg/L (the maximum reading on the YSI model 54A DO meter), at the 5 and 25cm depths at 16:00h. Maximum DO values always occurred in the top 75cm, generally between 12h and 16h. The minimum DO concentration was

0 mg/L, and occurred without any pattern of location, but never at the surface during day light hours. The mean concentration of DO for the water column at 8:00h was very close to the mean for the whole day.

The highest values of chlorophyll *a* were found in the top 200cm usually during high light intensities (i.e. before 16:00h). The lowest chlorophyll *a* values were found irregularly along the WSTR depths. There was no marked variation in chlorophyll *a* concentration of the WSTR column through the day in the 24-hour profiles. The 8:00h samples could therefore be considered representative for the day.

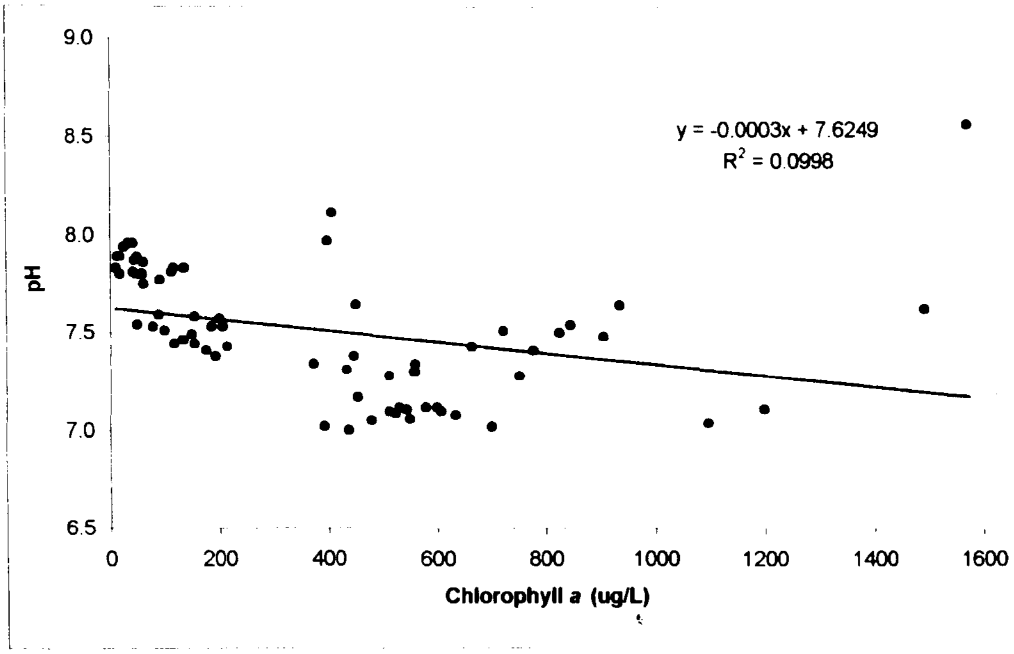


Figure 7.1- Variation of pH against chlorophyll *a* in the 24-hour profiles performed in the WSTR2E2 (667 kgBOD$_5$/ha.day).

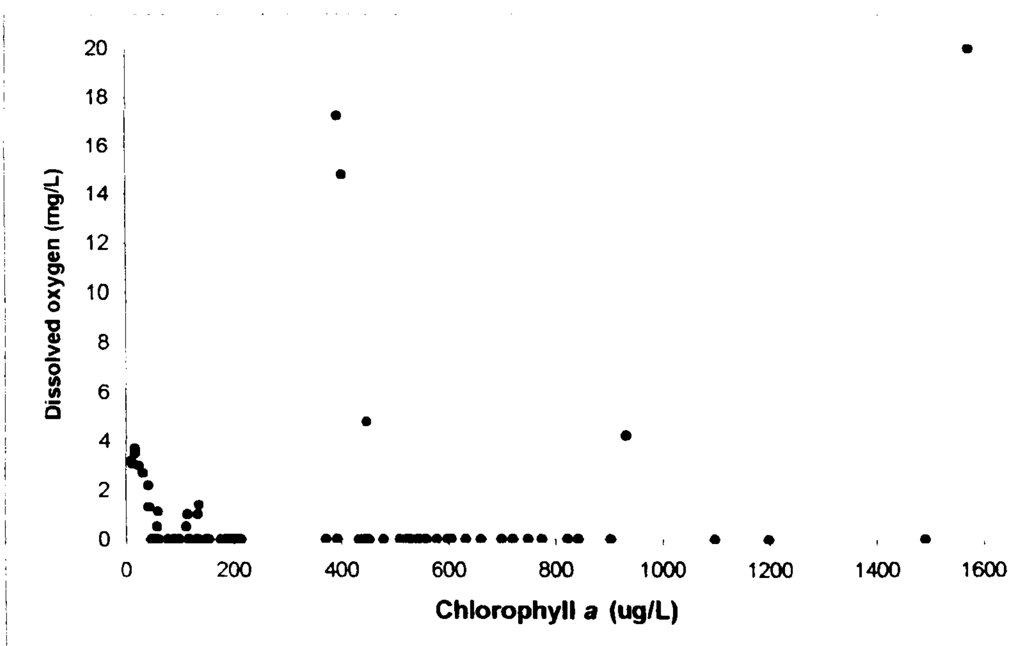


Figure 7.2- Variation of DO against chlorophyll *a* in the 24-hour profiles performed in the WSTR2E2 (667 kgBOD$_5$/ha.day).

Figure 7.3. Temperature variations in the 24h-hour profile - WSTR2E2 (667 $kgBOD_5/ha.day$) -56 days from the beggining of filling phase.

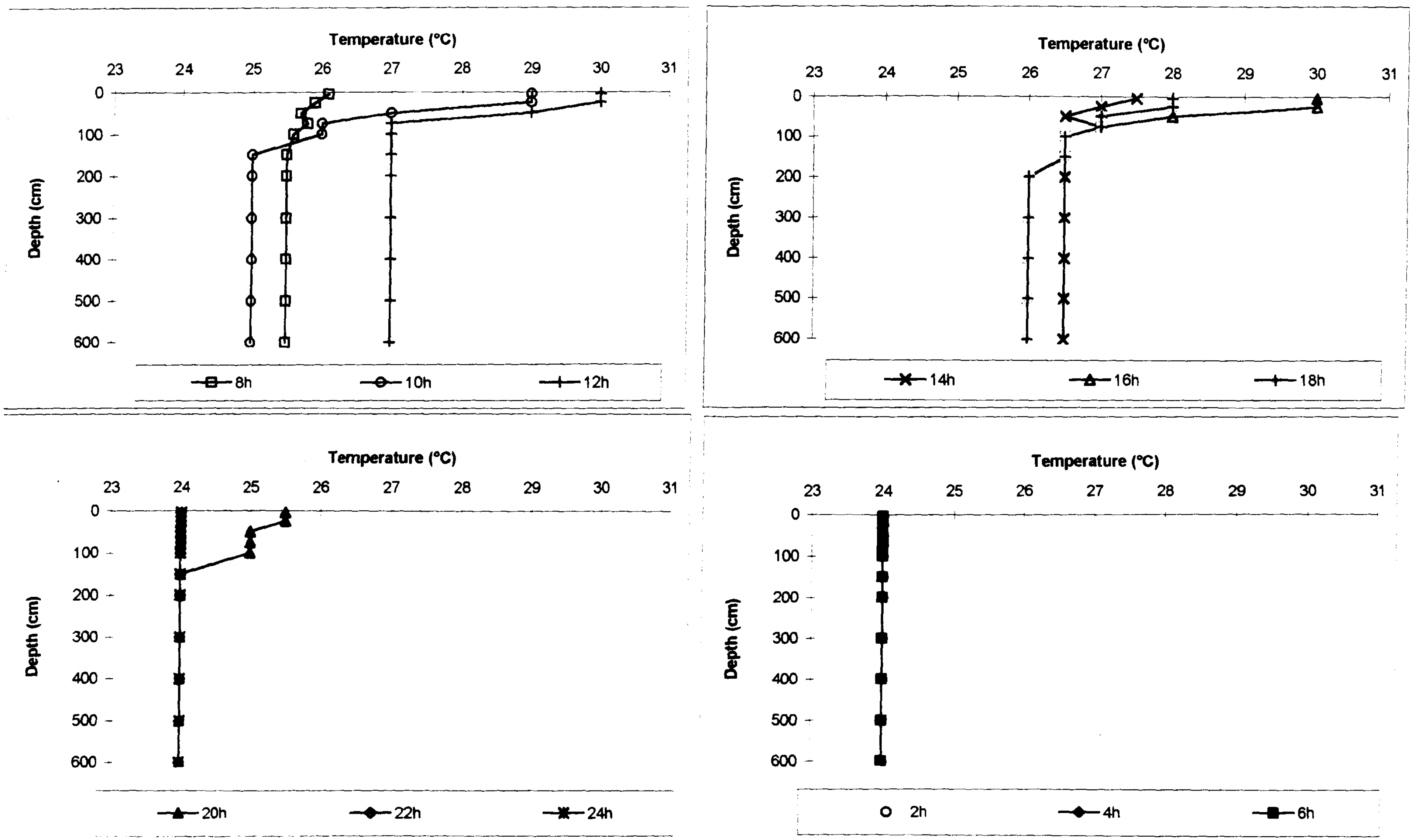


Figure 7.4. Temperature variations in the 24h-hour profile - WSTR2E2 (667 kgBOD$_5$/ha.day) -90 days from the beggining of filling phase.

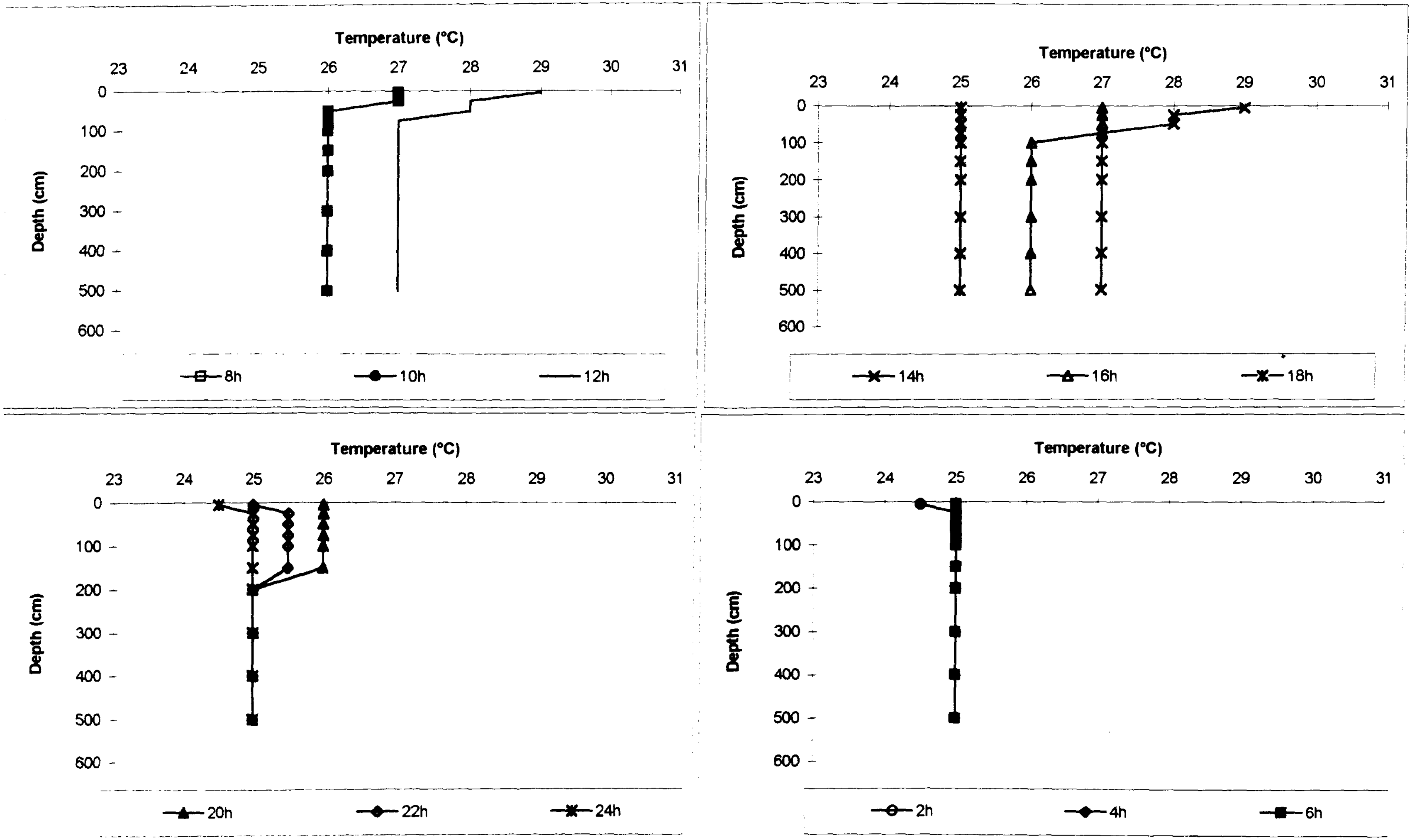


Figure 7.5. pH variations in the 24h-hour profile - WSTR2E2 (667kgBOD$_5$/ha.day) -56 days from the beggining of filling phase.

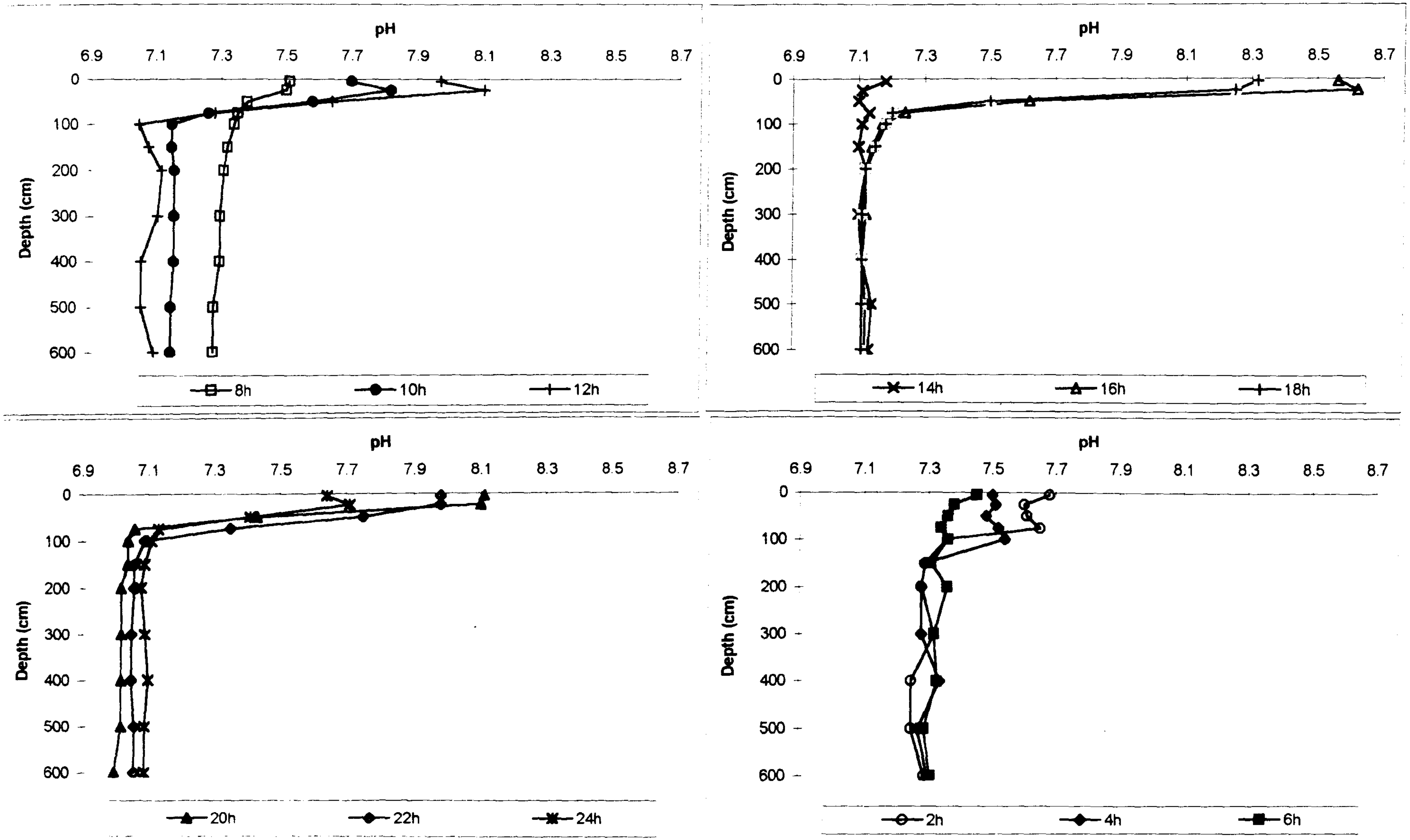


Figure 7.6. pH variations in the 24h-hour profile - WSTR2E2 (667kgBOD_5/ha.day) -90 days from the beggining of filling phase.

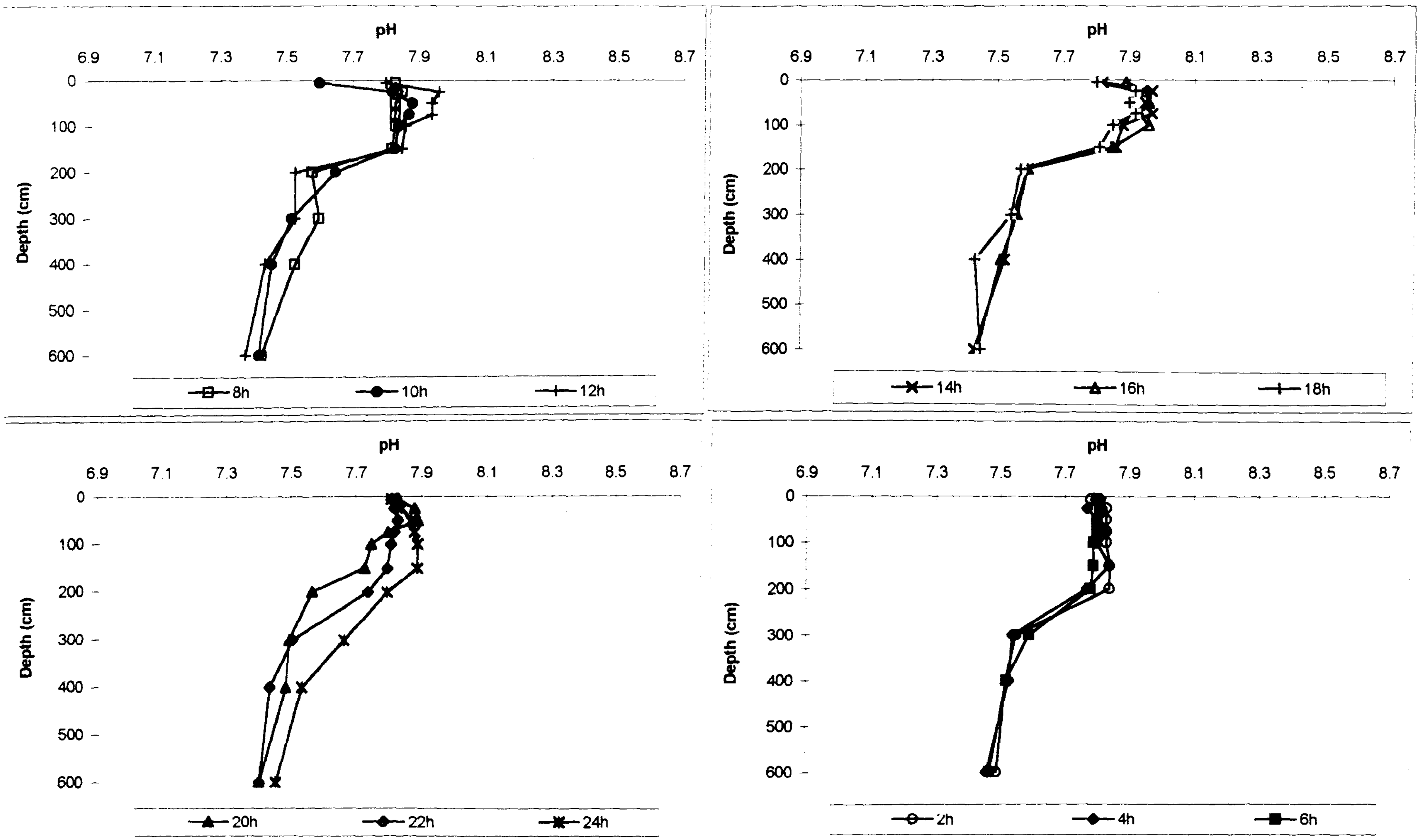


Figure 7.7. DO variations in the 24h-hour profile - WSTR2E2 (667 kgBOD$_5$/ha.day - 56 days from the beggining of filling phase)

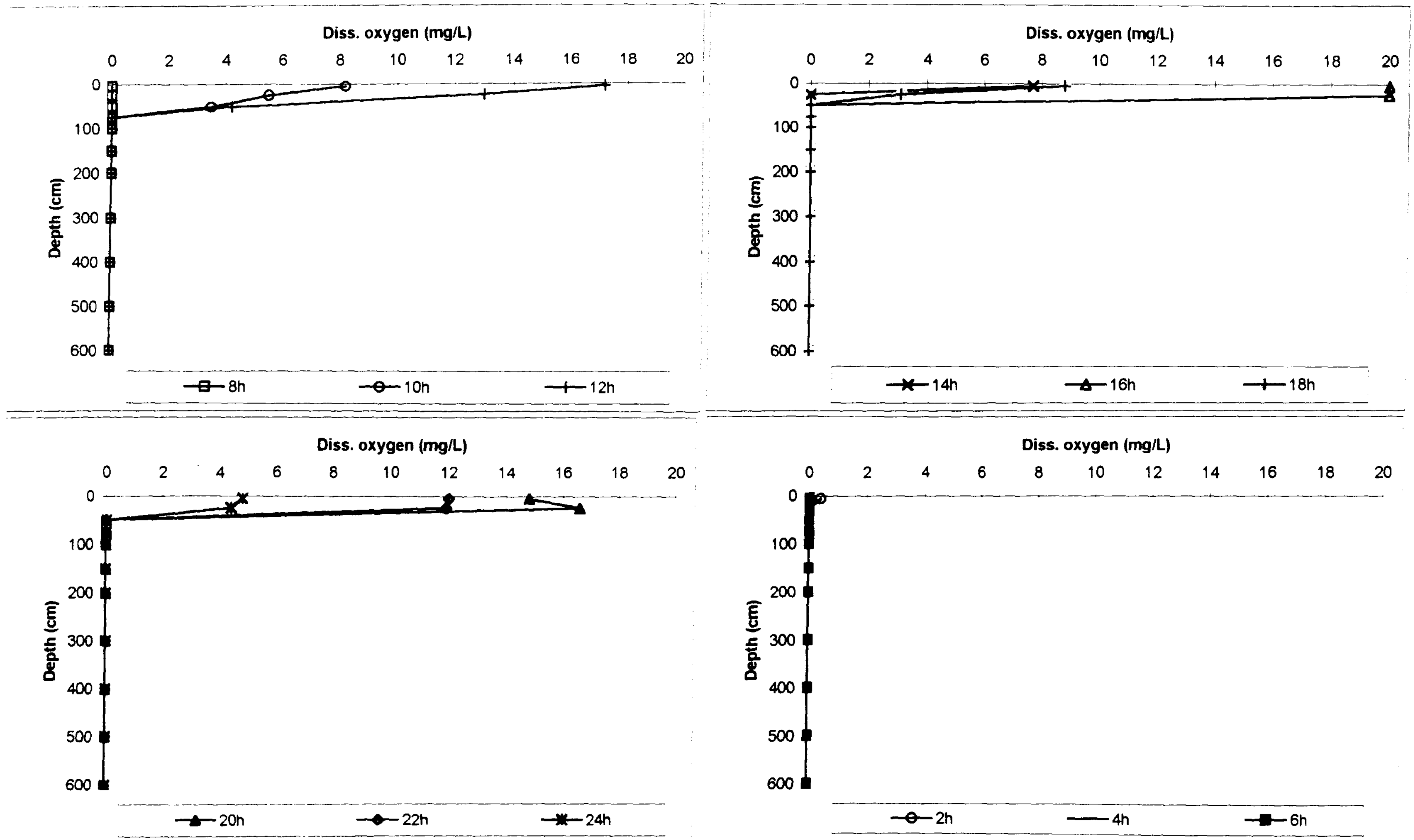
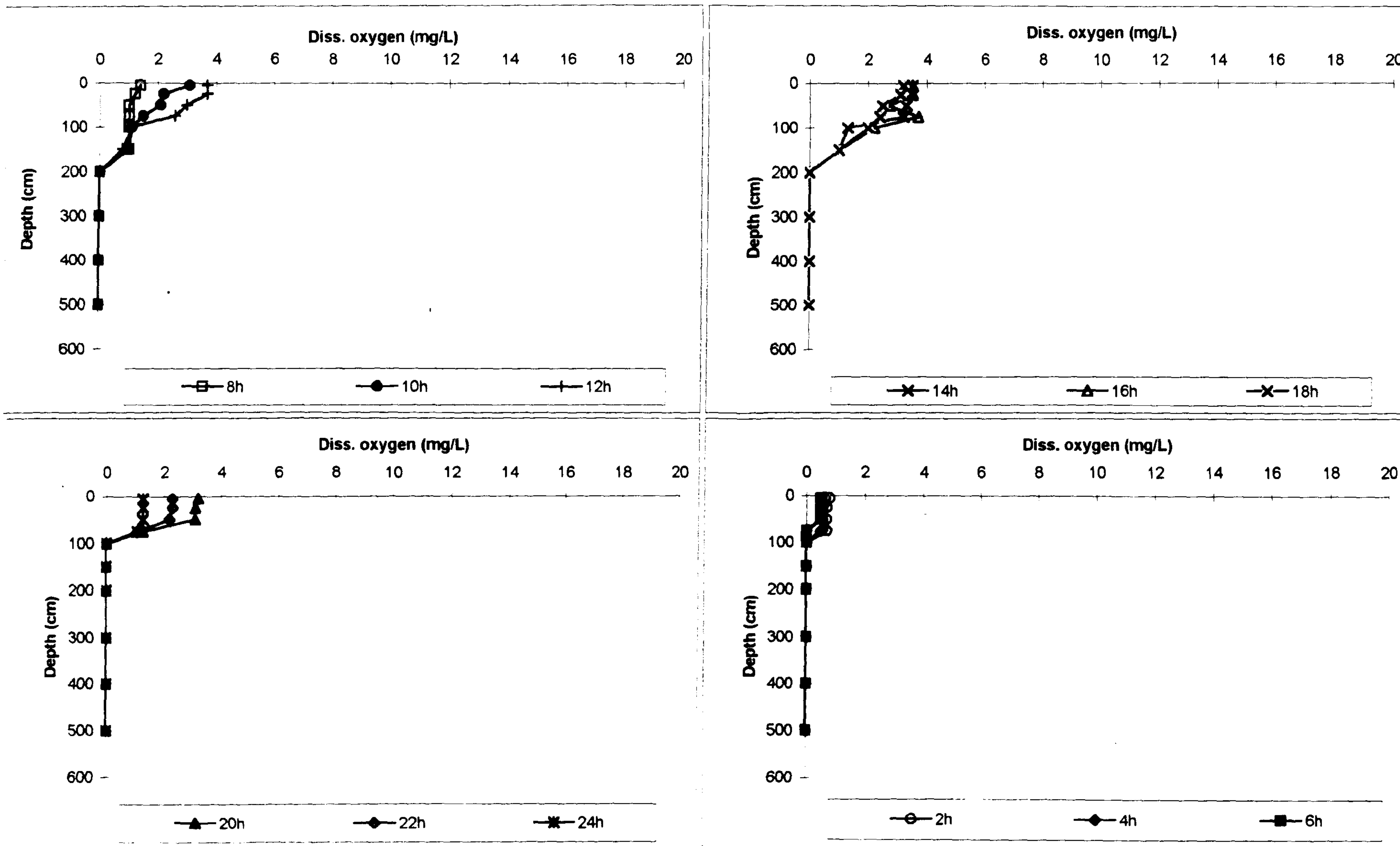
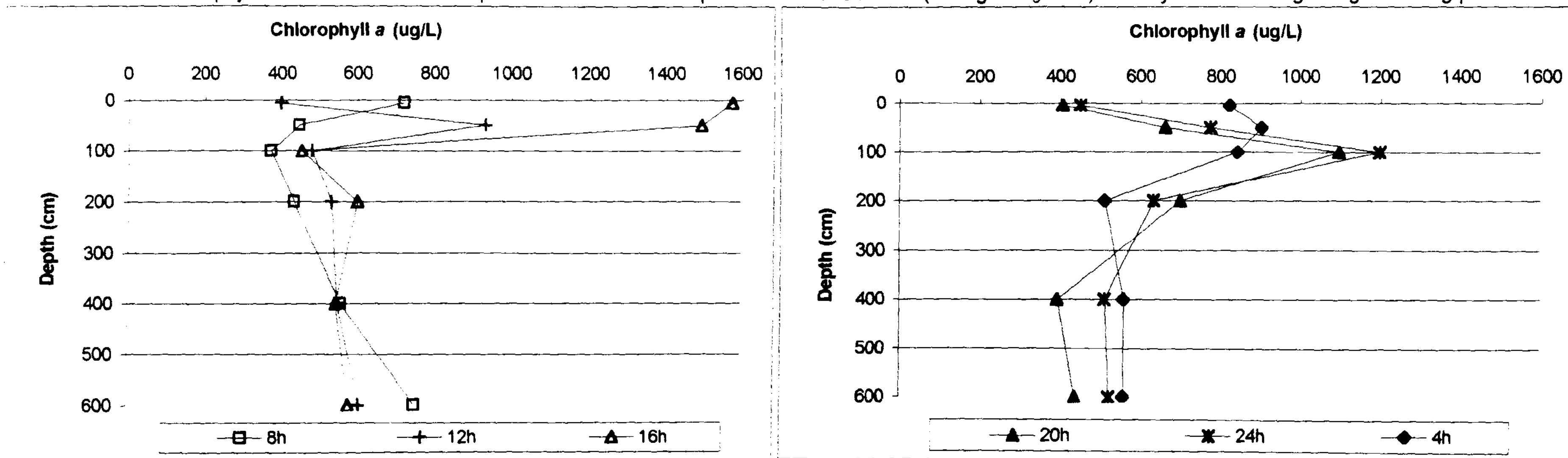


Figure 7.8. DO variations in the 24h-hour profile - WSTR2E2 (667 kgBOD$_5$/ha.day - 90days from the beggining of filling phase)



7.9a - Variation in chlorophyll *a* concentration with depth and time at the 24h profile in the WSTR2E2 (667kgBOD$_5$/ha.d) -56 days from the beginning of resting phase.



7.9b - Variation in chlorophyll *a* concentration with depth and time at the 24h profile in the WSTR2E2 (667kgBOD$_5$/ha.d) - 90 days from the beginning of resting phase.

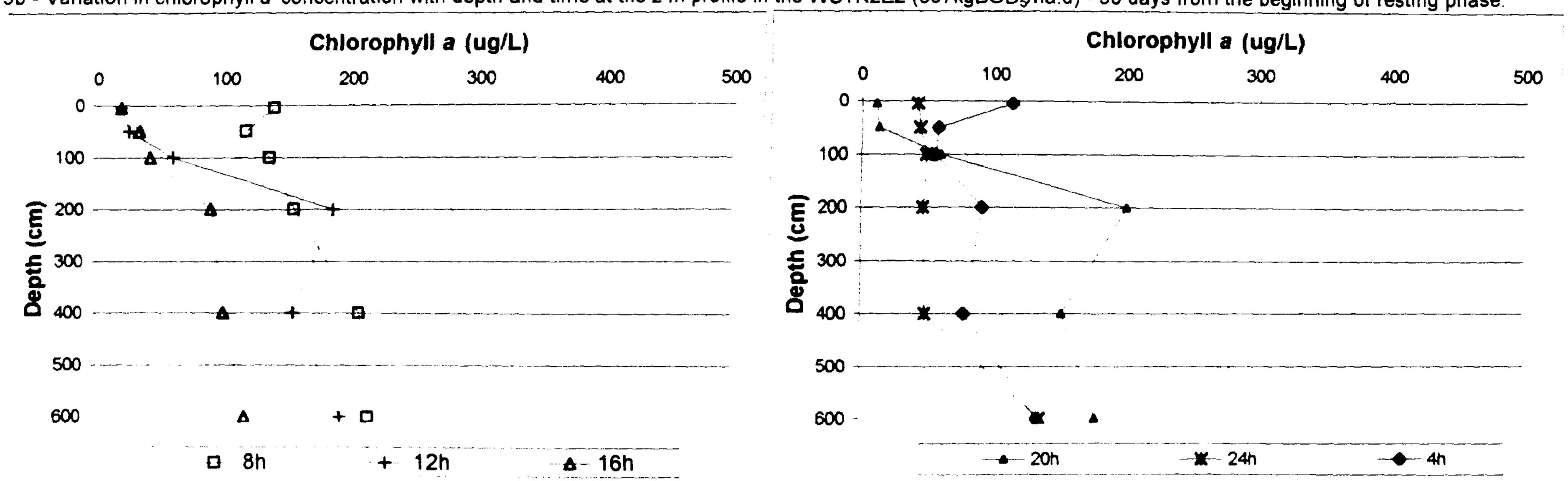


Figure 7.10a. Variation in chlorophyll *a* concentrations with time in the 24h-hour profiles in WSTR2E2 (667 $kgBOD_5/ha.day$) -56 days from the beggining of filling phase. The values are computed for the entire water column based on samples taken from several depths.

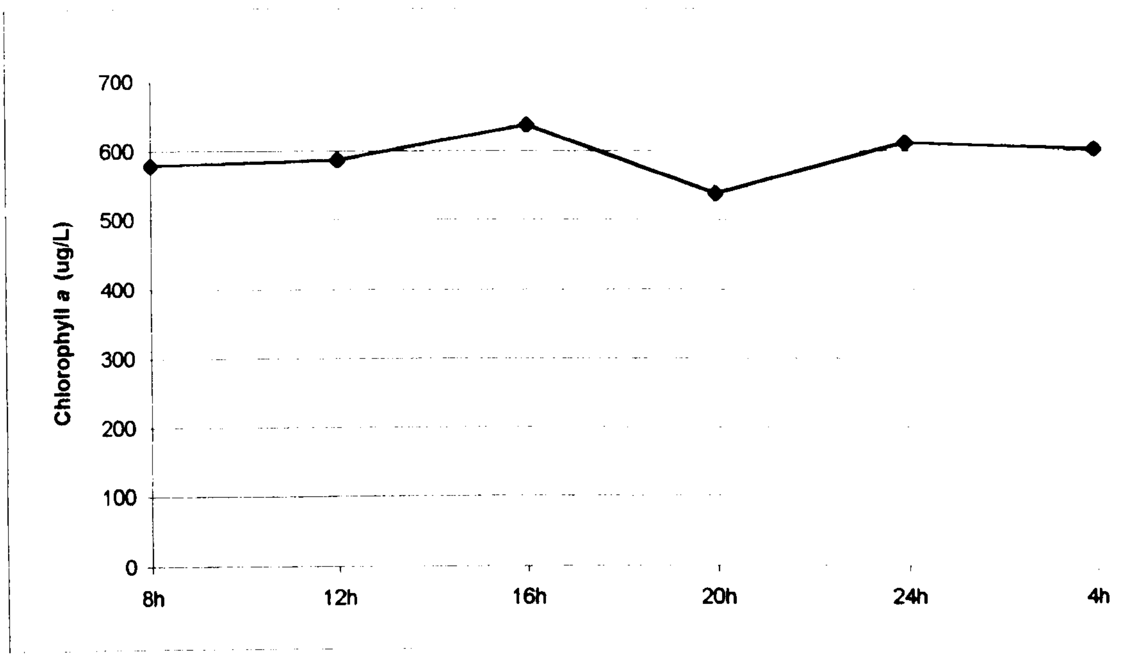
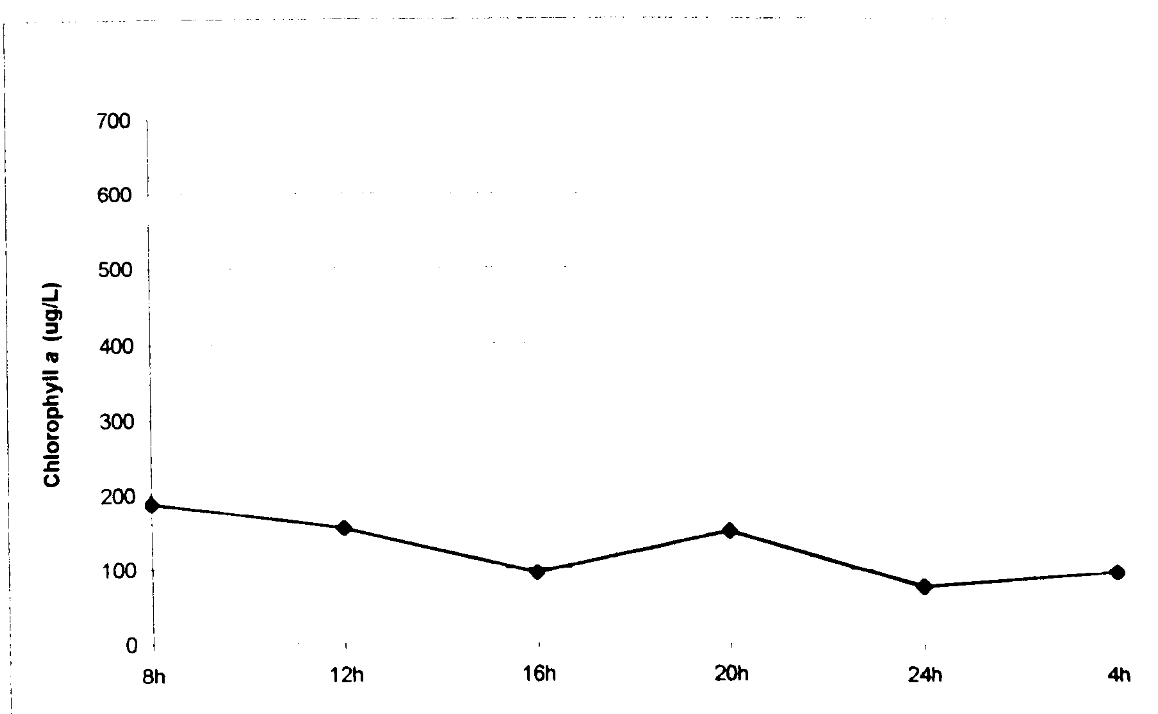


Figure 7.10b. Variation in chlorophyll *a* concentrations with time in the 24h-hour profiles in WSTR2E2 (667 $kgBOD_5/ha.day$) - 90 days from the beggining of filling phase. The values are computed for the entire water column based on samples taken from several depths.



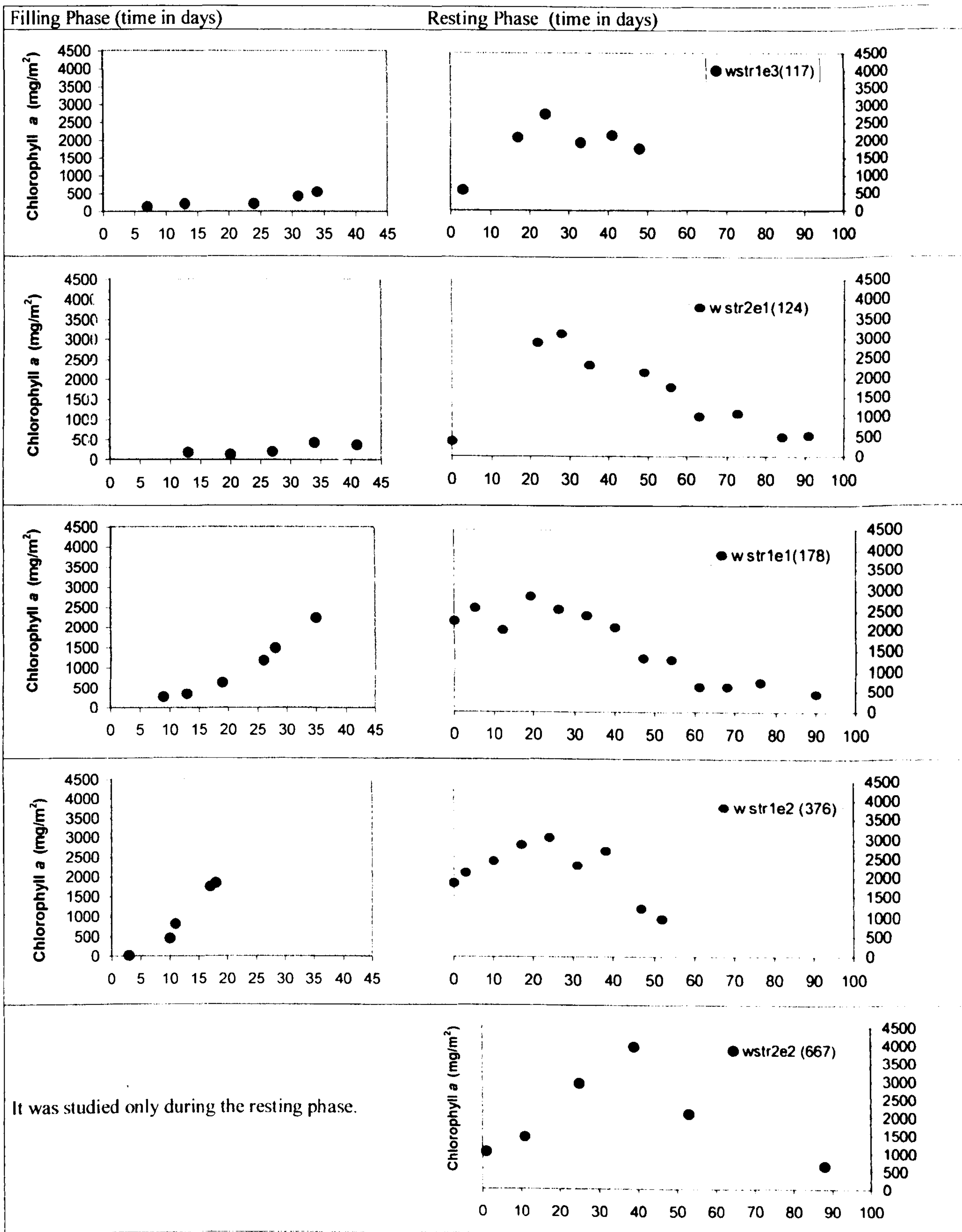
7.2. Parameter variation with time in the WSTR

As the WSTR were batch fed systems, they were filled and then left to rest for some time. Especially during the resting phase, there was variation in the parameters with time in the WSTR, so the use of the mean concentration for the whole experiment would not be appropriate. To describe the results of the various experiments more accurately, the mean concentration at several levels in the water column was calculated for each sampling day during the filling and resting phases.

Figure 7.11 and 7.12 showed the chlorophyll *a* data in scatter plots for the filling and resting phases of the WSTR filled with anaerobic pond effluent and raw sewage, respectively. The WSTR were the same size and some of the data represented different experiments in the same WSTR. The Chlorophyll *a* graphs were organized in order of increasing loading and consequently decreasing filling time, since surface organic loading increases proportionally to the decrease in the filling time.

The algal population started to develop during the filling phase, irrespective of the initial organic loading and filling source (APE or RW). During the filling phase the algal biomass did not reach the maximum concentration until approximately 20/40 days into the resting phase, except in one experiment (WSTR3E1) when algal peak was near day 60. Subsequently, the algal biomass decreased with time in all cases although the reason for this decrease was not clear. To investigate the reason for this algae decrease, regressions plots of chlorophyll *a* concentrations against NH_3, H_2S, NH_4 and orthophosphate, during the resting phase, were made to observe if the decrease in chlorophyll *a* was caused by toxicity (NH_3 or H_2S) or lack of nutrients (NH_4 and Orthophosphate) The regression plots showed no clear pattern between chlorophyll *a* and total ammonia, orthophosphate, NH_3 or H_2S that could explain chlorophyll *a* decrease due to toxicity or lack of nutrients (see figures 7.13 to 7.20).

Figure 7.11. Chlorophyll *a* against time in the filling and resting phases of the WSTR filled with APE.



7.12. Chlorophyll *a* against time in the filling and resting phases of the WSTR filled with RW

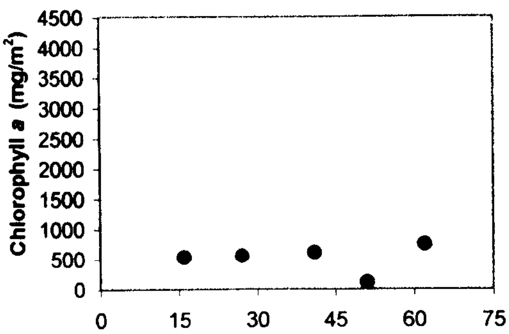
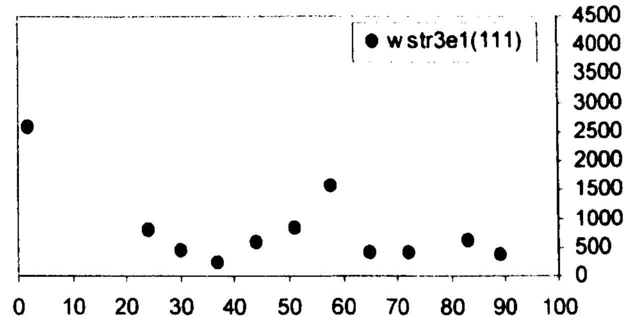
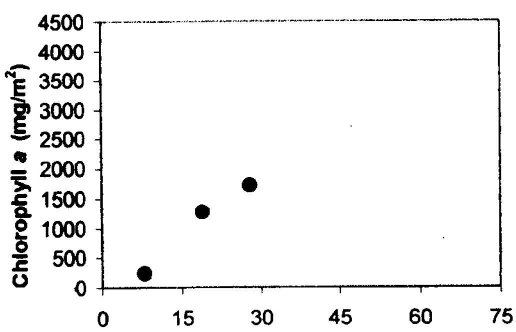
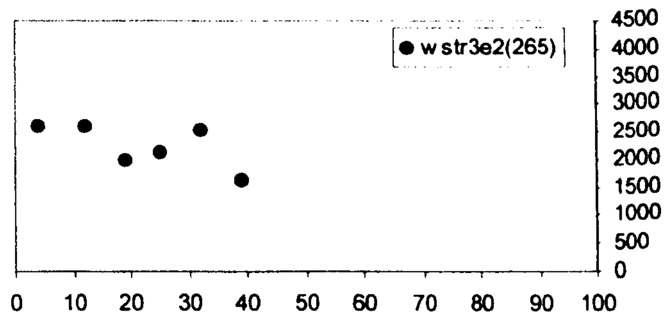
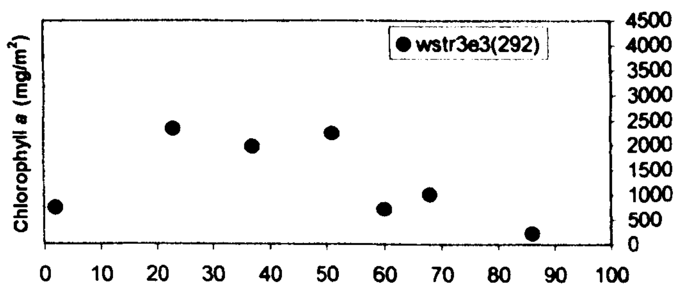
Filling Phase (time in days)	Resting Phase (time in days)
	
	
was studied only during the resting phase.	

Figure 7.13. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR1E3 (117)*

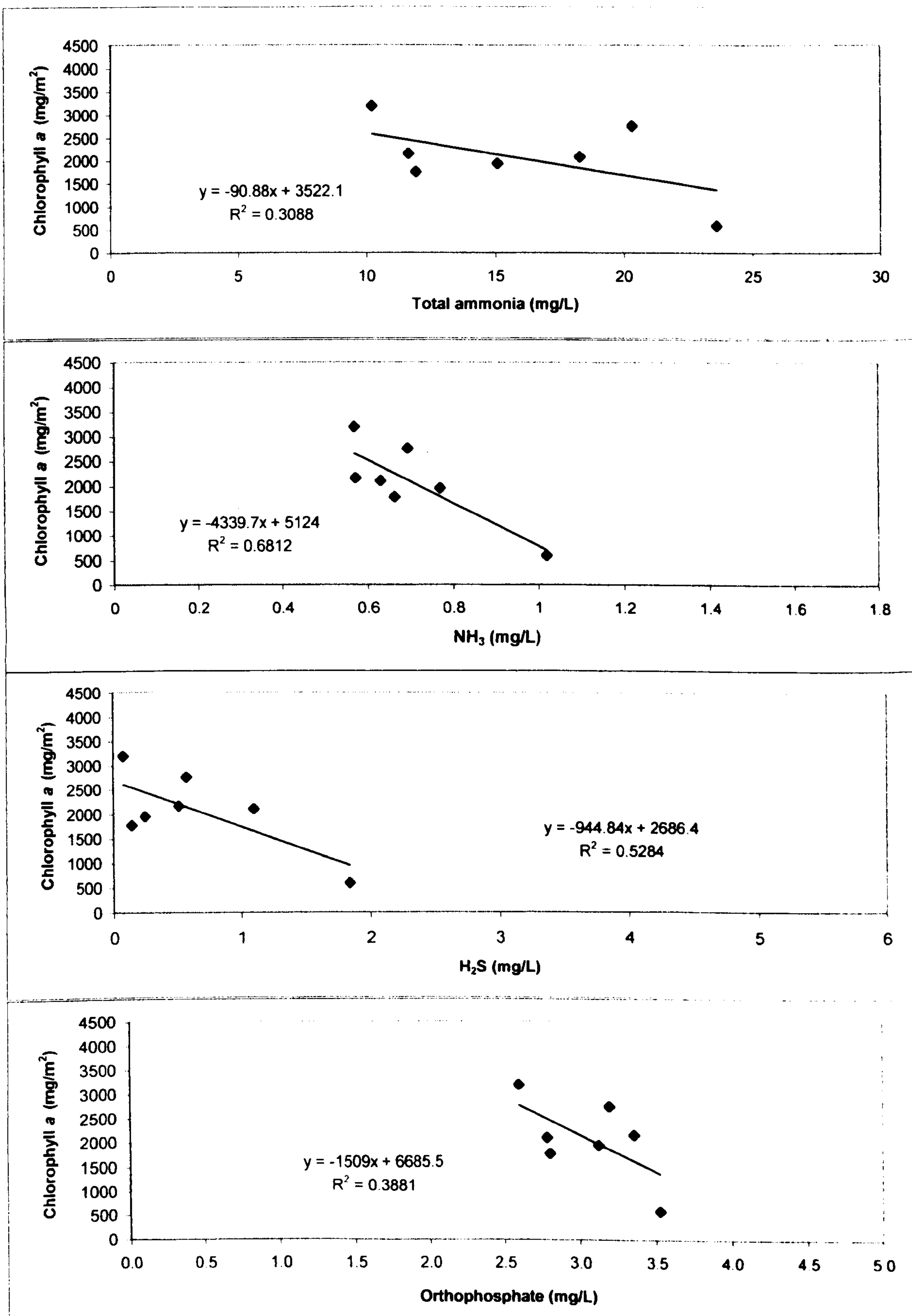


Figure 7.14. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR2E1 (124)*

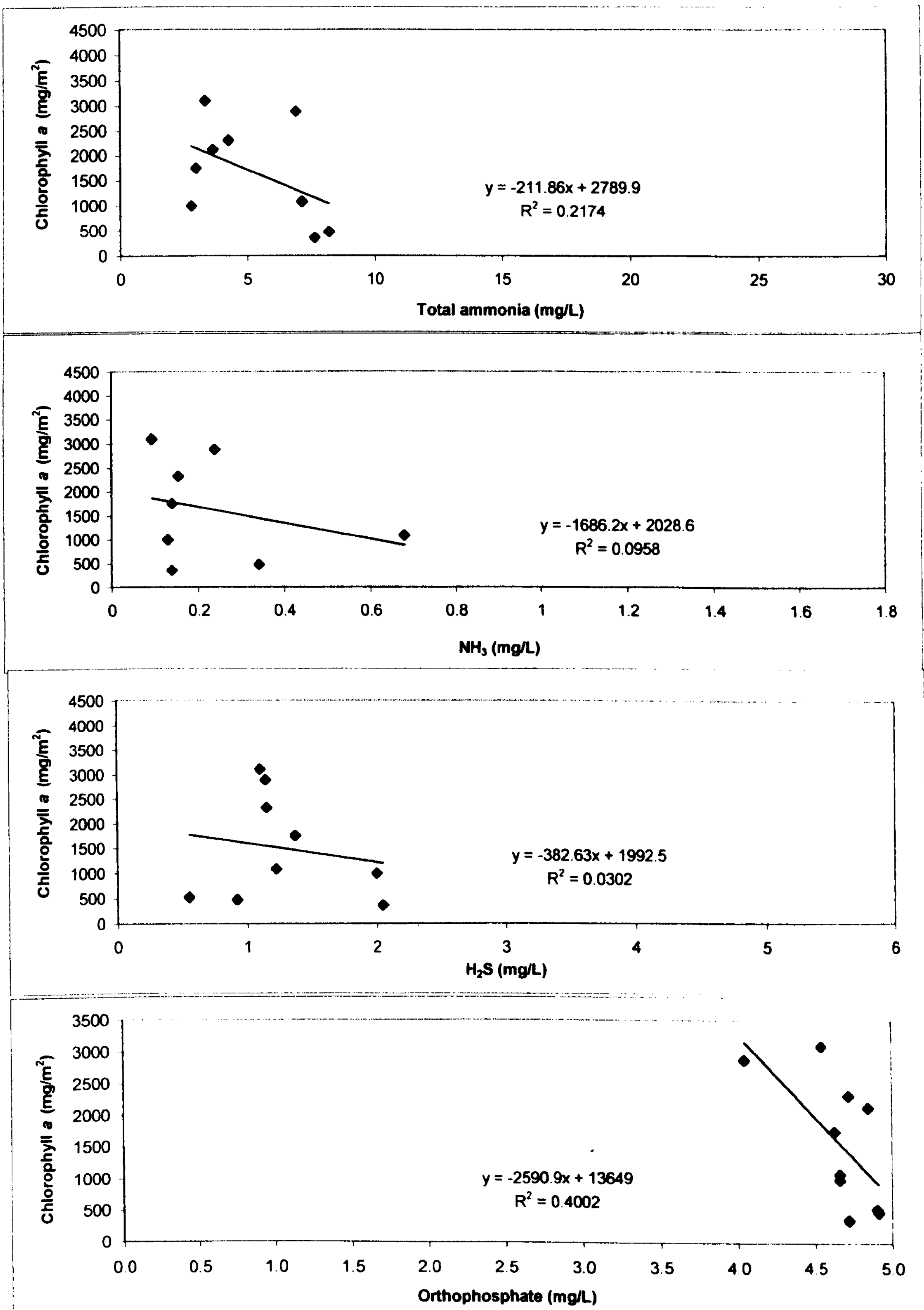
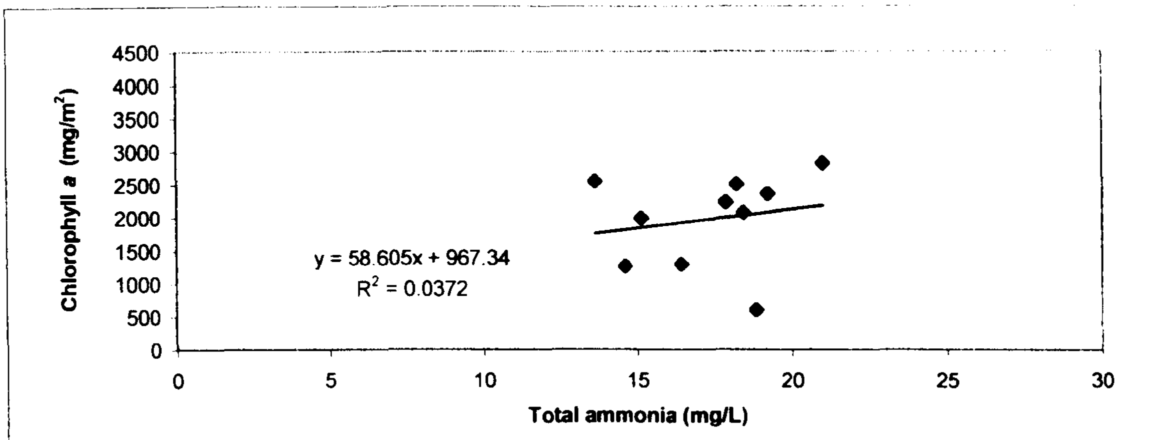
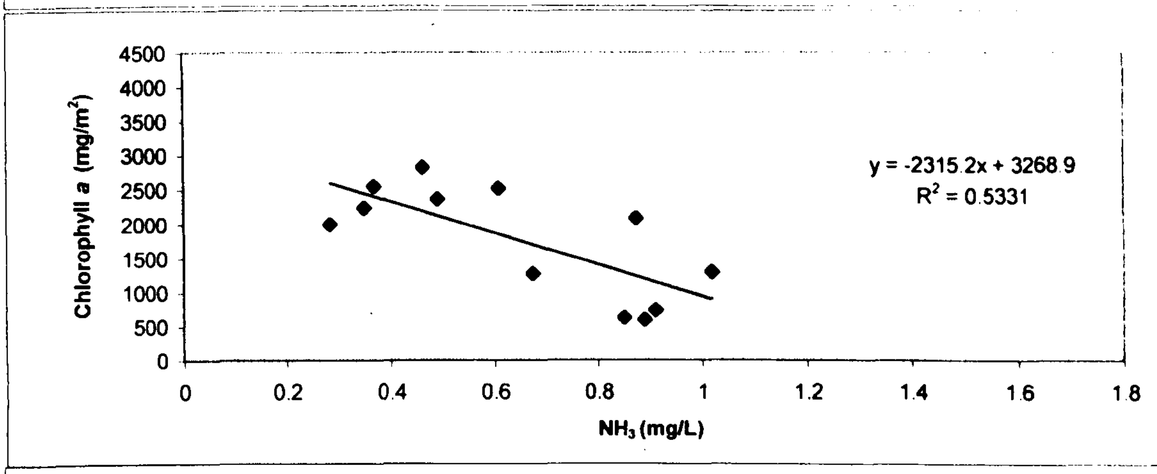
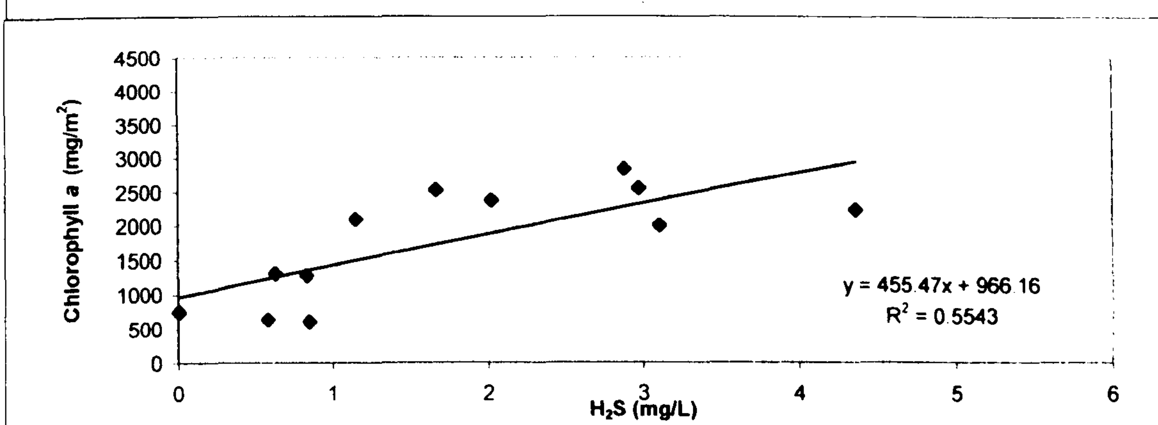


Figure 7.15. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR1E1 (178)*







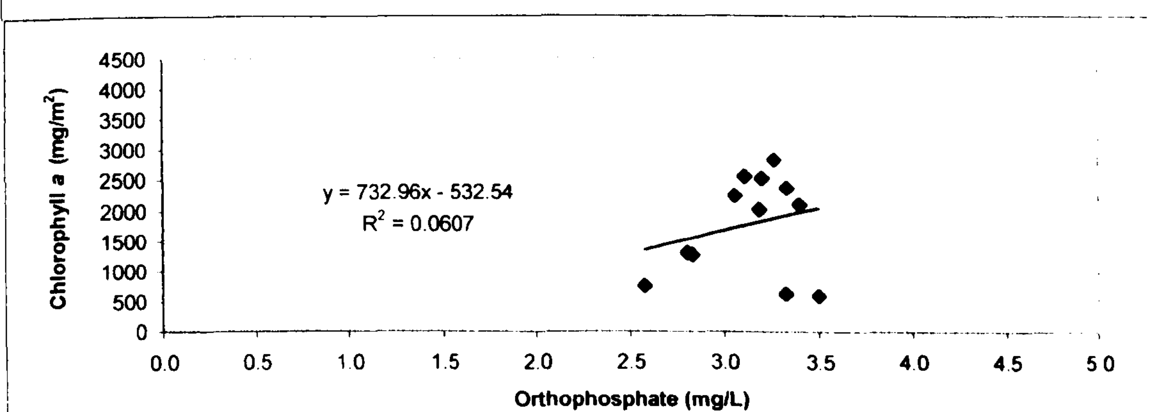


Figure 7.16. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR1E2 (376)*

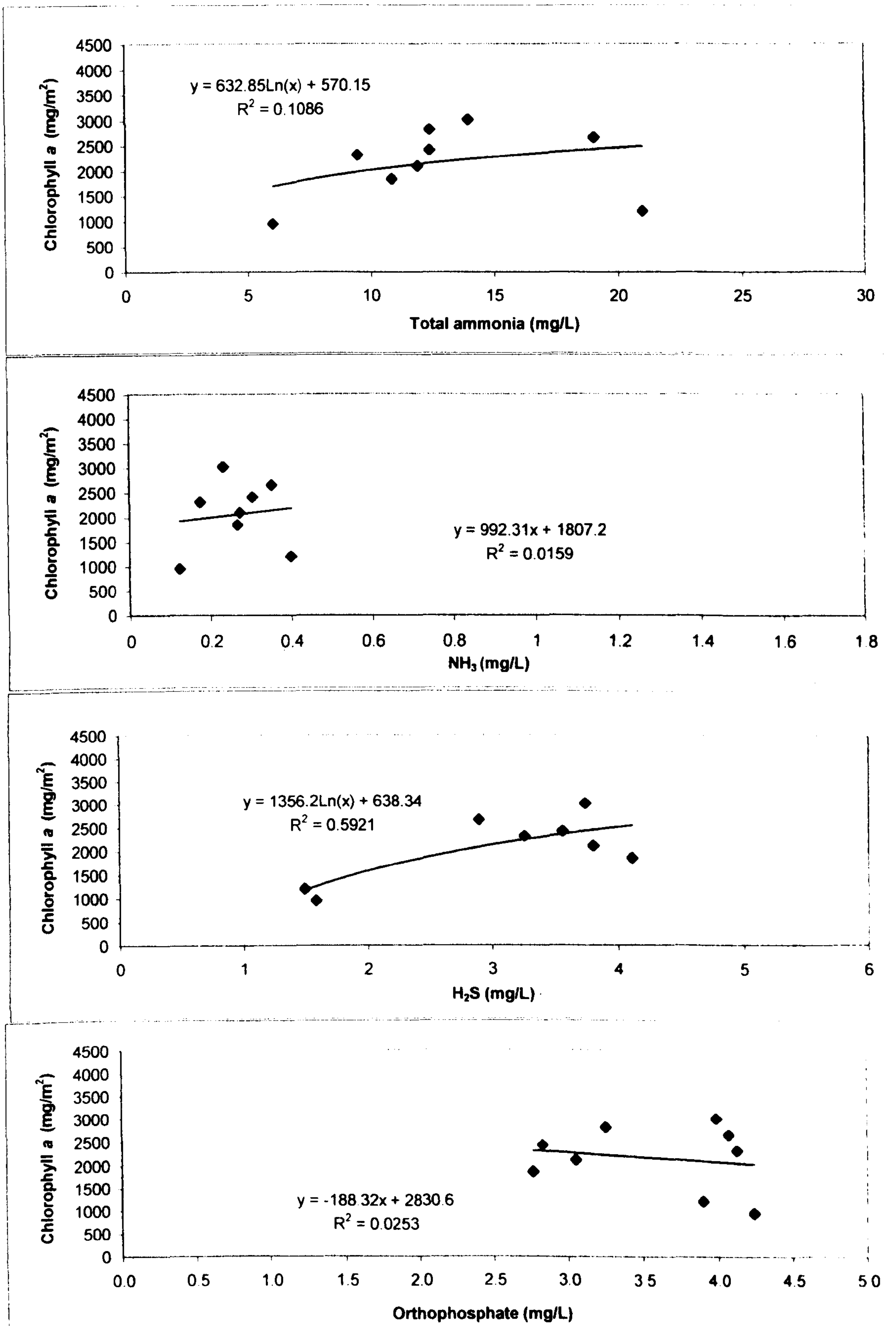


Figure 7.17. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR2E2 (667)*

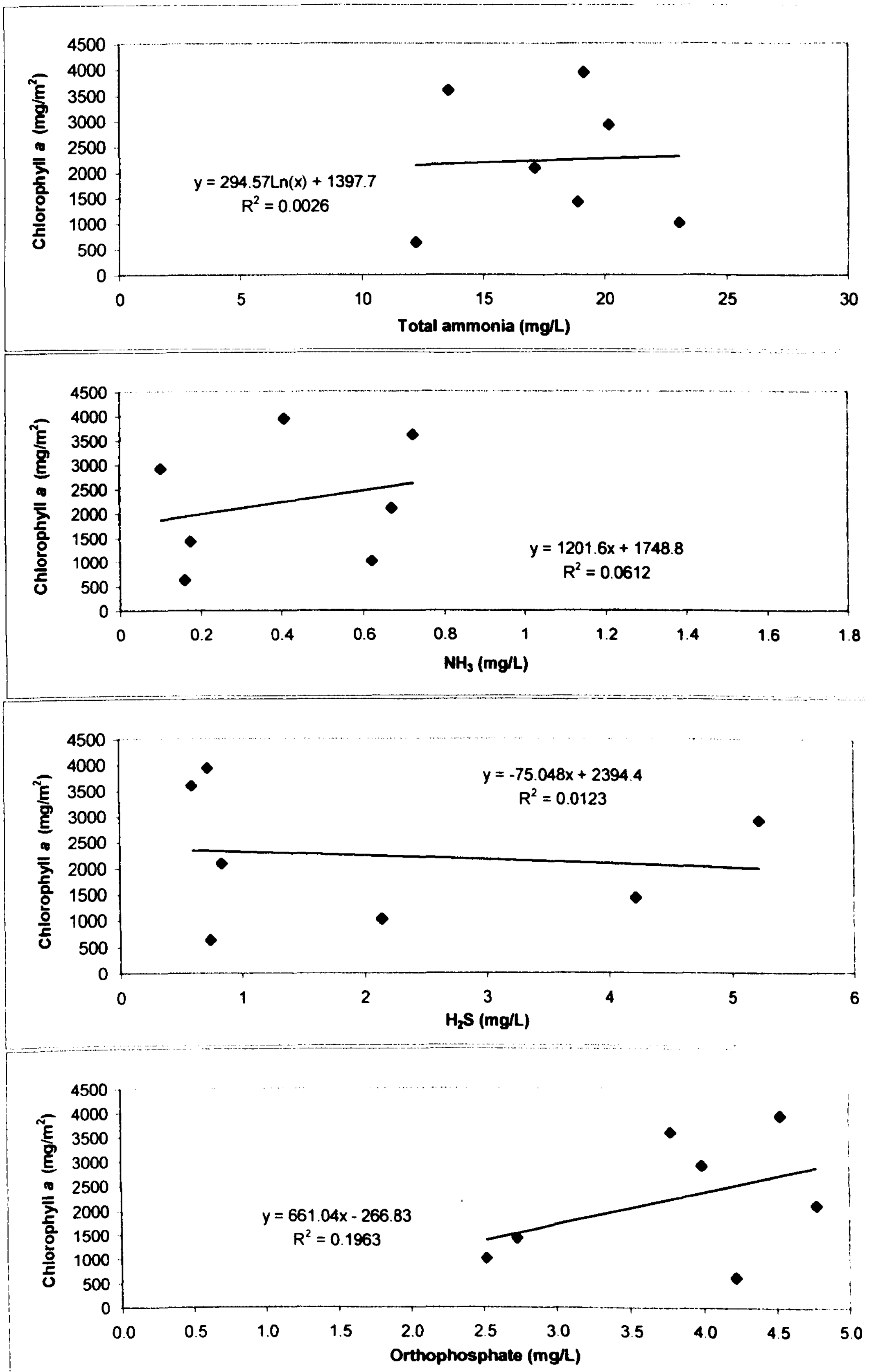


Figure 7.18. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR3E1 (111)**

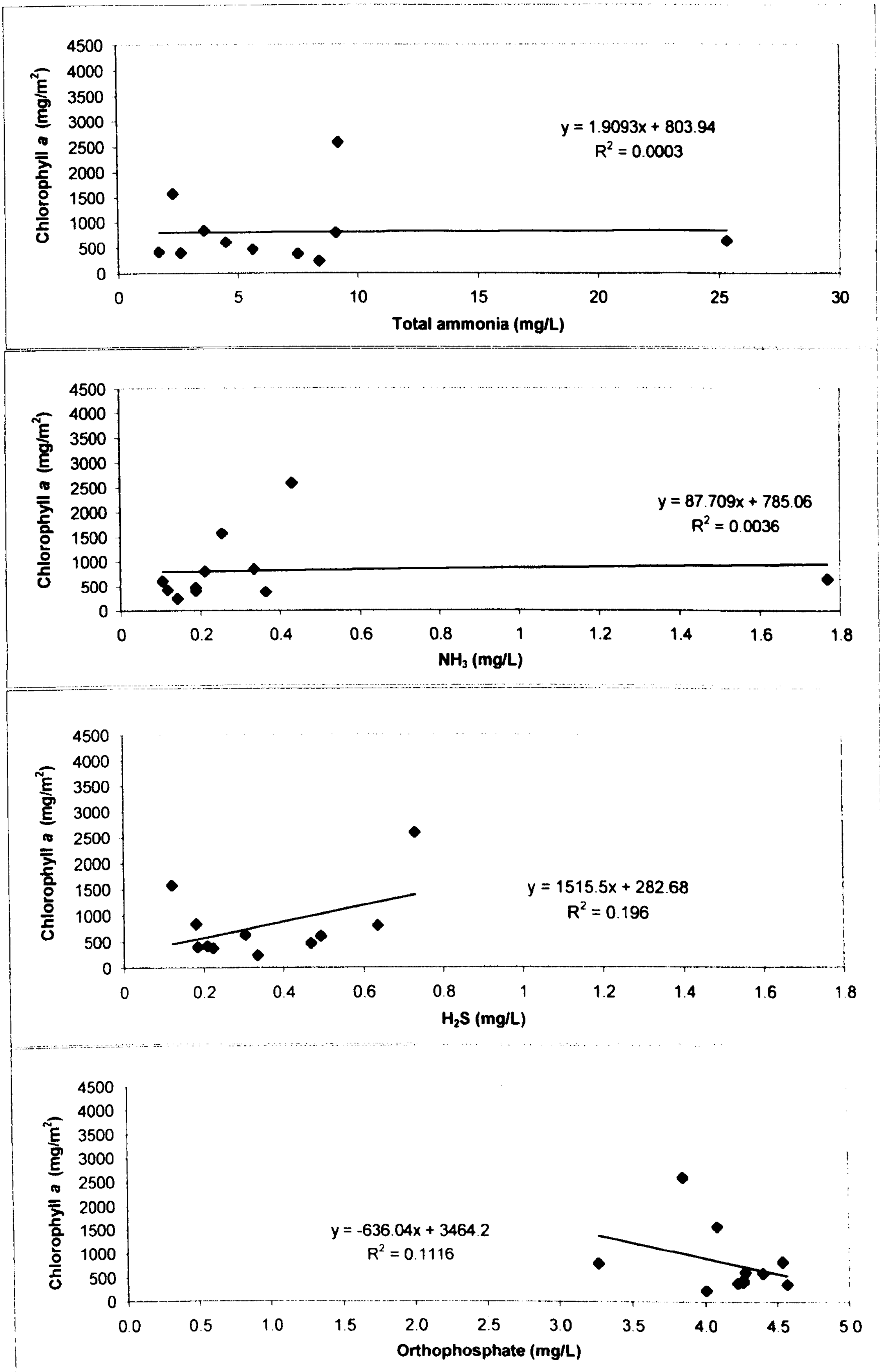


Figure 7.19. Regressions plots of Chlorophyll *a* against total ammonia,NH_3, H_2S and orthophosphate in the resting phase of WSTR3E2 (265)**

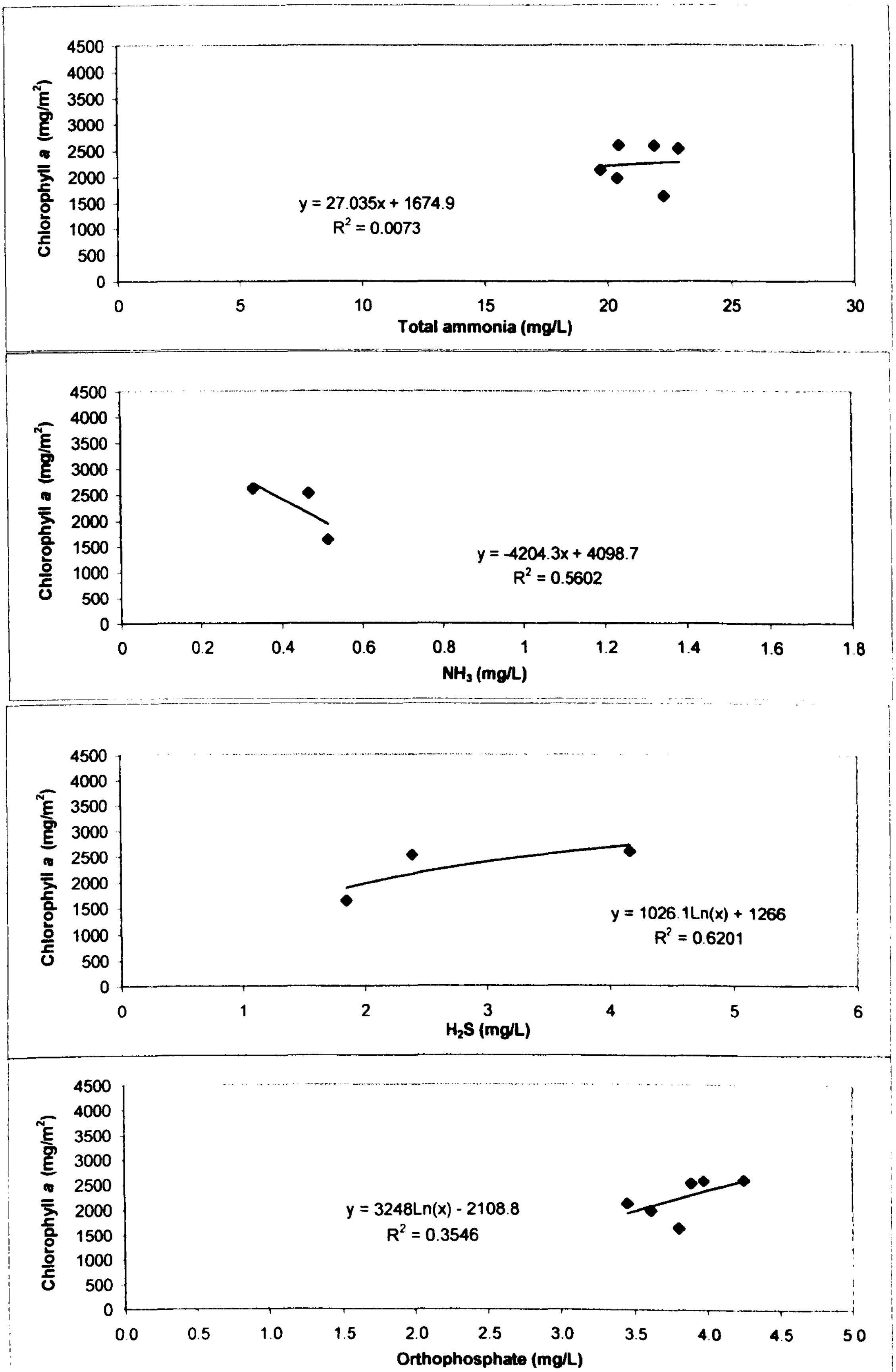
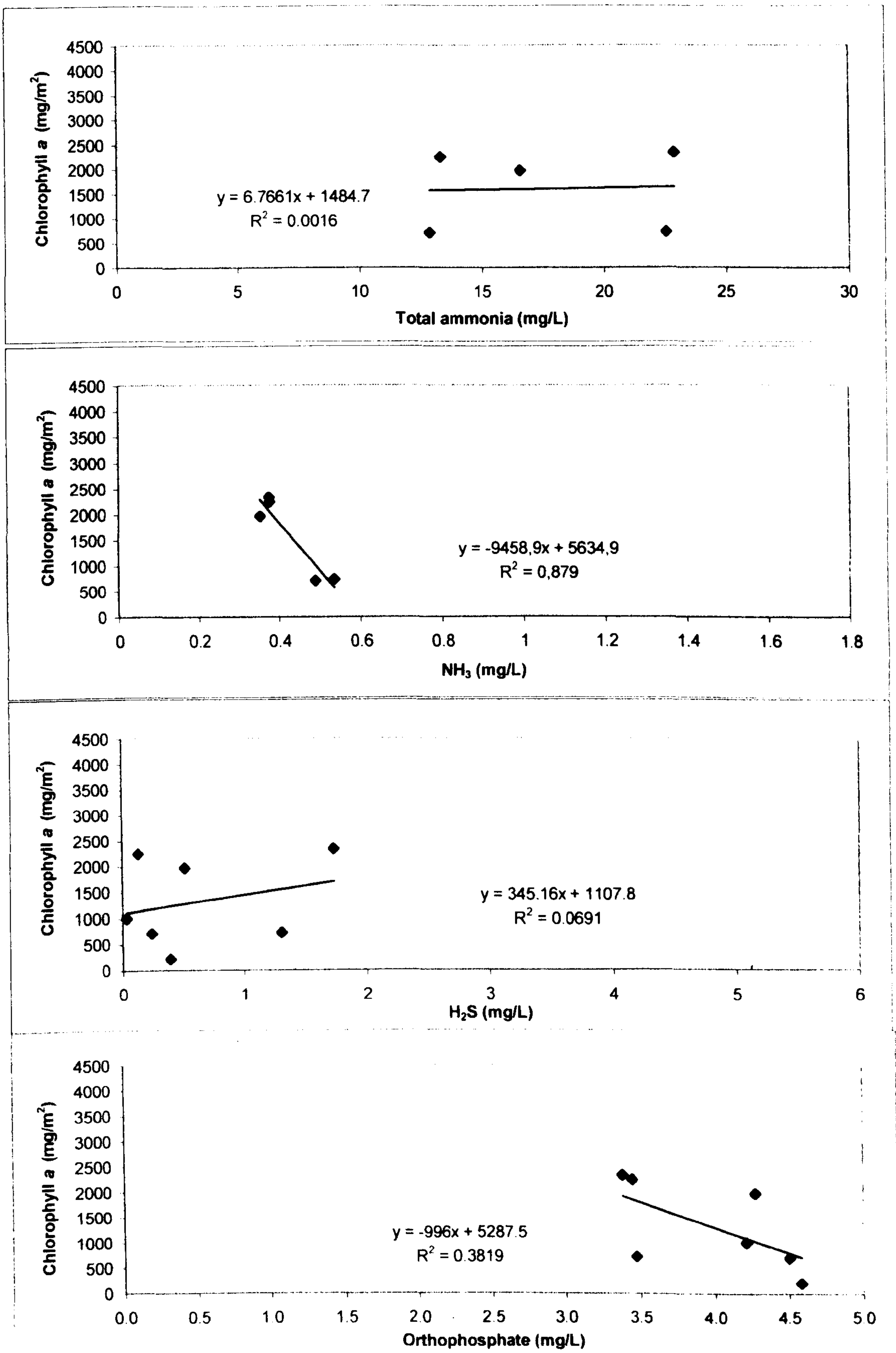


Figure 7.20. Regressions plots of Chlorophyll *a* against total ammonia, NH_3, H_2S and orthophosphate in the resting phase of WSTR3E3 (292)**



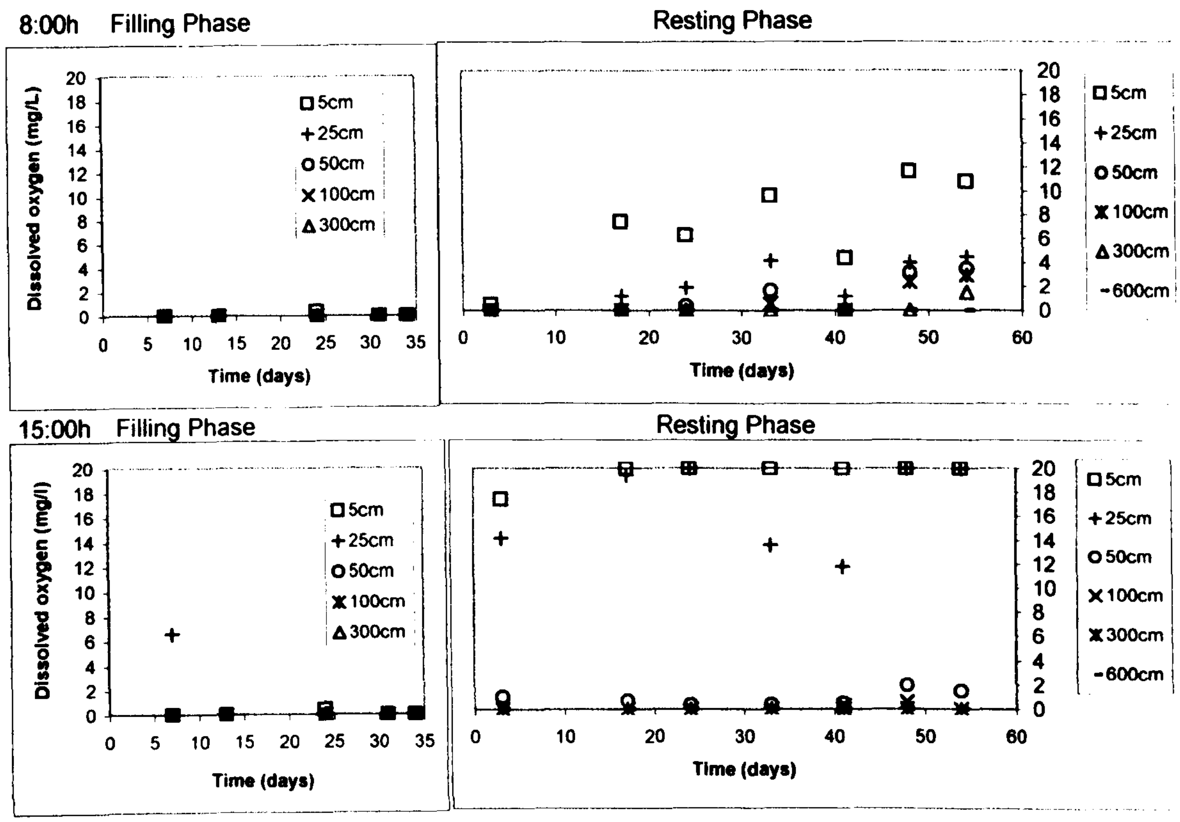
A weakness in these experiments was the lack of data on the fauna of the WSTR. The grazing by zooplankton could be a key factor in the decline in algal biomass with time. The highest chlorophyll *a* values measured did not change very much in the WSTR filled with the same source of wastewater, especially in the case of the WSTR filled with RS. In these WSTR, the highest chlorophyll *a* concentration was slightly lower than in the WSTR filled with APE and was in the range of 2337-2610 $kgBOD_5/ha.day$ and 2763 –3946 $kgBOD_5/ha.day$, respectively.

The WSTR were practically anaerobic during the filling phase (at 8:00h) irrespectively of whether they were being filled with APE or RS. At the beginning of the resting phase, aerobic conditions started to develop in the superficial layers of the WSTR. The greatest depth of oxygenation reached with time in the resting phase, was 100cm. The deeper layers always remained anaerobic.

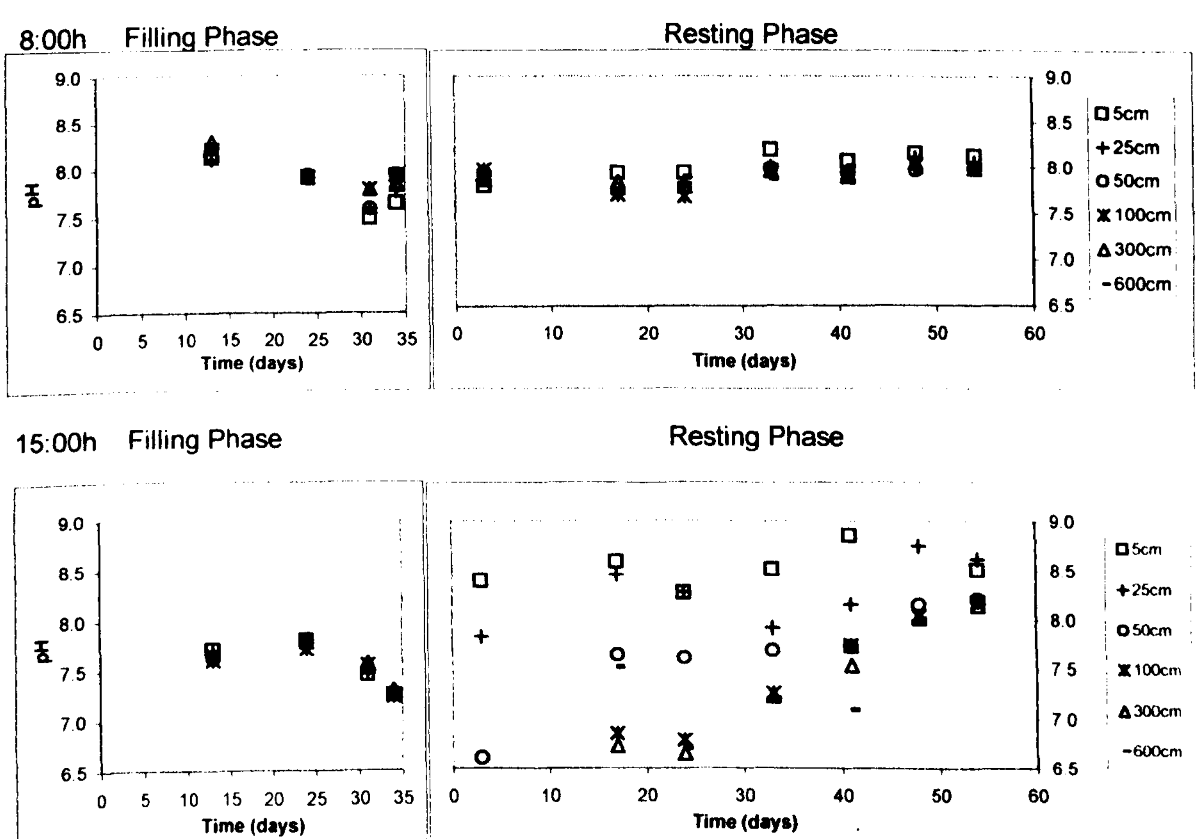
In the highest loaded WSTR, it took longer for the aerobic conditions to develop. The data obtained for DO at 15:00h showed that although in some of the WSTR the filling phase was not completely anaerobic, the highest DO concentrations were found in the resting phase always in the superficial layers (5 and 25cm). Even at this sampling time when algal photosynthesis is more intense the aerobic layers did not exceed 100 cm in depth. Typical data for DO in a WSTR are shown in figure 7.21.

The pH concentrations were higher in the upper layers and presented a general tendency to increase towards the end of resting phase. As was observed for DO, the highest pH values were found at 15:00h. Typical data for pH in a WSTR are shown in figure 7. 22.

7.21. DO concentration in the filling and resting phases at 08:00h and 15:00h in WSTR1E3 (117 kgBOD$_5$/ha.day).



7.22.pH in the filling and resting phases at 08:00h and 15:00h of WSTR1E3 (117 kgBOD$_5$/ha.day).



7.3. Nutrient removal in the WSTR

The WSTR are storage and treatment systems. Therefore, it is important to know the nutrient content in the effluent, which will be used for discharge into receiving water bodies or for irrigation. As was previously discussed in chapter IV, when discussing nutrient removal in the series of ponds, the use of treated wastewater provides both water and also nutrients for plant growth. e.g. nitrogen and phosphorus. However, high concentrations of nitrogen can be toxic to some crop plants. The value of total nitrogen is very important due to the changes in the nitrogen forms realized by bacteria. Nevertheless, ammonia is the nitrogen form used most commonly to characterize the sewage (Reis, 1995). Therefore this was the form of nitrogen analysed in these systems. Orthophosphate was the form of phosphorus analysed in these WSTR, since it is the form most rapidly assimilated by aquatic organisms and plants. The concentrations of nitrite and nitrate in the WSTR were low (less than 1 mg/L).

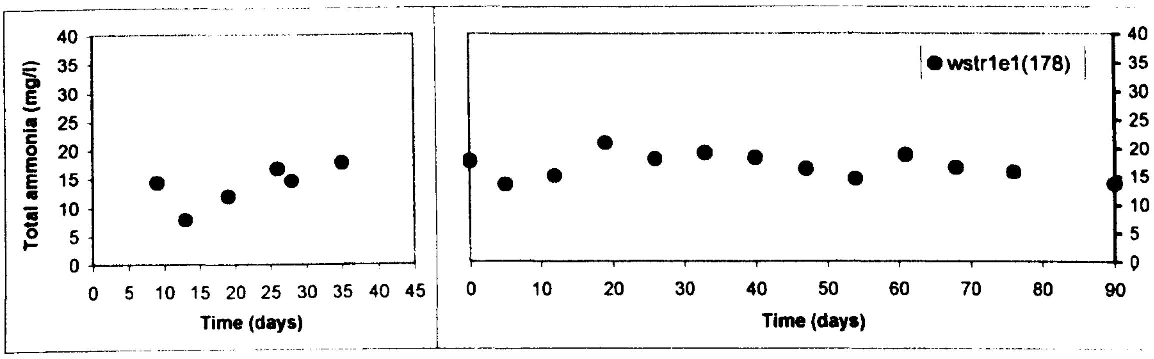
The ammonia fluctuations in the filling phase of the WSTR did not show a clear pattern, increasing with time in some experiments and decreasing in others. In the resting phase, the ammonia values remained practically constant with time in most of the experiments. Only in the WSTR loaded at 111 and 124 kgBOD$_5$/ha.d the CONAMA (1986) requirement of 5 mg/L of total ammonia was met in 44 and 28 days, respectively, in the resting phase. Typical ammonia data for the WSTR are presented in figure 7.23.

In most of the experiments, during the filling phase, a tendency for orthophosphate concentration to increase was observed. During the resting phase, the orthophosphate concentrations remained practically constant with time in most of the experiments. Typical orthophosphate data in the WSTR are shown in figure 7.24.

7.4 Assessment of the efficiency of WSTR

During the filling phase, the WSTR in this study remained largely anaerobic and were predominantly anaerobic reactors even during the resting phase, with an aerobic top layer during the day extending down to a depth of approximately 1m. In all cases the WSTR turned over at night and remained completely mixed from approximately 24.00h until 08.00h according to the temperature, dissolved oxygen and pH data. The

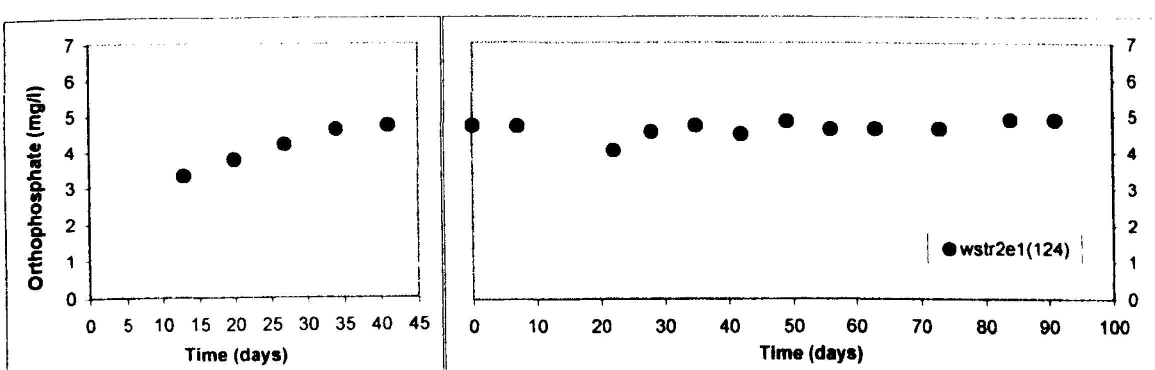
7.23. Total ammonia concentrations (arithmetic mean for the water column) in the filling and resting phases of WSTR1E1 (178 $kgBOD_5/ha.day$).



7.24. Orthophosphate concentrations (arithmetic mean for the water column) in the filling and resting phases of WSTR2E1 (124 $kgBOD_5/ha.day$).

Filling Phase

Resting Phase



WSTR can be loaded with raw wastewater or effluent from an anaerobic pond without obvious changes in performance, and surface BOD_5 loading rates similar to those applied to facultative ponds can be used without odour problems. In fact in many respects these batch-filled reactors behave like the primary and secondary facultative waste stabilisation ponds and standard equations to determine surface BOD_5 loading rates on facultative ponds can be used with WSTR during the filling phase.

Since the prime reason for using WSTR is to treat and store wastewater for subsequent reuse for the irrigation of crops, the microbiological quality of the effluent is more important than the BOD_5 and suspended solids providing the latter does not cause clogging of irrigation equipment or the need for expensive filtration. Previous work on these reactors has discussed effluent quality in relation to faecal coliforms and helminths (Pearson *et al* 1996; Athayde 2000) and showed that an effluent quality suitable for unrestricted irrigation according to WHO guidelines can usually be achieved after 28 days into the resting phase which is much shorter than the minimum storage period normally required for irrigation schemes employing the use of wastewater during the dry season. In terms of plant nutrients, the concentrations of ammonia and phosphate do not decrease with time during the resting phase in the WSTR and nutrient removal in terms of a wastewater treatment process is poor compared to WSP. In fact, in most experiments the effluent quality did not reach the CONAMA (1986) value of 5mg/L total ammonia by the end of the experiments, in some cases it was necessary 44 days or more. Phosphate concentrations also remained high, between 4 -5mg/L during the resting phase and thus discharge of WSTR effluent directly to surface waters rather than its use in irrigation where crops will assimilate the nutrients would require careful environmental evaluation. In short, WSTR are a poor alternative to WSP as a stand-alone treatment technology unless linked to irrigation whereupon their advantages become clear.

The controlling factor in terms of reuse is more likely to be the ammonia concentration of the effluent and the risk of over fertilisation or ammonia toxicity tothecrops.

7.5. Conclusions

•The study of daily variations in pH, DO, Temperature and Chlorophyll *a* showed that, although there were variations in these parameters throughout the day, the 08:00h sample was considered representative for the whole day;

•The highest water temperatures were recorded in the superficial layers between 12h and 16h, and the minimum temperatures at different depths after 18h;

•The cooling in the upper layers of the WSTR, at night caused complete mixing of the WSTR contents;

•The highest pH levels, in 24-hour daily profiles, were found in the superficial layers during the day; and the minimum pH in the deep layers at night;

•The maximum DO readings always occurred in the top 75cm, generally between 12h and 16h. The minimum DO concentrations occurred without any pattern of location, but never at the surface during day light hours;

•The highest values of chlorophyll *a* were found within the top 200cm, usually during periods of high light intensity (between 08:00h and 16:00h). The lowest chlorophyll a values were found irregularly along the WSTR depths;

•The algal population (expressed as chlorophyll *a*) started to develop during the filling phase, but only reached the maximum concentrations after approximately 20/40 days into the resting phase and then decreased again;

•The regression analysis showed no clear relationship between chlorophyll *a* concentration and total ammonia, orthophosphate, NH_3 or H_2S. The decrease in chlorophyll *a* concentration with time could therefore not be linked to toxicity or lack of nutrients;

•The WSTR were practically anaerobic throughout their depth during the filling phase at 08:00h, irrespective of whether they were filled being filled with APE or RS. The aerobic conditions started to develop in the superficial layers of the WSTR, at the beginning of the resting phase;

•The pH was higher in the upper layers of the WSTR and generally increased towards the end of resting phase;

•The concentrations of ammonia and orthophosphate remained practically constant with time throughout the resting phase in most of the experiments.

8.0. The algal population in the WSTR

This chapter will concentrate on the distribution of algal genera in terms of diversity (frequency data) and numbers (cell counts) in the WSTR filled with either APE or RW at different surface organic loads. The samples for algal identification and counts were obtained at the same time as those for physico-chemical analyses (i.e. between 08:00h and 09:00h).

The relative frequency of the algal genera present in the different experiments (organic loadings) of the WSTR were determined from presence and absence data recorded, for all depths in the water column, for each sampling day of the WSTR in the filling and resting phases.

8.1. Algal diversity

The algal genera present in the WSTR, irrespective of whether they were filled with APE or RW, and their classification into family, order and division are presented in table 8.1. In a total, 29 algal genera were found in the WSTR, the majority were non-flagellate algae (20) despite the depth of the WSTR (6.5m), which favours the genera capable of easy movement in the water column.

Tables 8.2 and 8.3 show the frequency of algal genera present in the WSTR under eight loading regimes, in the filling and resting phases, respectively. In the filling phase, the number of algal genera ranged from 8 to 16, and in the resting phase from 8 to 17. There was no strong variation between the number of algal genera in the filling and resting phases of the WSTR, possibly because the concentrations of NH_3 and H_2S which could be toxic to algae were not much higher in the filling phase than in the resting phase, and also the concentration of the nutrients total ammonia and orthophosphate were not much lower in the resting phase than in the filling phase. In all experiments, the number of non-flagellate genera was higher than the flagellate ones, in the filling and resting phases. One factor, which could have favoured the predominance of non-flagellate in these deep WSTR, was the fact that they turnover at night.

Table 8.1. Algae present in the WSTR, irrespective of being filled with APE or RW.

1- Cyanobacteria	**Chlorococcales**
Nostocales	**Oocystaceae**
Nostocaceae	*Chlorella* **
Nodularia **	*Ankistrodesmus* **
Anabaena **	*Oocystis* **
Rivulariaceae	**Scenedesmaceae**
Rivularia **	*Coelastrum* **
Chroococcales	*Scenedesmus* **
Chroococcaceae	**Palmellaceae**
Gomphosphaeria **	*Sphaerocystis* **
Anacystis **	**Zygnematales**
Oscillatoriales	**Desmidiaceae**
Oscillatoriaceae	*Closterium* **
Oscillatoria **	**Zygnemataceae**
Arthrospira **	*Zygnema* **
2- Euglenophyta	**Chaetophorales**
Euglenales	**Chaetophoraceae**
Euglenaceae	*Phytoconis* **
Euglena *	**Ulotrichales**
Phacus *	**Ulotrichaceae**
Lepocinclis *	*Ulothrix* **
3- Chlorophyta	4- Chrysophyta
Volvocales	**Centrales**
Chlamydomonadaceae	**Coscinodiscaceae**
Chlamydomonas *	*Cyclotella* **
Chlorogonium *	**Pennales**
Spondylomoraceae	**Naviculaceae**
Pyrobotrys *	*Navicula* **
Volvocaceae	**Fragilariaceae**
Pandorina *	*Fragilaria* **
Eudorina *	5- Cryptophyta
	Cryptochrysidaceae
	Rhodomonas *

* Flagellate algae
** Non-flagellate algae

Table 8.2 - Algal frequency (%) in the filling phase of the WSTR.

	INFLUENT SOURCE: ANAEROBIC POND EFFLUENT					INFLUENT SOURCE: RAW WASTEWATER		
PHASE	WSTR1E3	WSTR 2E1	WSTR 1E1	WSTR 1E2	WSTR 2E2	WSTR 3E1	WSTR 3E2	WSTR 3E3
ORGANIC LOADINGS	117	124	178	376	667	111	265	292
Cyanobacteria								
Chroococcales								
Chroococcaceae								
Gomphosphaeria **	-	-	14	-		-	-	
Anacystis **	-	49	28	74		82	22	
Oscillatoriales								
Oscillatoriaceae								
Oscillatoria **	-	100	76	100		92	9	
- Euglenophyta								
Euglenales								
Euglenaceae								
Euglena *	84	82	34	51		97	9	
Phacus *	-	-	14	-		-	-	
- Chlorophyta								
Volvocales								
Chlamydomonadaceae								
Chlamydomonas *	82	-	40	8		-	-	
Spondylomoraceae								
Pyrobotrys *	66	90	80	100		100	91	
Volvocaceae								
Pandorina *	-	-	8	-		8	-	
Eudorina *	-	-	14	-		-	-	
Chlorococcales								
Oocystaceae								
Chlorella **	100	67	100	90		90	100	
Ankistrodesmus **	-	-	6	-		-	-	
Oocystis **	-	-	12	-		-	-	
Scenedesmaceae								
Coelastrum **	8	10	50	36		44	70	
Scenedesmus **	-	-	20	-		-	-	
Palmellaceae								
Sphaerocystis **	13	-	-	-		-	-	

Table 8.2. continuation.

ALGAE	WSTR1E3	WSTR 2E1	WSTR 1E1	WSTR 1E2	WSTR 2E2	WSTR 3E1	WSTR 3E2	WSTR 3E3
Zygnematales								
Desmidiaceae								
Closterium **	13	-	32	-		-	-	
Zygnemataceae								
Zygnema **	-	46	-	87		38	-	
Chaetophorales								
Chaetophoraceae								
Phytoconis **	11	-	-	-		-	13	
4- Chrysophyta								
Centrales								
Coscinodiscaceae								
Cyclotella **	-	-	-	-		-	17	
Pennales								
Naviculaceae								
Navicula **	-	-	-	5		-	-	
Fragilariaceae								
Fragilaria **	-	23	-	8		-	-	
5- Cryptophyta								
Cryptochrysidaceae								
Rhodomonas *	-	8	10	54		21	-	
Non-flagellate genera	5	6	9	7		5	6	
Flagellate genera	3	3	7	4		4	2	
Total N° of genera	8	9	16	11		9	8	
Cyanobacteria	0	2	3	2		2	2	
Euglenophyta	1	1	2	1		1	1	
Chlorophyta	7	5	10	5		5	4	
Chrysophyta	0	1	0	2		0	1	
Cryptophyta	0	1	1	1		1	0	

• Organic loading expressed in $kgBOD_5$/ha.day.

• The $WSTR_2E_2$ and $WSTR_3E_3$ were researched only during the resting phase.

Table 8.3 – Algal frequency (%) in the resting phase of WSTR.

	INFLUENT SOURCE: ANAEROBIC POND EFFLUENT					INFLUENT SOURCE: RAW WASTEWATER		
ALGAE	WSTR1E3	WSTR 2E1	WSTR 1E1	WSTR 1E2	WSTR 2E2	WSTR 3E1	WSTR 3E2	WSTR 3E3
ORGANIC LOADINGS	117	124	178	376	667	111	265	292
1- Cyanobacteria								
Chroococcales								
Chroococcaceae								
Gomphosphaeria **	8	-	20	-	-	-	-	-
Anacystis **	-	26	78	87	32	18	29	19
Oscillatoriales								
Oscillatoriaceae								
Oscillatoria **	22	54	71	95	51	80	9	17
Arthrospira **	-	-	11	-	-	-	-	-
2- Euglenophyta								
Euglenales								
Euglenaceae								
Euglena *	86	50	39	59	4	57	38	10
Lepocinclis *	-	-	-	-	-	29	-	-
3- Chlorophyta								
Volvocales								
Chlamydomonadaceae								
Chlamydomonas *	-	54	60	-	62	22	70	43
Chlorogonium *	-	-	-	-	-	-	-	5
Spondylomoraceae								
Pyrobotrys *	69	54	97	100	59	30	100	92
Volvocaceae								
Pandorina *	-	-	43	22	4	-	-	-
Chlorococcales								
Oocystaceae								
Chlorella **	100	81	98	94	74	65	100	43
Ankistrodesmus **	-	-	12	-	-	-	-	-
Oocystis **	-	14	8	-	-	4	-	-
Scenedesmaceae								
Coelastrum **	47	11	60	15	24	2	42	18
Scenedesmus **	-	-	56	-	-	-	-	-

Table 8.3. Continuation

ALGAE	WSTR1E3	WSTR 2E1	WSTR 1E1	WSTR 1E2	WSTR 2E2	WSTR 3E1	WSTR 3E2	WSTR 3E3
Zygnematales								
Desmidiaceae								
Closterium **	3	-	7	-	-	-	-	-
Zygnemataceae								
Zygnema **	12	26	8	85	13	46	-	4
Chaetophorales								
Chaetophoraceae								
Phytoconis **	-	17	-	-	-	4	12	-
Ulotrichales								
Ulotrichaceae								
Ulothrix **	-	-	-	-	-	2	-	-
4- Chrysophyta								
Centrales								
Coscinodiscaceae								
Cyclotelia **	-	3	-	-	-	-	-	-
Pennales								
Naviculaceae								
Navicula **	-	-	5	-	-	-	-	-
Fragilariaceae								
Fragilaria **	-	7	-	3	-	-	-	-
5- Cryptophyta								
Cryptochrysidaceae								
Rhodomonas *	-	4	40	49	-	2	-	-
Non-flagellate genera	6	9	12	6	5	8	5	5
Flagellate genera	2	4	5	4	4	5	3	4
Total N° of genera	8	13	17	10	9	13	8	9
Cyanobacteria	2	2	4	2	2	2	2	2
Euglenophyta	1	1	1	1	1	2	1	1
Chlorophyta	5	7	10	5	6	8	5	6
Chrysophyta	0	2	1	1	0	0	0	0
Cryptophyta	0	1	1	1	0	1	0	0

• Organic loading expressed in $kgBOD_5$/ha.day.

The algal genera *Phacus* and, *Eudorina* were found only in the filling phase of the WSTR loaded at 178 kgBOD_5/ha.day and the genus *Sphaerocystis* in the filling phase of the WSTR loaded at 117 kgBOD_5/ha.day, all genera with low frequencies. Both WSTR were fed with APE. The algal genera *Arthrospira*, *Lepocinclis* and *Ulothtrix*, *Chlorogonium* were found only in the resting phase of the WSTR, also with low frequency. They were found in the WSTR loaded at 178, 111, 292 kgBOD_5/ha.day respectively. Thus, there was no reason to believe that of the algal genera recorded, any particular species could only exist in either APE or RW filled WSTR.

In the filling phase of the WSTR, the genera *Pyrobotrys* and *Chlorella* were present in all experiments with frequency higher than 50%, possibly because these chlorophytes can grow at high organic loadings (de Noyelles,1967; de Oliveira, 1990). The genus *Euglena* was also present in all experiments, but its frequency ranged from 9 to 97%. The genus *Coelastrum* was also present in all experiments, with a frequency ranging from 8 to 70%. *Oscillatoria* was present in 5 of 6 experiments with frequency ranging from 9 to 100%.

In the resting phase of the WSTR, the algal genera *Oscillatoria, Euglena, Pyrobotrys*, *Chlorella* and *Coelastrum* were present in all the experiments.

No clear pattern was seen between algal frequency and the increase or decrease in the initial surface loading in the WSTR during either the filling or resting phases. Between the Chlorophytes, the algal genera *Pyrobotrys* and *Chlorella* showed the highest frequencies in both the filling and resting phases. From the division Euglenophyta, the genus *Euglena* was the only one present in both the filling and resting phases. From the division Cyanobacteria, *Oscillatoria* showed the highest frequencies in both the filling and resting phases. The presence of the algal genera Pyrobotrys, Chlorella, Euglena and Oscillatoria in the WSTR of different surface organic loadings in both filling and resting phases suggested the ubiquitous nature of these algae. The algal divisions Chrysophyta and Cryptophyta were not well represented in the WSTR.

8.2. Algal biomass in the WSTR

As the frequency data gives only a general indication of the algal speciation and occurrence, the algal population in the WSTR was also analyzed in relation to algal biomass (in terms of cell numbers) of the seven most common algal genera: *Euglena, Oscillatoria, Scenedesmus, Chlamydomonas, Chlorella, Pyrobotrys* and *Ankistrodesmus*.

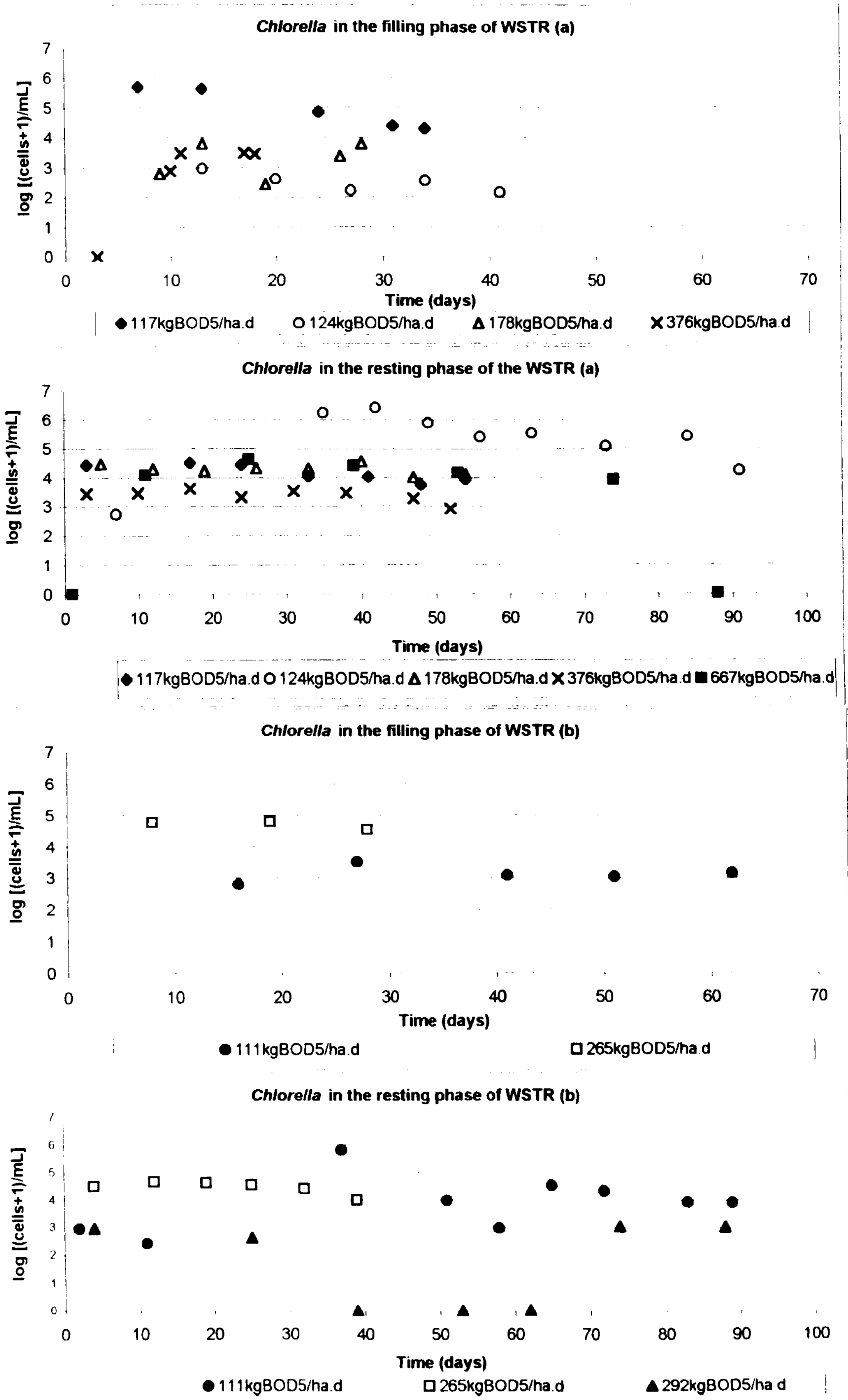
An attempt was made to evaluate any stratification in algal genera in the WSTR water column with depth. Since the reservoirs turnover at night (7.1) and no stratification had occurred by the sampling time of 08:00h – 09:00, water column samples for the eleven depths can be combined to give a mean value for algal counts at each sampling day in either the filling or resting phases. Due to dispersion in algal count results; the data were transformed into $\log_{10}$ [(cell + 1)/mL].

In the previous chapter (7.0), it was demonstrated, through regression plots of chlorophyll *a* against NH_3, H_2S, total ammonia and orthophosphate that the decrease in chlorophyll *a* concentration in the resting phase of the WSTR was not due to either toxicity or lack of nutrients.

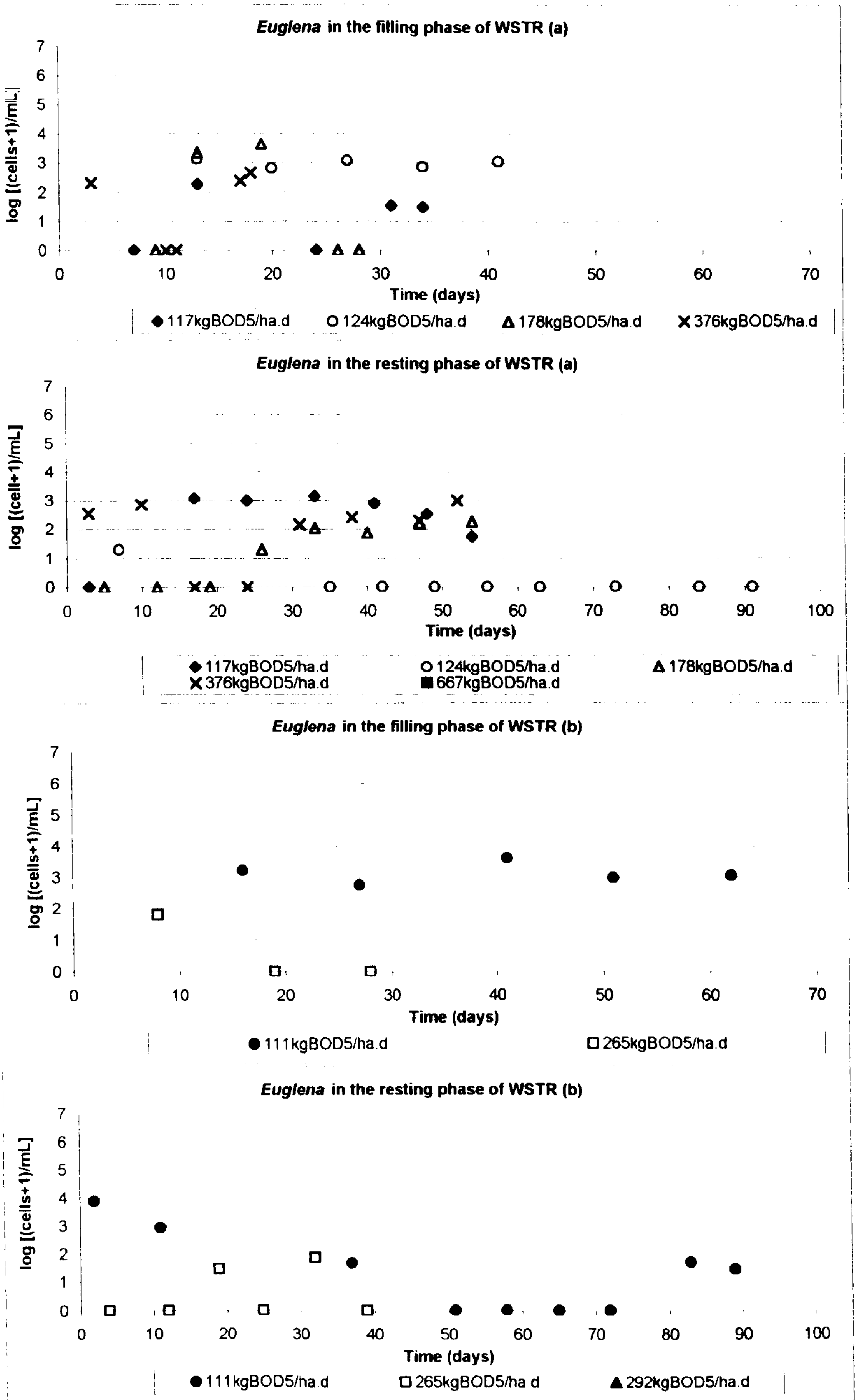
In this chapter, the biomass (in terms of numbers) of the seven genera mentioned above were plotted against time for both the filling and resting phases for the eight different organic loads applied to the WSTR, to investigate if the algal population increased or decreased with time. None of the seven genera showed a clear pattern with time in either the filling or resting phases. Also the algal biomass did not vary in relation to influent source i.e. APE or RW. See figures 8.1. to 8.7.

With a view to determining which parameter could be controlling each algal genera in the WSTR, correlations were made between the algal biomass for each genus and the concentrations of NH_3, H_2S, total ammonia and orthophosphate in both the filling and resting phases (tables 8.4 and 8.5). For the correlation analyse, only five genera of the seven mentioned above were considered because the algae *Scenedesmus* and *Ankistrodesmus* were present in only one of the eight experiments. The results showed that the algal genera were not affected by toxicity of NH_3 or H_2S or limited by nutrients (NH_4 and orthophosphate) in either the filling or resting phases, except the genus *Pyrobotrys* that had a negative and significant correlation with NH_3.

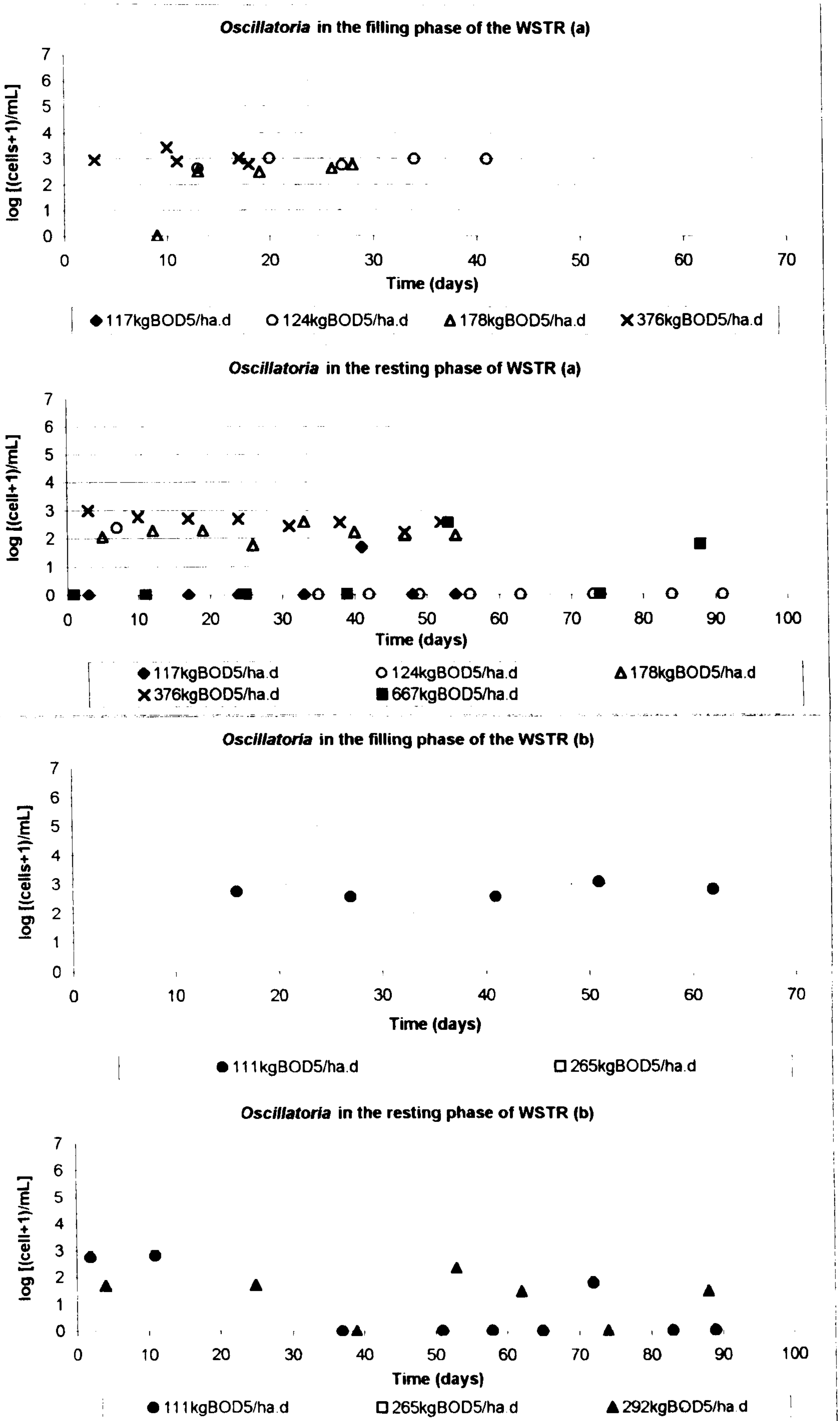
3.1. *Chlorella* in the filling and resting phases of WSTR filled with APE (a) and RW (b).



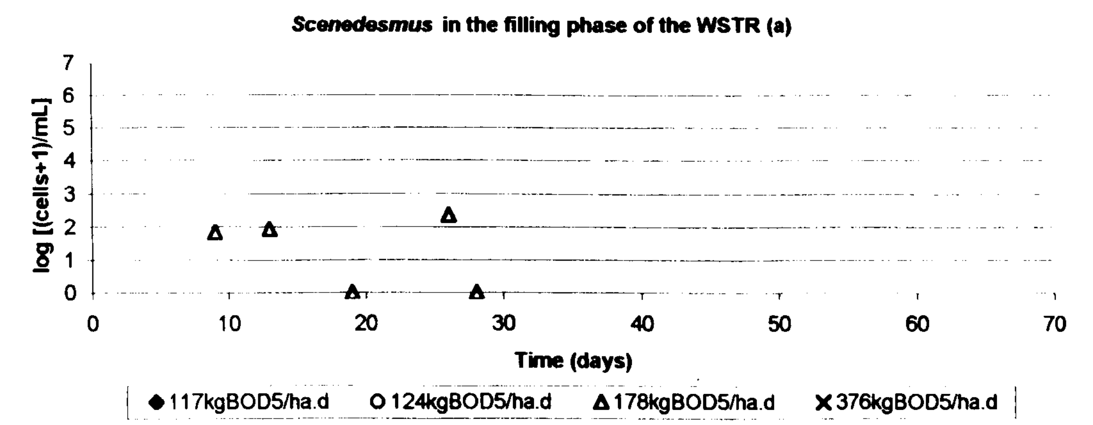
C.2 *Euglena* in the filling and resting phases of WSTR filled with APE (a) and RW (b)

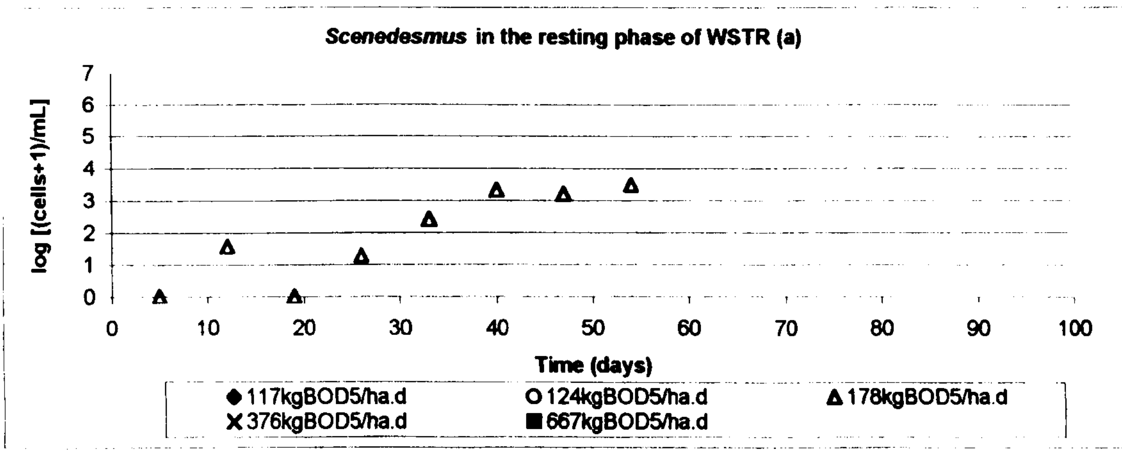


C.3 .*Oscillatoria* in the filling and resting phases of WSTR filled with APE (a) and RW (b)



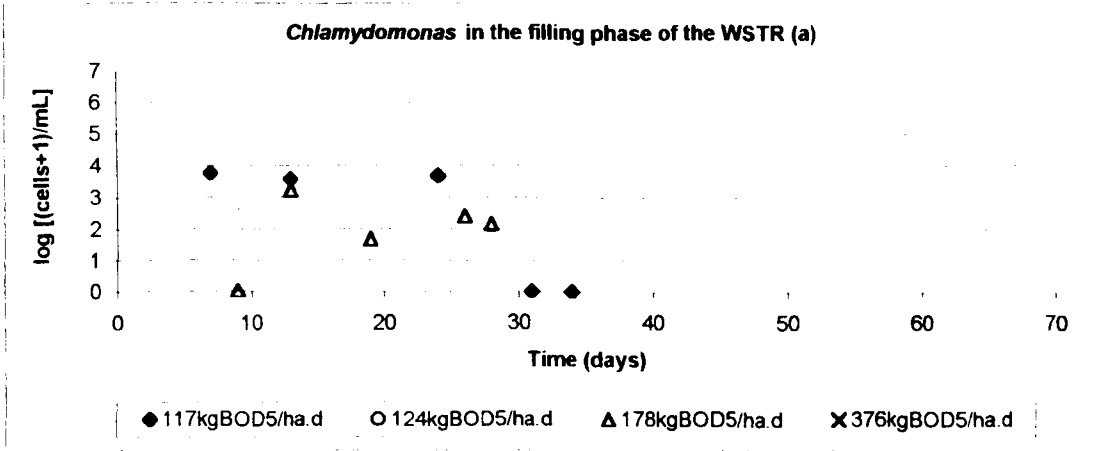
8.4.*Scenedesmus* in the filling and resting phases of WSTR filled with APE (a) and RW (b)

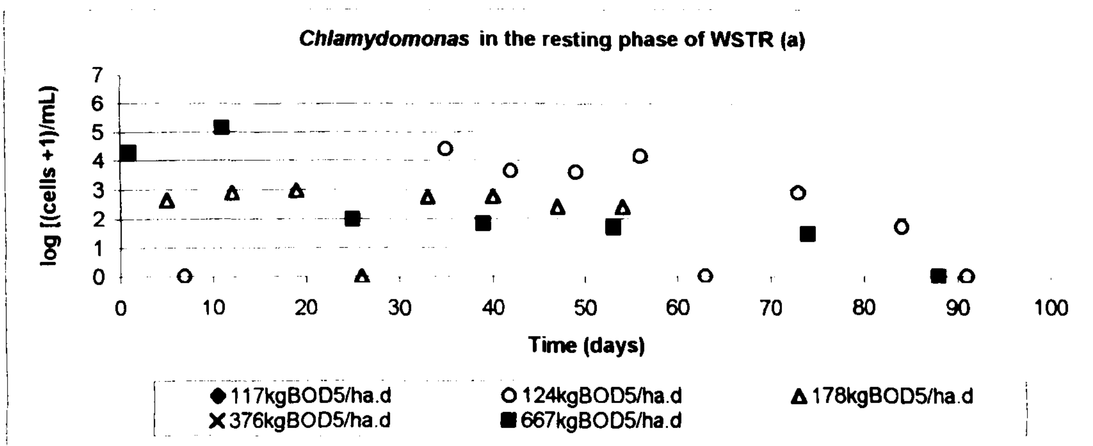


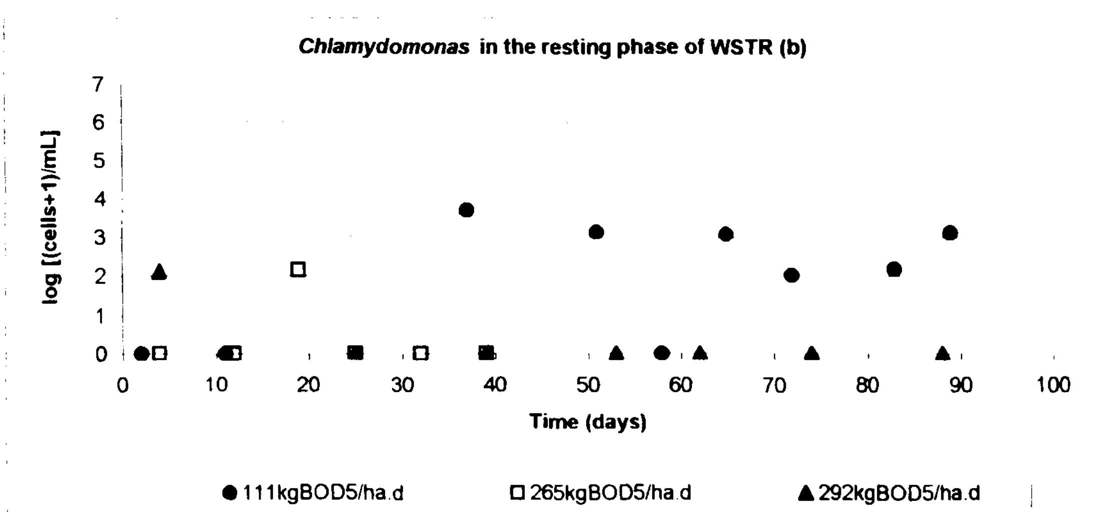


In the WSTR fed with APE, the genus Scenedesmus was present only at the surface organic loading of 178 $kgBOD_5$/ha.d. This genus was not found in any of the WSTR fed with RW.

ure 8.5.*Chlamydomonas* in the filling and resting phases of WSTR filled with APE (a) and RW (b)







Chlamydomonas was not found in the filling phase of the WSTR fed with RW.

Figure 8.6.*Pyrobotrys* in the filling and resting phases of WSTR filled with APE (a) and RW (b)

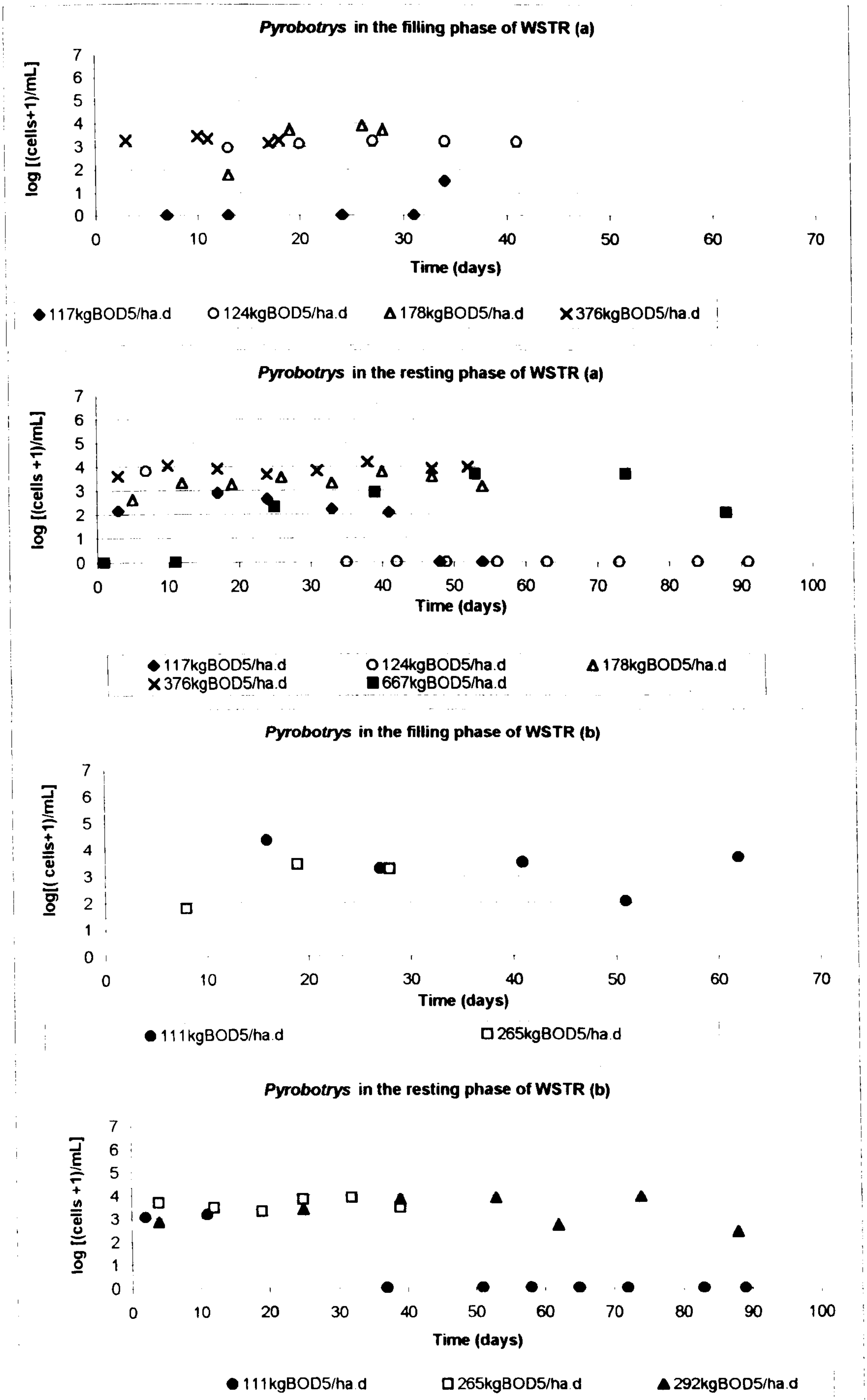
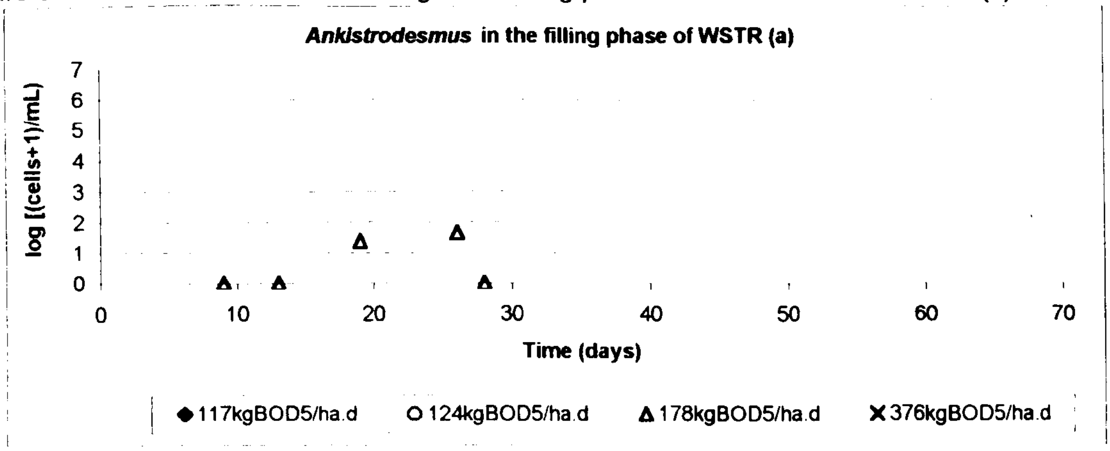


Figure 8.7. *Ankistrodesmus* in the filling and resting phases of WSTR filled with APE (a) and RW (b)



The algae Ankistrodesmus was found only in the filling phase of the WSTR loaded at 178 $kgBOD_5/ha.d$. This genus was not found in the resting phase of the WSTR fed with APE nor in any of the WSTR fed with RW.

Table 8.4. Correlation coefficients between some algal genera and NH_3, H_2S, total ammonia and orthophosphate in the filling phase of the WSTR.

	Chlorella	Euglena	Chlamydomonas	Pyrobotrys	Oscillatoria
NH_3	0.459*	-0.079	0.081	-0.513**	-0.005
H_2S	0.057	-0.344	-0.385	0.295	0.274
Total ammonia	0.211	-0.226	-0.492	-0.124	0.126
Orthophosphate	-0.133	0.196	-0.336	0.409*	0.525*

- Correlation was significant at the level of 5%;
- •Correlation was significant at the level of 1%;

Table 8.5. Correlation coefficients between certain algal genera and NH_3, H_2S, total ammonia and orthophosphate in the resting phase of the WSTR

	Chlorella	Euglena	Chlamydomonas	Pyrobotrys	Oscillatoria
NH_3	-0.021	0.256	-0.013	0.053	-0.196
H_2S	0.129	-0.110	0.196	0.326*	0.372*
Total ammonia	-0.207	0.207	-0.220	0.539**	-0.021
Orthophosphate	0.177	-0.495**	-0.168	-0.284*	-0.081

Abeliovich (1982) when discussing WSTR suggested that the algae that support the self-purification process in these systems are algal genera capable of competing for space and nutrients and are not very vulnerable to toxicity. As with exception of Pyrobotrys , appeared to be the situation here.

8.3.Algal population in WSP and WSTR systems

Waste stabilization pond systems and WSTR are operated differently in that; while pond systems receive sewage continuously, WSTR are fed and left to rest (batch systems). However, both the innovative WSP systems and the WSTR at Extrabes were filled with the same sewage source and were operated under the same environmental conditions (e.g. light intensity, temperature, etc).

In both sewage treatment systems, there was a predominance of the algal division Chlorophyta, possibly due to the high tolerance of this group to organic pollution.

The algal divisions Crysophyta and Cryptophyta were not well represented in either system. Representatives of the Cyanobacteria (i.e. Oscillatoria) and of the Euglenophytes (i.e Euglena) were also present at both systems.

In general, the genera *Euglena, Oscillatoria, and Pyrobotrys* showed resistance to high organic loadings, whereas *Ankistrodesmus and Scenedesmus* sensitivity to it. The genus Chlorella showed high frequencies even in the WSTR highest loaded. This finding is in accordance with Palmer (1969) who classified this chlorophyta among the top eight pollution tolerant genera. However, in the ponds this alga showed a different pattern increasing in frequency with the decrease in loading especially when the ponds worked under the lower loading regime.

The algal genera *Spirulina, Micractnium, Dictyosphaerium, Micrasterias, Spyrogyra, Gomphonema and Nitzchia* were found only in the pond system whereas *Lepocinclis, Sphaerocystis, Phytoconis and Ulothrix* only in the WSTR.

The results showed that WSP and WSTR systems are both hypertrofic environments, where phytoplankton plays a key role in the purification process. There was no strong difference between the main genera present in either WSP or WSTR. However, the algal succession with time was seen more clearly in the ponds. The genera tolerant to high loading predominated in the first ponds of the series, whereas the most sensitive ones in the last ponds. In the WSTR, no clear relation between the genera observed and time in either the filling or resting phases was seen.

8.4. Algal diversity as an indicator of WSTR performance

Since algal diversity and the numbers of algal genera present in WSTR were very similar to those found in WSP, it was anticipated that the identification of algal types present in WSTR might be similarly used to gauge changes in their performance under different loading regimes and as indicators of potential effluent quality during the resting phase. Analysis of the diversity data failed to produce any clear correlation between surface organic loading, algal genera present and potential effluent quality as was the case with WSP.

Similarly changes in algal numbers for the genera *Chlamydomonas* and *Scenedesmus* in relation to surface organic loading and the degree of purification that was demonstrated in the WSP could not be found in the WSTR. It had been anticipated that the changes in diversity and specific algal biomass concentrations through the pond series would be similarly found with time during the resting phase in the WSTR as purification proceeded and organic load decreased. The reasons for these differences between the ponds and the reservoirs was not clear although it must be remembered that whilst ponds are continuous flow reactors WSTR are batch filled ones.

Thus unlike the situation in ponds and based on the data collected in the WSTR in this study, algal diversity and changes in abundance of specific genera were no help in evaluating WSTR performance. There is therefore room for further and more detailed research into algal genera and species in WSTR but given the time and effort required to collect this type of data caution should be attached to studying the algae as part of the routine monitoring of these types of reactor.

8.5. Conclusions:

- Twenty-nine algal genera were found in the WSTR, irrespective of being filled with APE or RW, the majority were non-flagellate algae (20);
- There was no marked variation between the number of algal genera in the filling (8-16) and in the resting phases (8-17);
- The non- flagellate genera predominated in both the filling and resting phases;
- There was no reason to believe that of the algal genera recorded, any particular genus could only exist in either APE or RW filled WSTR;
- In the WSTR filling and resting phases, the algal genera *Pyrobotrys*, *Chlorella*, *Euglena*, *Coelastrum* and *Oscillatoria* showed the highest frequencies;
- In general, the algal division Chlorophyta predominated in the WSTR;
- The algal divisions Chrysophyta and Cryptophyta were not well represented in the WSTR;
- In neither the filling nor the resting phases, could any clear pattern be seen between algal frequency and increase or decrease in the WSTR initial surface organic loading,
- There was no clear reason that could explain the algal biomass variation of the genera *Euglena*, *Oscillatoria*, *Scenedesmus*, *Chlamydomonas*, *Chlorella*, and

Ankistrodesmus with time in either the filling or resting phases, only *Pyrobotrys* showed a significant negative correlation with NH_3 in the filling phase.

Chapter 9. General Discussion and Conclusions

Current waste stabilisation pond technology and wastewater storage and treatment reservoir design provides effective wastewater treatment at low cost and in a way that is sustainable for many poorer and less developed countries of the world. However their application in less developed countries does not mean that these technologies should be perceived as less efficient and inferior to electro-mechanical systems such as activated sludge just because they are cheaper to construct and less complicated to operate and maintain. Indeed there has been somewhat of a renaissance in interest in the use of WSP systems in industrialised countries as a way of treating wastewater cheaply and efficiently particularly where reuse of the treated wastewater can be linked to agricultural reuse. It is important not to forget that in many countries over 70% of the water used goes to agriculture for irrigation (UNDP, 1996) and the reuse of suitably treated waste water for agricultural use can thus be an important component of the water cycle particularly in water-short regions.

The efficient natural mechanisms of biological disinfection occurring in WSP and WSTR (Pearson *et al*, 1987, Pearson *et al* 1996; Curtis et al, 1992; Davies-Colley 2000) mean that they can be easily designed to produce effluents with a microbiological quality capable of meeting WHO guidelines (1989), for reuse in agriculture and aquaculture without the need for complex disinfection technologies as is the case with electro-mechanical systems.

However, increasingly stringent standards for the effluent quality of wastewater treatment systems discharging into freshwater and marine environments to protect these environments from pollution and degradation means that emphasis is also being put on nutrient removal as well as BOD and pathogen removal efficiencies by treatment systems.

Ponds nevertheless require much larger land areas than conventional wastewater treatment systems and this frequently militates against their use where land availability is limited and the opportunity cost of land is thus high.

It is therefore essential to reduce the land take of WSP and WSTR systems whilst maintaining their efficiency or indeed improving it so as to increase their application and suitability. Much current research in this field is currently being directed towards these ends.

BOD removal efficiency in the individual primary facultative ponds was similar in both the shallow (1.25 m depth) and the deeper (2.3m depth) ponds and they gave comparable BOD_5 effluent quality for the same surface organic loading. Thus the increased retention time (HRT) in the deeper facultative ponds does not apparently enhance BOD_5 removal and therefore the additional cost of excavation to construct deep facultative ponds would have no justification. On the other hand where the profile of land would mean lower construction costs if the facultative ponds were deeper, this would not have a deleterious effect on BOD removal efficiency.

Although single pond systems are not recommended even for very small communities, effluent quality would be directly proportional to surface organic loading. The data presented here showed that at a temperature of 25°C, a single lagoon of 1.25m depth and a surface organic loading of 225 $kgBOD_5/ha.d$ gave an effluent quality of approximately 40 mg/L BOD_5 (total). This represents a removal efficiency of in excess of 80% which is at the very top end of BOD_5 removal efficiency recorded for primary facultative ponds.

The data also showed that loadings as high as 500 $kgBOD_5/ha.d$ can be applied to primary facultative ponds at a temperature of 25 °C without a loss of efficiency. It would seem therefore that current recommended design surface organic loadings for primary facultative ponds could be increased from what seems a rather conservative value of 350 $kgBOD_5/ha.d$ (Yanez 1992; Mara and Pearson 1998;) to 500$kgBOD_5/ha/d$ for tropical systems where mean air temperatures of the coolest months equal or exceed 25°C. This would represent a 30% saving in land area for the facultative pond of a pond series.

The deep facultative ponds (2.30m) exhibited much lower k_b values for FC die-off than the shallow ones (1.25m). This correlated with poorer FC removal and thus poorer bacterial pathogen die-off in the deep ponds compared to the shallow ones even taking account of the increased hydraulic retention time provided by the deeper ponds for the same surface organic loading.

FC die-off rates (k_b) were affected by surface organic loading being better at lower organic loadings compared to high ones. However, in engineering design terms this is not so significant as primary facultative ponds are basically designed at the head of a pond series with the main role of BOD removal whilst FC removal is the main prerogative of the subsequent maturation ponds of the series.

The data presented in chapter 4 established that the shallow pond series (depth 1.0m) taken, as a whole, were more efficient at both BOD_5 removal and faecal coliform removal than the deeper series (2.2m). However, both shallow and deep pond series showed that a significant negative correlation existed between pond effluent BOD_5 and log FC concentration when these parameters were plotted against mean theoretical HRT. The increased overall HRT per unit area resulting from the use of the 2.20m deep pond series compared to the 1.00m deep ponds more than compensated for their reduced efficiency. It also allowed a significant saving in land area (>32%) compared to the shallow pond series when achieving the same effluent quality. This means that the accepted practice of building facultative ponds to a pond liquid depth of between 1.5 and 2.0m and maturation ponds to between 1.0 and 1.25m may place unnecessary constraints on pond design and furthermore uses more land area than is necessary. It is important to remember that the cost of land can be an expensive component of overall pond construction costs (Arthur 1983). The use of these deeper ponds also raises another issue important in semi-arid and water-short countries and that is that deeper pond series will result in a reduction in water loss through evaporation, as the surface area to pond volume ratio will be smaller. However, whether ponds should be increased in depth beyond 2.2m would require further study.

The trend of more efficient removal efficiency in shallow ponds was further accentuated in the innovative system with its very shallow maturation ponds (1m – 0.39m depth) and although differences in geometry between this system and the five pond series described in Chapter. 4 precluded a direct comparison the advantages shown by deeper ponds in terms of saving land area may not hold when considering these ultra shallow ponds. The data showing different K_b for different types of ponds do however support the views held by certain workers that design equations for determining K_b for FC removal should be different for different types of ponds (Oakley *et al* 2000). This has been based on the observations that the first-order rate constant for faecal coliform removal varied with organic loading on the pond as well as temperature (León and Moscoso, 1996) a fact supported by this study. Pearson *et al* (1995) had also observed that K_b varied with maturation pond depth and surface organic loading and was higher in maturation ponds than facultative ponds and anaerobic ponds. Sáenz (1985) showed a preference for the use of the Wehner and

Wilhem equation for designing maturation ponds (Wehner and Wilhem, 1956) that places emphasis on the inclusion of a dispersion number in the design equation for maturation ponds to take account of the degree of mixing in the ponds. Sáenz determined the dispersion number for use in the Wehner and Wilhem equation based on temperature and taking account of pond depth, width and length. This dispersion model has been used extensively although not exclusively in Central and Latin America to design maturation ponds.

This more complicated approach to pond design has its critics and certain design manuals (Mara and Pearson, 1998) have instead based designs for maturation ponds on the Marais equation (Marais 1974). This assumes a completely mixed regime in each of the pond reactors and dispenses with values for dispersion and thus degrees of mixing. Pearson *et al* (1996) demonstrated that although differences in K_b values were obtained for different pond types using the Marais equation for determining K_b, nevertheless the application of the theoretical K_b based on temperature in a completely mixed reactor to a series of five ponds for a design temperature of 25°C in northeast Brazil gave theoretical values for final effluent FC concentrations very close to the actual values measured. This study has shown that in both deep and shallow pond series there is a significant inverse relationship between effluent FC concentration and HRT using the Marais equation and tends to undermine the necessity to use more complicated approaches invoking the degree of mixing in the ponds as defined by the dispersion number when designing these systems. Indeed the use of theoretical dispersion numbers based on proposed design length to width to depth measurements before the ponds are constructed may be naïve, since current modelling of dispersion in pond systems would appear to be complex and sometimes unpredictable (Brissaud et al 2000; Shilton et al, 2000; Soler et al 2000). It also seems to place unnecessary constraints on pond physical design and this may create difficulties particularly on sites where land is at a premium (Pearson *et al*, 1996).

Flexibility would appear to be the key to designing the most appropriate pond system at any location and the data in figure 5.1 suggests that there is rapid die-off of FC at low surface organic loading and this correlates with high pH and good algal activity. Thus given that shallow maturation ponds had higher pH's than the deeper ponds there is an argument for making the last pond in the series which is also the one with the lowest organic loading shallow i.e. 30-60cm in depth to promote FC removal. This would not significantly increase the overall land area occupied by the system since the previous ponds would be kept deep. This suggestion requires further investigation.

The shallow innovative system with maturation ponds ranging between 1-0.39m deep gave higher ammonia removal rates than the other two deeper five-pond series. But in all cases the lower the organic loading on the pond systems the higher the nitrogen (as ammonia) removal obtained. Similar results were obtained with phosphorus removal i.e. the shallow ponds gave better removal results and in fact in the 2.20m ponds no orthophosphate removal was observed.

Unlike BOD_5 removal and FC removal the loss of efficiency in terms of nutrient removal in deeper ponds was not compensated for by the increased HRT. Therefore, the use of shallow ponds particularly shallow maturation ponds would increase nutrient removal and the correlation between increased nutrient removal and high pH's fits the pattern of increased ammonia volatilisation and phosphorus precipitation as calcium phosphate and hydroxyapatite at alkaline pH. Thus in terms of the best pond design for nutrient removal these data would suggest that using very shallow final maturation ponds (60cm or less) might prove to be an acceptable trade-off, as the increase in land-take might be compensated for by more efficient removal of nitrogen (ammonia) and phosphorus. This concept would be compatible with the idea of a final, very shallow, maturation pond also giving improved FC removal whilst not adversely affect BOD removal and it therefore warrants further study.

The poor efficiency of phosphorus removal, in conventional pond systems, has led to the suggested use of a pond system followed by activated sludge (ASP), the so-called PETRO (Pond Enhanced Treatment and Operation) system. In this system the ponds remove 70% of the BOD and the ASP considerably enhances phosphorus removal over the maximum of 30% obtained in conventional pond systems (Shipin *et al* 2000). Whilst in conventional ASP systems operated for maximum P removal, the important bacterial genus involved in luxury P uptake is an *Acinetobacter* species, in the PETRO system the pond microalgal genera *Chlorella*, *Euglena* and *Chlamydomonas* were implicated.

The need to store and treat wastewater during the wet or non-growing season for subsequent use in crop irrigation has increased the use and research into WSTR systems originally developed in Israel over 20 years ago (Shelef, 1991). More recently the concept of combining the use of WSP with WSTR to facilitate a more flexible irrigation regime has been proposed and implemented (Mara and Pearson, 1999; Shelef and Azov, 2000).

This study has shown that in many respects these batch-filled reactors can be designed, in terms or permissible surface organic loading rates, as primary and secondary facultative waste stabilisation ponds. Thus standard equations to determine surface BOD_5 loading rates on facultative ponds can be used with WSTR during the filling phase. WSTR can be loaded with raw wastewater or effluent from an anaerobic pond without obvious changes in performance. Indeed the proposed increase in organic surface loading for facultative ponds is applicable to WSTR without risk of odour.

The results from this study also showed that these tropical WSTR mixed completely (i.e. turned over) at night and this contrasted with results in Spain where Soler *et al* (2000) studying deep "batch-filled" WSP (and thus comparable to WSTR) showed that their systems showed prolonged stratification. They modelled they system as if it was two completely mixed reactors one sitting above the other, i.e. one reactor representing the water volume above the thermocline and the other the water volume below it. The implication for overall design is as yet not clear. However, one difference is immediately obvious and that is that all the water in the tropical system irrespective of depth will be of similar quality unlike the unmixed temperate WSTR. Thus potentially, draw-down of the tropical system could occur more rapidly if irrigation strategy so dictates, provided that the nightly turnover of the WSTR contents does not lead to the re-suspension of viable helminth eggs from the sludge layer into the water column so contravening WHO guidelines. The current study did not consider helminth eggs because of logistical problems although previous work indicated that this resuspension of viable eggs was not likely to be a problem (Pearson *et al* 1996).

A detailed investigation into the removal and die-ff of FC in these WSTR was the subject of a different study (Athayde 2000) and will not be discussed in detail here. However, certain broader issues require comment. Previous work on these reactors has discussed effluent quality in relation to faecal coliforms and helminths (Pearson *et al* 1996; Athayde *et al*, 2000) and showed that an effluent quality suitable for unrestricted irrigation according to WHO guidelines can usually be achieved after 28 days into the resting phase which is much shorter than the minimum storage period normally required for irrigation schemes. Nutrient removal in the WSTR was poor compared to WSP, in fact in most experiments the effluent quality did not reach the Brazilian standard for discharge into receiving waters (CONAMA, 1986) of 5mg/L total ammonia even after 44 days or more in the resting phase. Phosphate concentrations also remained high, between 4-5mg/L during the resting phase and thus discharge of WSTR effluent directly to surface waters rather than its use in irrigation where crops will assimilate the nutrients would require careful environmental evaluation. Therefore WSTR is a poor alternative to WSP as a stand-alone treatment technology unless linked to irrigation where the combination of plants and soil filtration provide tertiary treatment of the effluent drainage water in a similar way to wetland systems (Gschlosz *et al* 1998) and whereupon the advantages become obvious.

The controlling factor in terms of reuse is more likely to be the ammonia concentration of the effluent and the risk of over fertilisation or ammonia toxicity to the crops rather than the concentrations of pathogens or BOD. The relatively high and persistent concentrations of sulphide did not appear to seriously affect algal growth in the WSTR and thus are unlikely to be toxic to crop plants even if the sulphide was not oxidised in the irrigation system before reaching the crops as is most likely.

The presence of sulphide in the water column of WSTR although potentially toxic to fauna and flora has the advantage of ensuring that heavy metals that could reach the treatment system in industrial wastewaters will be precipitated out and thus will not represent a toxic threat in subsequent reuse strategies. Laboratory studies have shown that algal genera such as *Chlorella, Scenedesmus* and *Chlamydomonas* common in WSTR are capable of sequestering heavy metals within their cells (Lawson *et al* 1996; Chong *et al* 2000). This would constitute a risk of heavy metal concentration in the food chain notably in the case of reuse in aquaculture, however, the chemical precipitation of heavy metals by sulphide in the WSTR is likely to preclude or minimise this risk.

The use of algal diversity and the relative abundance of different algal genera as a means of rapidly predicting potential changes in WSP performance and as a visual method for classifying pond types (Pearson *et al*, 1987) was applied to WSTR. The genus *Chlamydomonas* was most abundant in highly loaded ponds and decreased in numbers as the organic loading dropped through the series. In contrast *Scenedesmus*, behaved in an opposite way decreasing in numbers as organic loading increased. In this study there was no clear pattern suggesting a higher frequency of flagellate genera in ponds of high organic loading in contrasts to earlier findings (Pearson *et al*, 1987).

It is likely that even within a single genus, algal species will show considerable differences in their sensitivity to environmental conditions and thus one failure of this study might have been the inability to identify algae down to the species level. However, since algal species are known to change morphologically in response to environmental conditions a more rigorous taxonomic approach based on molecular studies (DNA profiles) of pond algae may represent and interesting new approach to this subject.

The algal diversity and numbers of genera present in WSTR were very similar to those found in WSP. Therefore it was anticipated that the identification of algal types present in WSTR might be similarly used to gauge changes in their performance under different loading regimes and also as indicators of improving effluent quality during the resting phase. Analysis of the diversity data failed to produce any clear correlation between surface organic loading, algal genera present and potential effluent quality as was the case with WSP.

Similarly changes in algal numbers for the genera *Chlamydomonas* and *Scenedesmus* in relation to surface organic loading and the degree of purification that was demonstrated in the WSP could not be found in the WSTR. It had been anticipated that the changes in diversity and specific algal biomass concentrations through the pond series would be similarly found with time during the resting phase in the WSTR as purification proceeded and organic load decreased. The reasons for these differences between the ponds and the reservoirs was not clear although it must be remembered that whilst ponds are continuous flow reactors WSTR are batch filled ones.

In contrast to the situation in WSP, the study of algal diversity and changes in abundance of specific genera were no help in evaluating WSTR performance. There is therefore room for further and more detailed research into algal genera and species in WSTR using a molecular approach as mentioned previously. But given the time and effort required to collect this type of data, caution should be attached to studying the algae as part of the routine monitoring of these types of reactor.

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