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Bacterial Die-Off in Waste Stabilization Ponds Receiving Domestic Wastewater in Dar es Salaam, Tanzania

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BACTERIAL DIE - OFF IN WASTE STABILIZATION PONDS RECEIVING DOMESTIC WASTEWATER IN DAR ES SALAAM, TANZANIA

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ABSTRACT

In this work, the bactericidal action of direct solar radiation was investigated. The effect of other parameters namely hydraulic detention time, pond depth, pH and dissolved oxygen on the reduction of bacteria were also investigated. a pilot plant was constructed at the University of Dar es Salaam in Tanzania for these investigations.

Bacterial reduction was observed to proceed with increasing direct solar radiation intensity. The mortality rate of faecal coliforms used as test organisms was observed to be higher in samples incubated near the pond surface and decreased rapidly when the samples were incubated at greater depths in the pond. Faecal coliforms were generally found to be reduced rapidly in shallow ponds and at high solar intensity. Some other factors namely high pH values and dissolved oxygen concentration near and above saturations value were also observed to contribute to the bacterial die-off.

Furthermore, faecal coliforms were found to be reduced more rapidly in ponds with longer hydraulic retention times. The mortality rate followed Chiks's law very closely. However, it was noted that even with over 99 % reduction in faecal coliform number, the amount remaining is alarmingly high.

1 INTRODUCTION

Waste stabilization ponds frequently are looked on as presenting the sanitary engineer with an alternative method of sewage purification. Depending on the specific needs of the community, wastewater stabilization ponds may be used for either partial treatment or complete treatment in conjunction with conventional primary or secondary process.

The use of wastewater ponds is accompanied with several benefits. For example, they may provide opportunities for reuse of water and nutrients. They are also used as buffer and back-up treatment systems to increase the reliability of other forms of wastewater treatment.

Wastewater lagoons have specific relevance in developing countries where more costly techniques are prohibitive: costs are low, operation is simple and maintenance is cheap. Furthermore, waste treatment lagoons are not very sensitive to shock loadings. They also form a reliable tool in pollution prevention especially in tropical areas where climate is favourable for biological treatment of domestic effluents.

Unfortunately, wastewater lagoons have fallen into disfavour because of land requirements especially in city areas, high concentration of organics in the effluent, and dependence on environmental factors. Many of the problems result, however, from a lack of understanding of the basic biochemical mechanisms involved, improper operation and overloading.

Waste stabilization ponds should be considered as a complex system encompassing the existence of several living organisms, particularly algal-bacterial inter-relationship. These micro-organisms are largely responsible for purification of wastewater in stabilization ponds.

The growth of an algal mass culture in a natural environment depends on many physical, chemical and biological factors. Some of these parameters can be controlled while others are essentially uncontrollable. In general, physical factors are least controlled. Solar radiation, being the principal driving force for photosynthetic reaction in the pond, is the most important physical factor and man's ability to control this parameter is minimal. Since temperature depends to a large extent on light intensity in nature, any variations in the luminous energy also would affect the growth of algae by affecting the temperature. Of the physical factors, mixing is a critical factor under tropical conditions.

Algal growth proceeds in the presence of nitrogen, phosphorous and inorganic carbon in the form of carbon dioxide and HCO_3-from bacterial oxidation of organic matter and influent alkalinity. Because of intense photosynthetic activity during the daylight hours, oxygen supersaturation may result in the ponds. Variation of pH is also common during the daily cycles because of utilization of CO_2 by

algae, thereby causing dissociation of HCO_3- to produce a hydroxyl ion. As pH rises above 9, bacterial activities begin to diminish, causing a reduction in Co_2 production and thereby limiting subsequent algal growth.

Though the performance of waste stabilization ponds in terms of reduction of organisms is known, the mechanism of this reduction is still unclear. Several hypotheses have tried to explain causes of bacterial reduction including the high pH levels in the ponds, toxic extracellular compounds produced by algae, and antibacterial substances produced by algae just to mention a few.

Due to the complex ecosystem existing in waste stabilization ponds, it is thus necessary to study the physical, biochemical and environmental factors which influence the behaviour of stabilization ponds in order to provide ponds which will be as economical and effective as possible. Retaining sewage effluents in ponds has a number of consequences which pose questions on the design and performance including the following.

- what detention time is optimum for removal of pathogenic organisms, suspended solids and organic material

- what depth of ponds (minimum in case of weed growth and maximum in case of performance) is suitable

- what effects will algae have on the performance of the ponds when discharged to natural waters, and what operating conditions are required to optimize growth of algae

- what are the levels of reduction of nutrients and what are the removal mechanisms of these nutrients.

The effect of solar intensity, pond depth and hydraulic detention time on the bacterial die-off were investigated by the author under tropical weather conditions and the outcome of research is presented in this report.

FEATURES, STABILIZATION PROCESS AND ALGAL ACTIVITIES IN WASTEWATER PONDS

2.1 Types of Ponds

Waste stabilization ponds are primarily of 3 types:

(a) anaerobic ponds: These are so heavily loaded with organic matter that they are completely anaerobic, except possibly at the surface. They are used for decomposition of complex organic wastes before further treatment in facultative ponds. Anaerobic ponds are often 2.5 to 4.0 m deep since they do not depend on photosynthetic algal action.

(b) facultative ponds which are aerobic in upper water layers and anaerobic in the deeper layers.

(c) aerobic ponds which are shallow ponds (about 0.3 to 0.5 m deep) which contain dissolved oxygen throughout the whole water body at all times. Their major application is in algal culture and harvesting rather than ordinary waste treatment (Arceivala, 1973).

Maturation (or polishing) ponds are aerobic or facultative ponds whose primary function is the destruction of faecal bacteria although they are also responsible for the quality of final effluent. The quality of final effluent and the overall efficiency is controlled by the number and size of ponds (Marais, 1963; Meiring et al, 1968), detention time and depth of ponds (Marais, 1963, Marais, 1974).

2.2 Pond Depth

Pond depth normally varies between 1 to 2 m although deep ponds up to 5 m have been used (Wachs and Berend, 1968 cited by Arceivala, 1986). However, the common depths for maturation ponds is in the range of 1.0 to 1.5 m (Marais, 1963).

Although shallower ponds are preferred due to high rate of bacterial die-off, the depths less than 1.0 m are unfavourable due to vegetation growth which would attract mosquito breeding (Gloyna, 1971; Drews and Denysschen, 1978) and also reduce the effective volume of pond, consequently reducing hydraulic detention time.

In deeper ponds light cannot penetrate the liquid because of excessive liquid depth and excess turbidity caused by algae and scum mats.The photosynthetic action of algae will thus be inhibited, consequently reducing the efficiency of the ponds. For example, some products of bacterial degradation of the organic matter in the waste inflow on which algae feed will remain in the bulk of the liquid and appear in the effluent. Furthermore, reduction of pathogenic organisms will be minimized as a result of limited algal growth and inadequate exposure to direct sunlight.

2.3 Detention Time

Algae require sufficient time in a pond to grow and multiply through binary fission. Each pond compartment should provide a minimum retention time to avoid premature cell washout (Arceivala, 1986). Detention time is also required to achieve a desired level of coliform and organic material removal.

Marais (1966) considered that 7 days was the minimum desirable retention time in an individual pond because of short-circuiting in ponds with shorter retention time. Sufficiently long retention time of about 5 to 10 days should be provided to allow parasites to sink to the bottom of the ponds and die-off there (INFU, 1980).

2.4 Stabilization Process

Natural purification of water by wastewater stabilization ponds begins immediately after wastewater enters a pond. Settleable solids, suspended solids and colloidal particles either settles to the bottom of the pond by gravity or may be precipitated by action of soluble salts due to rise in pH (Canter et al 1969, Oswald, 1973). Soluble materials are oxidized by bacteria. Settled organic matter is converted to inert residual and soluble substances diffuse into the bulk of the water above, where further decomposition is carried out by bacteria. Figure 1 shows a schematic model of stabilization pond ecosystem.
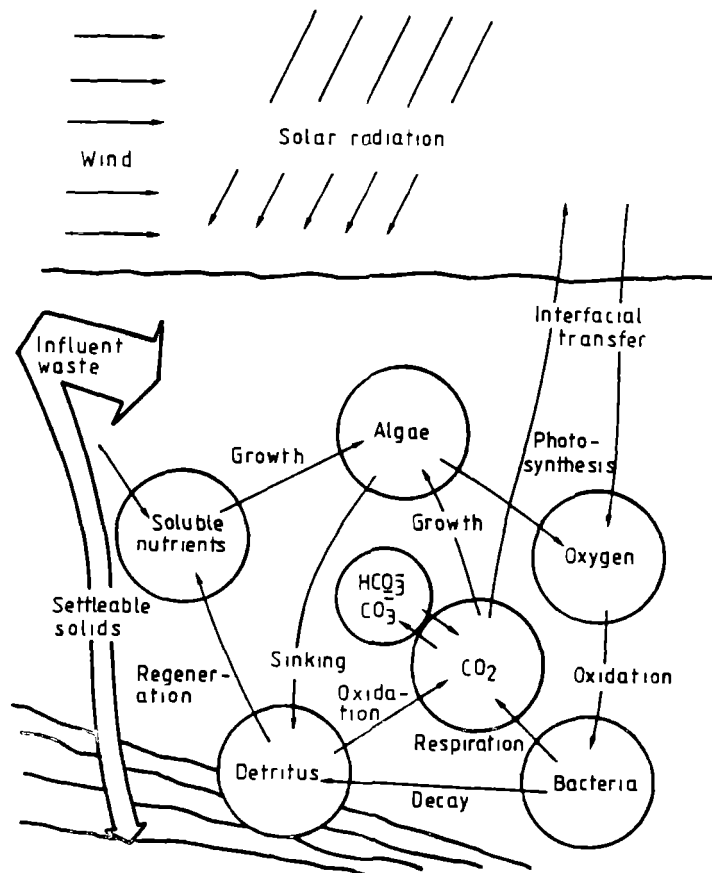
Figure 1. Schematic model of stabilization pond ecosystem (Fritz et al, 1979).

For aerobic or facultative pond to operate effectively oxygen supply is needed for metabolism of aerobic, heterotrophic bacteria and algae respiration. The sources of oxygen in wastewater stabilization ponds are (Mäkelä, 1977 and Fritz et al, 1979):

- photosynthetic oxygen production

- atmospheric oxygen through the pond surface

- chemically bound oxygen mainly in nitrated and sulphates

- dissolved oxygen in influent which is usually less than 5 mg/l.

The most important source is photosynthetic oxygen production.

2.5 Importance of Algae in Waste Stabilization Ponds

Algae are unicellular or multicellular, autotrophic, photosynthetic protists (Metcalf & Eddy, 1979). They are microscopic green plants containing chlorophyll which in the presence of light utilize the simpler end products of bacterial degradation of organic matter to produce more algae simultaneously releasing oxygen.

Algae in wastewater ponds are valuable due to their ability to produce oxygen through the mechanism of photosynthesis which is in turn used by bacteria in the decomposition of organic matter. In the presence of light, respiration and photosynthesis can occur simultaneously in algae. However, the respiration rate is low compared with the photosynthesis rate, resulting in a net consumption of carbondioxide and production of oxygen. In the absence of light, algae respiration continues, while photosynthesis stops, resulting in a net consumption of oxygen and production of carbondioxide (U.S.EPA, 1983).

Equations 1 and 2 below represent simplified biochemical reaction for photosynthesis and respiration (Metcalf & Eddy, 1979):

$$\text{Photosynthesis} : CO_2 + H_2O \xrightarrow{\text{light}} CH_2O + O_2 \qquad (1)$$
$$\text{new algae cell}$$

$$\text{Respiration} \quad : CH_2O + O_2 \longrightarrow CO_2 + H_2O \qquad (2)$$
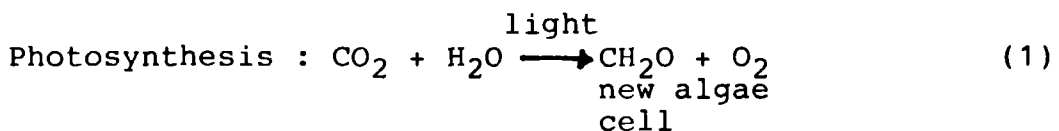
The part of total photosynthetic oxygen production of assimilation of algae is used for respiration of algae. The remaining part which is available for bacterial respiration and decomposition is about 6 to 10 g O_2/m^2 x d in tropical regions and 4 to 6 g O_2/m^2 x d in temperate zones (Mäkelä, 1979).

The growth of algae will also cause a rise in pond pH which will accelerate death of coliforms (Parhad and Rao, 1974)

and cause precipitation of polyvalent cations and anions such as calcium, magnesium and orthophosphate (Golueke and Oswald, 1965). Sobsey (1971, cited by Oswald, 1973) found that inactivation of poliovirus in the ponds can be effected by a combination of algae and bacteria.

Other beneficial use of algae include stripping of nutrients (Borchardt and Azad 1968, Gates and Borchardt, 1964) and for livestock feeding (INFU, 1980).

2.6 Stoichiometry of Algae and Bacterial Systems

A quantitative chemical relationship exists between the organic wastes treated and the micro-organisms produced. The decomposition of organic material by heterotrophic bacteria (decomposers) and the ingestion of organisms by animals (consumers) result in the return of soluble inorganic components to the water. The decomposition may be expressed by equation 3 (Gloyna, 1971):

$$C_aH_bN_cO_dP_e + (a+ \frac{b}{4} + \frac{d}{2} - \frac{3}{2}c + 2e)O_2 = aCO_2 + \frac{b}{2}H_2O + cNO_3^- + ePO_4^{3-} \qquad (3)$$
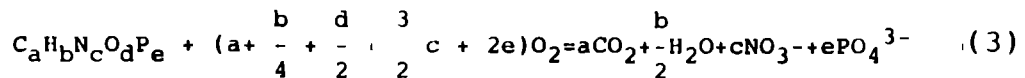
$C_aH_bN_cO_dP_e$ can be expressed by the empirical formula $C_{106}H_{180}O_{45}N_{16}P$ or $C_{106}H_{263}O_{110}N_{16}P$.

Conversely, the algae, acting as autotrophic organisms, produce organic material from inorganic substances as shown by equation 4 (Arceivala, 1973):

$$106\ CO_2 + 90\ H_2O + 16\ NO_3^- + PO_4^{3-} + light \longrightarrow C_{106}H_{180}O_{45}N_{16}P + 154.5\ O_2 \qquad (4)$$
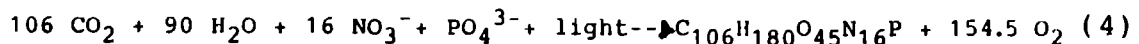
Equation 4 shows that phosphorous and nitrogen present in wastewater, as well as significant amounts of carbondioxide constitute suitable nutrients for the development of algal growth. Other trace elements such as copper, iron, molybdenum (Metcalf & Eddy, 1979), potassium, manganese, calcium and magnesium (Neos and Varma, 1966) are also very noteworthy.

2.7 Algae Species in Waste Stabilization Ponds

Algae are mainly divided into three major groups, based on the colour imparted to the cells by the chlorophyll and other pigments involved in photosynthesis. Green algae include unicellular, filamentous, and colonial forms. Brown algae are unicellular and flagellated, and include the diatoms. Certain brown algae are responsible for toxic red blooms. Red algae include a few unicellular forms, but are primarily filamentous. The most common algae in waste stabilization ponds are green and brown algae, with red algae occurring infrequently (US. EPA, 1983).

The most common algae generally found in stabilization ponds in tropical areas are Chlorella, Oscillatoria, Chlamydomonas and Euglena (Canter and Malina, 1976).

2.8 Changes in Algae Species

Blue-green algae are more efficient at obtaining low concentrations of CO_2 than green algae, and under alkaline conditions, blue-green algae are predominant (King, 1970). Shapiro (1973) in his experiment with algae from lake Emily, U.S.A., concluded that addition of CO_2 or lowering of pH stimulated a shift from blue-green algae to green algae.However, when pH was raised, blue-green algae would predominate once again probably because of their ability to absorb CO_2 more rapidly.

The comparative advantages of blue-green algae over green algae in CO_2 uptake kinetics under adverse conditions could stem from those blue green algae containing indistinctly layered gelatinous sheaths or which are of filamentous construction. These characteristics would give them a very high surface area to volume ratio, and allow for fast diffusion of free carbondioxide into the cells (Ip et al, 1982).

Apart from effect of carbondioxide concentration, the type of algae present in wastewater ponds is dependent on sunlight, temperature and nitrogen content (Parker, 1979).

2.9 Growth Pattern of Algae

The population of any environment is controlled by the physical, chemical and biological factors in the environment (Pipes, 1961 cited by Abid, 1983). The most important physical factors affecting growth of algae are light intensity and temperature.Inter-relationship among the different organisms is mentioned as the biological factor of greatest significance while nutrition and pH were emphasized as major chemical factors (Goldman, 1979).

The increase in algae population follows a characteristic pattern presented graphically in Figure 2 in which the following phases may usually be recognized (Fogg, 1965 cited by Abid, 1983):

1) a lag phase,in which no increase in population occurs

2) an exponential growth phase, in which cell multiplication is rapid and numbers increase in geometric progression

3) a phase of declining growth

4) a phase in which the population remains stationary, and

5) a death phase.
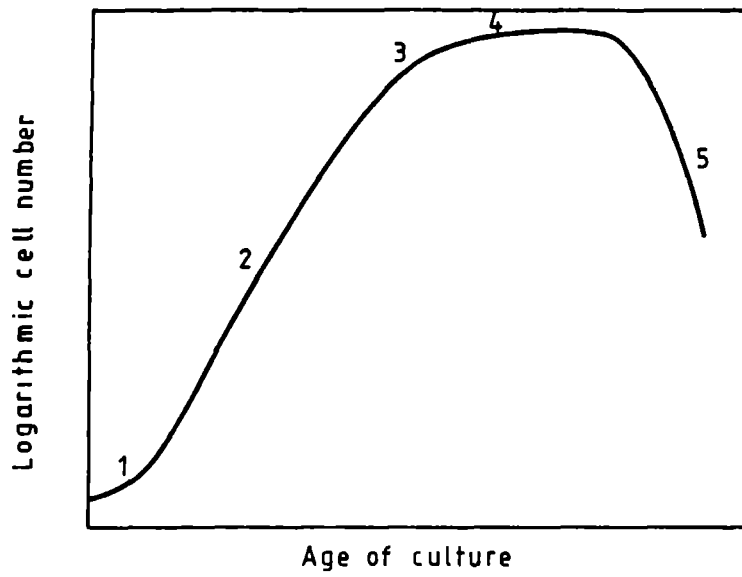
Figure 2. The characteristic pattern of growth shown by unicellular algae in a culture of limited volume (After Fogg, 1965 cited by Abid, 1983).

3 FACTORS AFFECTING GROWTH OF ALGAE

3.1 Influence of Biological Factors on Growth of Algae

3.1.1 Algae-Bacterial Symbiosis

Waste stabilization is a complex system comprising the existence of several living species, especially the inter-relationship of algae and bacteria which bring about an ecological pattern. Algae and bacteria are not parasitic to each other but may live with one another effectively.

The growth pattern of algae and bacteria will depend upon the nature or organic and other matter in the incoming wastewater and on environmental conditions (Arceivala, 1973). Figure 3 shows the algae - bacteria relationship found in a pond.
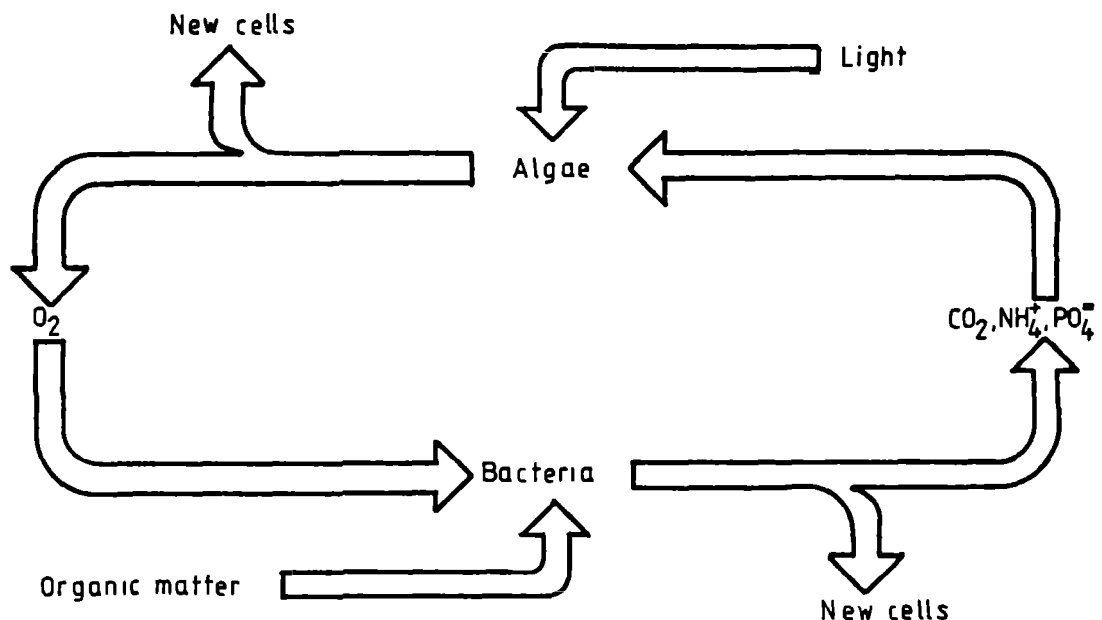
Figure 3. Symbiosis of algae and bacteria in stabilization ponds (INFU, 1980).

Humenik and Hanna (1971) found out that a very close association exists between the algal and bacterial cells in numerous and large floc particles. The majority of bacterial cells were attached to the larger algal cells.

Because the concentration of carbondioxide and oxygen are maximum at the bacterial and algal cell surfaces, respectively, favourable conditions for growth apparently occur when these cells are in close proximity (Humenik and Hanna, 1971).

Inorganic carbon present as a result of wastewater alkalinity was not sufficient to satisfy the algal carbon requirements, and thus the algae depended on carbondioxide from bacterial respiration.

Algae may also benefit from incomplete metabolism products or nutrients and growth factors released by the bacteria, and similarly the bacteria may utilize vitamins and growth factors that leach from algal cells (Humenik and Hanna, 1971).

3.1.2 Algae Repression by Bacteria

However, algal growth can be repressed by bacteria, depending upon species and environmental factors. For example, bacterial-mediated algal inhibition may be due to cell-wall lysis upon direct contact with several types of myxobacteria (Shilo, 1970; Gromov et al, 1972), toxic substances released by bacteria into the surrounding medium (Berland et al, 1972; Reim et al, 1974) and low molecular weight compounds liberated by bacteria suppress algal growth (Granhall & Berg, 1972; Shaaris & Morrison, 1976, all cited by Berger et al, 1979).

Berger et al (1979) found that Arthrobacter produces hydroxylamine-N which was the major factor for Chlorella inhibition.

3.2 Influence of Physical Factors on Growth of Algae

The principal climatic factors which affect pond performance are solar radiation, temperature, wind, evaporation and rainfall. Of the physical factors, mixing is a critical factor under all environmental conditions (Marais, 1970).

3.2.1 Effect of Light

The intensity and spectral compositions of light penetrating a pond surface significantly affects all resident microbial activity. The available light determines, to a large degree, the level of photosynthetic activity and hence, oxygen production (US. EPA, 1983).

3.2.1.1 Influence of Light Intensity

In general photosynthetic activity increases with increasing light intensity until the photosynthetic system becomes light saturated as shown by Figure 4.
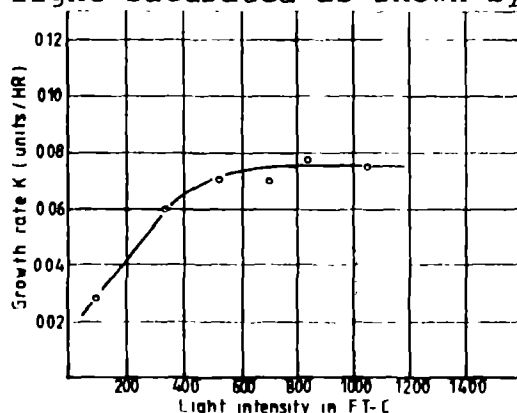
Figure 4. Growth rate of a mixture of Chlorella and Scenedesmus as a function of light intensity (Gates and Borchardt, 1964).

Shrivastava and Sharma (1984) found that the pond dissolved oxygen and production follow closely the light intensity. However, the magnitude of diurnal oxygenation is probably influenced more by variation in algal populations than by changes in light intensity. The algal-bacterial masses are usually not homogeneous, so the design calculations based on light penetration alone may be misleading (Gloyna, 1971).

Algae have a range of light intensity tolerance in which photosynthetic rate is independent of light intensity. Pipes (1961, cited by Abid, 1983) reported that photosynthetic rate is independent of light intensity between 19.25 and 1925 cal/cm^2 x d.

3.2.1.2 Influence of Pond Depth and Biomass Concentration

The quantity of light penetrating the pond surface to any depth depends on the presence of dissolved and particulate matter as well as water absorption characteristic. As the concentration of algae increases, light penetration decreases so that the phytoplankton becomes self-shading and thereby restricting photosynthesis in the lower layers (US. EPA, 1983; Moss, 1982).

Figure 5 shows the relationship of light transmission against depth at various biomass concentration.
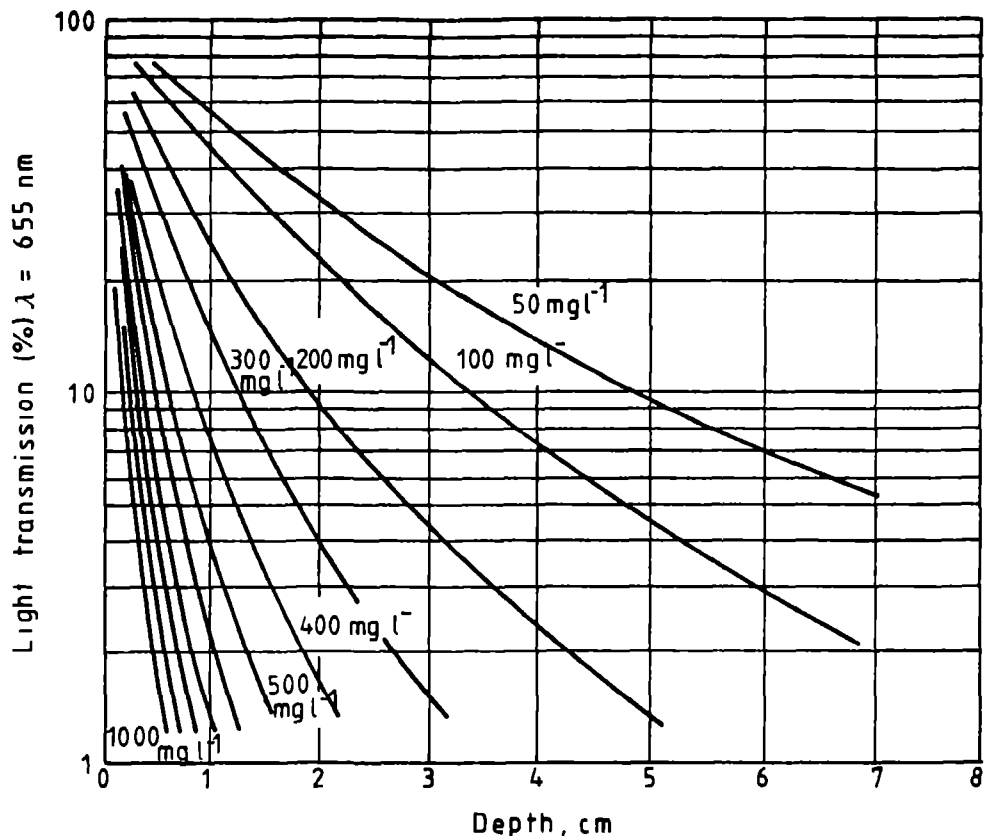
Figure 5. Light transmission vs vessel depth at various biomass concentration (Ip et al, 1982).

From Figure 5 the following conclusions can be drawn:

(i)   for a given depth, the percentage of light transmission decreases rapidly with increasing algal biomass concentration. Thus, it is necessary for sufficient agitation to occur to maintain a reasonably effective light intensity (Ip et al, 1982).

(ii)   for a given algal biomass concentration a higher incident light intensity results in a larger or deeper light zone with sufficient intensity for algal photosynthesis. This explains why algae growing in algal ponds flourish under strong solar radiation (sunlight can be up to 21,600 lx (2000 ft-candles) (Ip et al, 1982) even though surface saturation light intensity for algae growth is less than 5400 lx (500 ft-candles) (Goldman, 1979) and inhibition to photosynthesis occurs when the solar radiation intensity exceeds the saturation value by 9720 lx (900 ft-candles) (Ryther, 1956 cited by Ip et al, 1982).

3.2.1.3 Spectral Composition

The spectral composition of available light is also crucial in determining photosynthetic activity. The ability of photosynthetic organisms to utilize available light energy primarily depends upon their ability is determined by the specific photosynthetic pigment of the organism which are mainly chlorophylls and the phycobilins (US. EPA, 1983).

In general, the highest and lowest wavelengths (reds and blues) are absorbed most rapidly, most strongly by dissolved organic substances and particles and the water itself. The middle wavebands (yellows and greens) penetrate deepest until they themselves are absorbed (Moss, 1982).

Green light penetration which penetrates deeper is used by red algae for their metabolism. At the surface green algae are found where red light is available for their metabolism (Seppänen, 1986).

3.2.1.4 Photoperiod

Ip et al (1982) found that for a completely mixed algae-sewage system with a detention time of 7 days and a light intensity of 3240 lx (300 ft-candles), photoperiod appeared to be the primary factor limiting growth. When the photoperiod was increased from 6 to 12 h biomass concentration increased by an average of 180 %, that is, doubling photoperiod gave almost three time higher algal concentration (Ip et al, 1982).

3.2.1.5 Efficient Utilization of Light Energy

Another important factor is the efficient utilization of light energy by algae. Different species of algae have different capacity of utilization of light energy (Mäkelä, 1979 and Seppänen, 1986).

3.2.2 Effect of Temperature

The pond liquid temperature is probably the parameter which has the greatest bearing on pond performance and is usually 2 to $3^{\circ}C$ above the ambient temperature. The periods of cloud are seldom a problem in tropical and subtropical regions because solar insolation during the day exceeds saturation light intensity of algae in the pond (Arthur, 1983; Feachem et al, 1978).

3.2.2.1 Source of Heat

Solar radiation is a major source of heat generally resulting in a temperature gradient with respect to depth. The other major source of heat is the influent (US. EPA, 1983 and Klock, 1972). In sewerage systems having no major inflow or infiltration problems, the influent temperature is higher than that of pond contents. Evaporation, contact with cooler groundwater, and wind action are influencing cooling of pond contents (US. EPA, 1983).

3.2.2.2 Effect of Temperature on Biological Activities of Algae

Temperature affects photosynthetic oxygen production as well as other biological reactions. Optimum oxygen production for some species of algae is obtained at $20^{\circ}C$, and limiting lower and upper values appear to be about $4^{\circ}C$ and $37^{\circ}C$ respectively, although it is known that higher temperatures can be tolerated and some algae have been observed to grow quite well under a cover of clear ice (Gloyna, 1971).

Fitzgerald and Rohlich (1958, cited by Abid, 1983) stated the growth of Chlorella and perhaps of other algae, usually reached a maximum at temperature between $25^{\circ}C$ and $30^{\circ}C$. Green algae show most efficient growth and activity at temperature near $30^{\circ}C$ to $35^{\circ}C$ (US. EPA, 1983) while blue-green algae blooms near $23 - 26^{\circ}C$ and are seldom found at water temperature below $15^{\circ}C$ (Hammer, 1964, by Vincent & Silvester, 1979).

Shrivastava and Sharma (1984) found that maximum respiration occurs in the afternoon, reaching its lowest in the morning, corresponding to minimum pond temperature, an indication that respiration is a function of pond temperature.

3.2.2.3 Temperature and Carbon Source

Temperature affects algal production by means of both biological and physical-chemical effects. Microbial growth rate normally increases with temperature to a maximum in the early growth phase where carbondioxide and/or other carbon sources are not limited. Increasing concentration of algae leads to a higher demand for carbon nutrient sources.

Ip et al (1982) found that the effect of increased temperature was to decrease the algal biomass concentration by 25 - 30 % when the temperature was raised from 15 to

30°C. This can be explained by the fact that temperature has a marked effect on the concentration of various potential sources of carbon (ip et al, 1982).

Loewenthal and Marais (1976, cited by Ip et al, 1982) found that an increase in dissolved carbondioxide of approximately 67 % occurs for any given partial pressure when the temperature is reduced from 30 to 15°C. Thus, increased concentrations results at lower temperatures, particularly in the case of dissolved carbondioxide.

3.2.2.4 Interaction of Temperature and Light Intensity

Algal growth is also influenced by the interaction of temperature and light intensity.

Figure 6 shows the influence of temperature on culture growth rate at various light intensities.

The figure shows that saturation intensities increase with increasing temperature within the range of temperature studied (Gates and Borchardt, 1964).
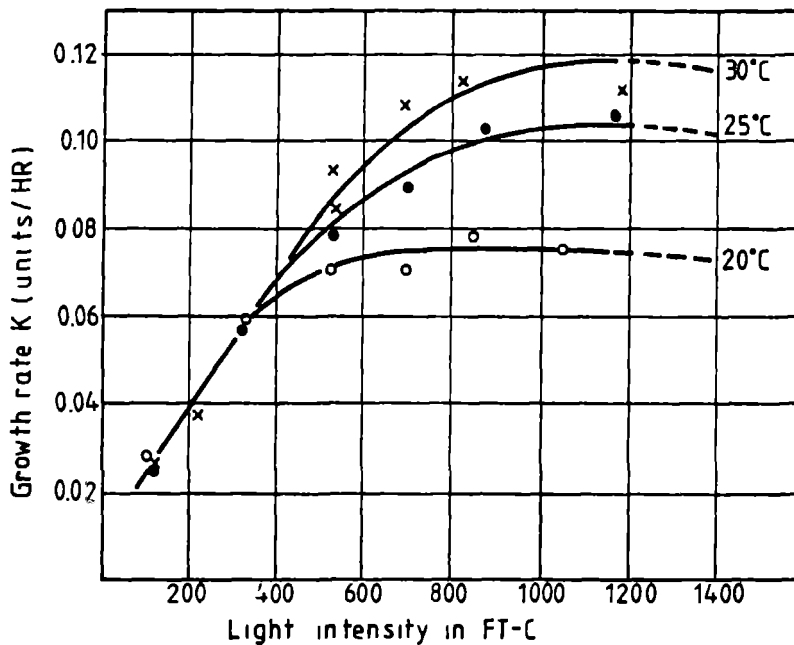
Figure 6. Growth rate of a mixture of Chlorella and Scenedesmus as a function of light intensity at various temperatures (Gates and Borchardt, 1964).

3.2.2.5 Interaction of Temperature and Phosphorous

The amount of phosphorous present in the wastewater at a particular temperature also influences growth of algae.

Figure 7 depicts the effect of temperature on the growth of algae (predominantly Scenedesmus) and the critical concentration of PO_4^{3-} required for growth. It is clear that the higher phosphate concentrations are required to grow

the same mass of protoplasm at lower temperatures and, conversely, much lower phosphate concentration are required at higher temperatures. Such a relationship may well explain the sudden blooming of algae a warm spring day (Borchardt and Azad, 1968).
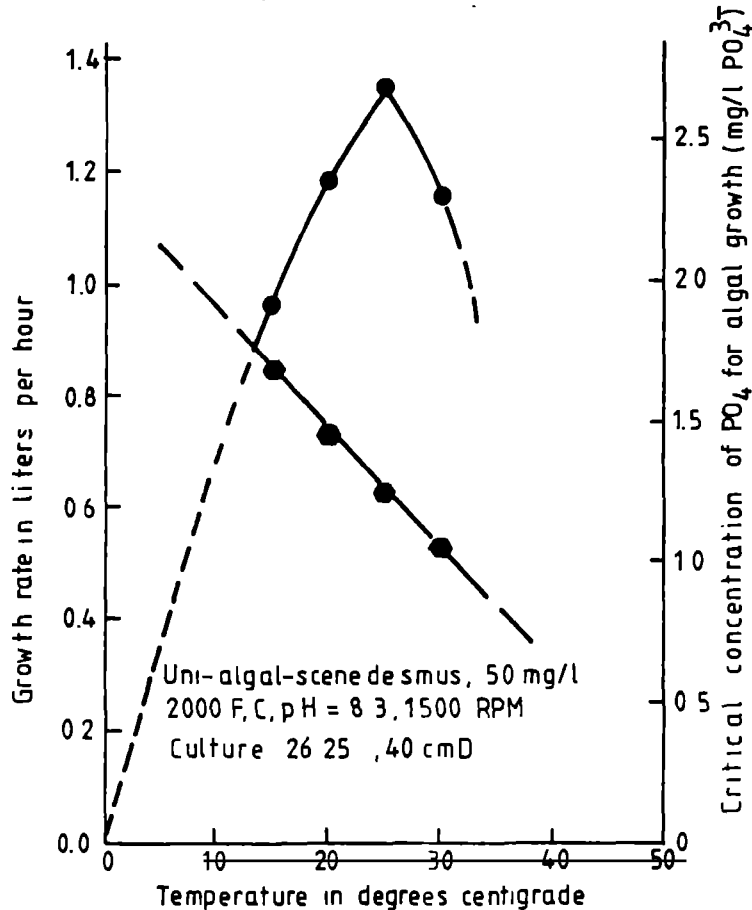
Figure 7. Effect of temperature on growth rate of algae (Ft.C x 10.75 = 1x, Borchardt and Azad 1968).

3.2.3 Effect of Mixing

In tropical zones, the upper water layer will heat up rapidly. Without the circulation of the water caused by the activity of the wind, a discontinuity in temperature at a depth of about 50 cm occurs. Some of the algae sink down from the warm water region (more than $35^{\circ}C$), forming a light barrier (INFU, 1980).

Mixing is required in order to enhance the "flashing light effect", a biochemical phenomenon by which photosynthesis rate of an algal cell are increased when the cell is transferred back and forth from light to dark in a time sequence corresponding to the light and dark biochemical reactions (Rabinovitch & Govindjee, 1968, cited by Goldman, 1979).

However, mixing is required for several other reasons to prevent thermal stratification, to provide uniform cell exposure to light, and to breakdown diffusion gradients of essential nutrients which could develop at the cell surface in intense mass cultures. Mixing can also, to some degree, enhance CO_2 transport from the atmosphere; but, because of

the very low concentration of CO_2 in the atmosphere (0.03 %), the transport gradient is always small and CO_2 transport is ineffective unless very turbulent mixing is employed (Goldman, 1979).

If stratification persists, non-motile algae below the thermocline cannot enter the photic zone (top 15 to 30 cm of pond) and die due to lack of light (Marais, 1970). Thermal stratification can also cause short-circuiting, resulting in reduced effluent quality (Barson, 1973, cited by Canter & Malina, 1976). This phenomenon will immediately cut down the rate of photosynthesis and therefore also the production of oxygen and finally restrict the stabilization of wastewater by bacteria.

3.3 Influence of Chemical Factors on Algal Growth

3.3.1 Effects of Nutrients on Algal Growth

Growth of micro-organisms is controlled by the availability of essential nutrients such as carbon, nitrogen, and phosphorous and a variety of other substances required in small quantities . These nutrients may be classified as inorganic or organic. Nitrogen and phosphorus represent the inorganic nutrients, while organic carbon compounds represent the organic nutrients (US. EPA, 1983).

The nutrient constituents of the aquatic environment is responsible for algal growth. The main category of interest are macronutrients, nitrogen and phosphorous. Through the activities of algae, the nutrients are removed from the solution and concentrated in algal cells. When the nutrient levels have been reduced to a point where scarcity of one element becomes limiting, the rate of growth will decrease appreciably.

Figure 8 shows the relationship between limiting nutrient concentration and algal biomass.
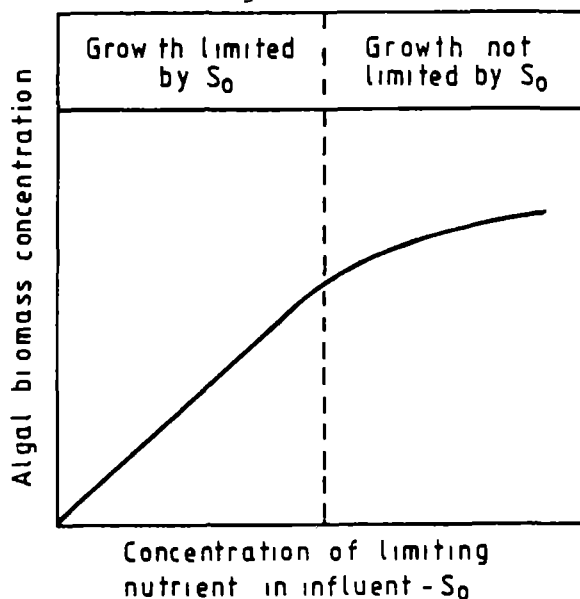
Figure 8. Relationship between limiting nutrient concentration and algal biomass under limiting and non-limiting conditions (Goldman, 1976).

All algal forms cannot use every nitrogen and phosphorous compound found in wastewaters, and may lead to certain imbalances. For example, some organic nitrogen compounds and some polyphosphates, are not suitable nutrients for algae unless they are first acted on by bacteria (Gloyna, 1971).

3.3.1.1 Nitrogen Forms and Requirement

Typical municipal wastewaters contain from 15 to more than 50 mg N/l. About 60 % is is ammonia form and the remaining is essentially in the organic form. The partition of ammonia nitrogen as ammonia gas (NH_3) or ammonium ions (NH_4+) strongly depends on pH and temperature. At pH 7, only ammonium ions are present and at pH 12, only dissolved NH_3 gas is present. Most of the organic nitrogen is initially associated with the particulate matter in untreated wastewater (Reed, 1985).

Nitrogen may become a limiting nutrient for primary productivity involving algae in waste stabilization ponds (Ferrara and Avci, 1982; US. EPA, 1983). In waste stabilization ponds, biological oxidation of organic material releases carbondioxide. This can increase the algal growth rate to the point where nitrogen becomes limiting (Goldman et al, 1974).

Ferrara and Harleman (1980) examined data from Corinne, Utagh, and Eudora ponds in United States and concluded that nitrogen was deficient nutrient when customary ratios for cell composition are used.

Antia et al (1963, cited by Goldman, 1976) found that the N:P ratios of phytoplankton populations to be in the range 12 - 22 due to wide variability in the phosphorus content of algal cells (Goldman, 1976).

Goldman (1976) found that the N:P ratios in wastewaters suggest that nitrogen is a limiting nutrient when customary ratios for cell composition were used.

3.3.1.2 Phosphorous Forms and Requirement

Phosphorous may occur in wastewaters in the form of organic phophate found in organic matter and cell protoplasm, as complex inorganic phosphate such as sodium tripolyphosphate and hexametaphosphate used in agriculture, water treatment processes and in cleaning compounds; and as inorganic orthophosphate (PO_4^{3-}), the final breakdown product in the phosphorous cycle and the form in which phosphorous is most readily available for biological utilization (Nesbitt, 1969).

The concentration of phosphorous in domestic wastewater may differ from place to place depending on the quantity of water consumed by the population and may range from 9 to 18 mg/l total phosphorous (Srinath and Pillai, 1972).

Municipal wastewater is normally quite rich in phosphorous (US. EPA, 1983). The weight of phosphorous incorporated in growing algal systems is only a small fraction of the weight of carbon and nitrogen in the algae which suggests that phosphorous is not a limiting factor (Abid, 1983).

Srinath and Pillai (1972) found that there is a close correlation between the phosphorous content of water and the amount of growth of algae. The amount of phosphorous intake by algae was far below the concentration in wastewaters indicating phosphorous in non-limiting factor.

Moreover, algae have been shown to be able to store phosphate internally in the condensed form (probably meta- or polyphosphate) and use it for continuing growth in the absence of external supplies (Borchardt and Azad, 1968). For example, Microcystis aeruginosa, a blue-green algae species, has been maintained in the laboratory for several weeks in phosphorous free medium simply by first allowing cells to absorb large quantities of phosphate before inoculating then into phosphorous free medium (Shapiro, 1970). This argument further suggests that even during periods when inadequate phosphorous is supplied to wastewater lagoons, phosphorous is unlikely to be a limiting factor.

3.3.1.3 Carbon Sources and Requirement

One of the major nutrients required by algae is carbondioxide. Where organic matter id decomposing, carbondioxide is used by algae which in turn produces oxygen needed by bacteria. Most of the species of algae use only free carbondioxide in photosynthesis (Neilsen, 1955 cited by Gloyna, 1971), but there is some indication that a few algae use bicarbonate ion (Osterlind, 1948 cited by Gloyna, 1971).

Massive algal blooms always are associated with excessive amounts of decomposable organic matter. The large amounts of carbondioxide required for fast growing massive algal blooms of blue-green algae cannot come from atmosphere and/or dissolved carbonate salts via the normal physical-chemical Processes. At most, about 1 mg/l of free carbondioxide accumulated over a period of many hours to days can be expected (Kuentzel, 1969).

Natural establishment of equilibria to restore free carbondioxide that is removed by algal growth takes time since the movement of carbondioxide in and out of a water interface is a slow process. On the other hand, the replacement of free carbondioxide by carbonate salts in slightly alkaline media is a relatively slow process. Thus, the available free carbondioxide from natural inorganic sources (at pH of 7.5 to 9) probably never exceeds 1 mg/l and it becomes available at a rather slow rate (Kuentzel, 1969).

Maximum blooms as high as 211,550 cells/ml, 56 mg/l dry weight, most of which grew in one day has be reported by Mackenthun et al (1968). This indicated that some 110 mg/l

of carbondioxide must have been delivered to algae during their growth period and which cannot be supplied by inorganic sources. The supply can only be attributed to high logarithmic growth rates of bacteria under favourable conditions which can deliver high amounts of carbondioxide required for algal bloom development (Kuentzel, 1969).

Young and King (1980) used A.nidulans and found that there exists a positive interaction between light, phosphorus and carbondioxide as regulators of algal carbon fixation.

3.3.2 Effect of pH on Growth of Algae

pH of pond depends upon carbon dioxide concentration in the medium which in turn varies inversely with the rate of production.

All organisms have a pH tolerance range in which the actual pH value has little effect on their metabolism. Most of organisms have a pH tolerance between 6.0 and 9.0 and optimum range between 7.0 and 8.0 (Abid, 1983). The growth of Chlorella, for instance, if optimum at pH range of 6.0 to 6.5 (Krauss, 1964 cited by Abid, 1983) whereas the growth rate of Scenedesmus ceases at pH range below 5.5 and above 10 (Gates and Borchardt, 1964).

If concentration of carbondioxide is limiting, the utilization of bicarbonate for photosynthesis might result in the pH of a media rising to as high as 11 or more, which might reduce the growth in cultures of limited volume (Fogg, 1965 cited by Abid 1983).

4 ALGAL INFLUENCE ON BACTERIAL DIE-OFF

4.1 Bacterial Reduction Levels

Many researchers have reported reductions in the coliform numbers through wastewater stabilization ponds. Joshi et al (1973) reported that a total of 99.99 % reduction was obtained in indicator organisms (Coliforms, S. faecalis and Escherichia-Coli), in a 3 cell pond system of one acre (about o.4 hectare) each in India. Low coliform indices ranging from 80 to 99 % have been repeatedly reported (Parker, 1962). Sidio et al (1961) reported up to 99 % removal of coliforms with complete removal of Salmonella.

Marais (1974) reported 99.91 % faecal bacteria reduction when four ponds in series each with detention time of 2.5 days were used and 95 % reduction for a single pond of 10 days retention time in South Africa.

However, although reduction percentages are very impressive bacteria may not be eliminated completely (Geldreich, 1966 cited by Davis and Gloyna, 1972; Parhad and Rao, 1974). Geldreich (1966) pointed out that 1 to 10 % of coliforms remaining in the effluent may constitute 4×10^6 to 10×10^6 coliforms per 100 ml.

4.2 Direct Influence of Algae on Bacterial Die-off

Several hypotheses have been put forward as to the cause of great reductions of enteric and pathogenic organisms in wastewater stabilization ponds. The potential causes include:

(a) Production of toxic unicellular products by algae attributing the high rate of bacterial die-off in waste stabilization ponds (Caldwell et al, 1946 cited by Parhad and Rao, 1974; Merz et al, 1962). Chlorella has been found to liberate extra-cellular fatty acids such as chlorellin (Merz et al, 1962) that seem to have a marked antibacterial activity according to Pratt et al (1944) and Speehr et al (1949) (all cited by Amin and Ganapati, 1972).

Extracellular products are defined as soluble substances liberated from healthy cells, as distinct from substances set free by injured cells or by autolysis or decomposition of dead algae (Fogg, 1962 cited by Abid, 1983).

However, Parker (1962) found no evidence to support the view that the release of bactericidal substances from algae was responsible for reduction of coliform counts. Vela and Guerra (1965) reported rapid die-off patterns of Shigella, Proteus and Streptococci when exposed to Chlorella but reported Salmonella typhi and Salmonella paratyphi grew well in the presence of Chlorella. Parhad and Rao (1974) found that Escherichia-Coli and Chlorella can grow together provided pH level is near neutral.

(b) Oswald (1960, cited by Abid, 1983) found that pH
     changes in algal-bacterial cultures was directly
     proportional to algal concentration. Mohanrao (1973)
     found that increase in pH due to photosynthetic
     activity of algae was the main cause of reduction of
     indicator organisms such as coliforms, Escherichia-
     Coli and S.faecalis in waste stabilization ponds in
     India.

     Parhad and Rao (1974) studied the growth pattern of
     Esherichia-Coli and algae in wastewater ponds and
     found that the increase in pH accompanies reduction of
     Escherichia-Coli. Coliform concentration in a
     stabilization pond may be reduced during the periods
     of high pH levels, but the bacteria are never
     eliminated completely. The pH of the wastewater
     decreases during the evening and night and there is
     continual influx of coliforms into a stabilization
     pond, thus minimizing effects of increased pH (Parhad
     and Rao, 1974).

(c) Some quantities of organic carbon must be available in
     the ponds for bacteria to survive. Coliform
     reduction was found to be associated closely with BOD
     removal, indicating that coliforms are removed because
     of their inability to complete successfully for
     nutrients (Gann et al, 1968; Skerry and Parker, 1979)
     and due to microbial antagonism (Polprasert et al,
     1983).

     However, McGrew and Mallette (1962) found that
     Escherichia-Coli and some bacteria of intestinal
     origin could survive at concentration levels of
     glucose less than 5 mg/l.

(d) High oxidation-reduction potentials established in
     algal cultures also contribute to bacterial die-off
     (Maksimova and Fednko, 1965, cited by Abid, 1983).

(e) Ohgaki et al (1986) found out that coliphages are
     adsorbed in oxidation ponds under aerobic conditions.
     The photosynthesis by algae under solar radiation
     increases dissolved oxygen concentration and
     coliphages are adsorbed in part to microbial
     particulate under aerobic conditions. During the
     night, under conditions without sunlight, the
     dissolved oxygen is diminished to zero and coliphages
     are disrobed from particulates under anaerobic
     conditions.

4.3 Other Factors Influencing Pathogens Die-off

Apart from direct influence of algae on indicator and
pathogenic organisms die-off, many other ecological
parameters should be taken into account. The comprehensive
model should include parameters such as temperature,
sedimentation, water quality, sunlight intensity, hydraulic
detention time, substrate degradation rate, sunlight
duration, aerobic and anaerobic nature of pond, depth and

pond dispersion number (Amin and Ganapati, 1972; Bowles et al, 1979; Funderburg et al, 1978, Polprasert et al, 1983; Sarikaya and Saatci, 1987; Skerry and Parker, 1979; Tarig and Aziz, 1975; Niemi, 1976; Wright et al, 1979; Moeller and Calkins, 1980). Other factors which influence the die-off include dilution and mixing, aggregation and presence of toxic substances (Gloyna, 1971).

4.3.1 Effect of Temperature, Aerobic and Anaerobic Nature of Pond and Detention Time

Marais (1974) presented a consolidation theory for a kinetic model for reduction of faecal bacteria in stabilization ponds incorporating the effect of temperature on the death rate. The die-off rate constant k is very sensitive to temperature and is approximately $k = 2.6 (1.19^{T-20})$ ($T^{\circ}C$). It is presumed in this relationship that the ponds are mixed and aerobic or facultative and valid between 5 to $21^{\circ}C$. Above $21^{\circ}C$, with low wind velocities, periods of stratification occur causing the lower liquid depth of the pond to be anaerobic. There is decline in k value under anaerobic conditions resulting into high rate of faecal organisms survival (Marais, 1974).

Figure 9 shows that decrease in pond temperature causes a significant increase in faecal coliform survival in Blayney polishing ponds (mean depth = 1.16 m) in Australia.
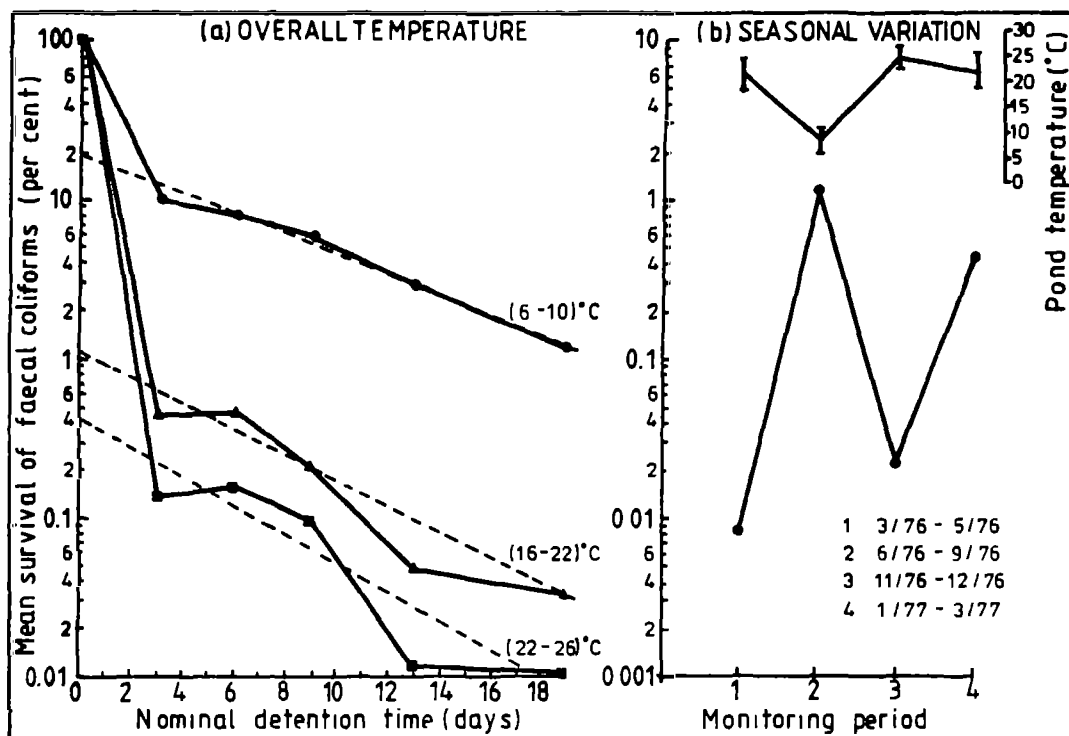
Figure 9. Influence of temperature on faecal coliform survival - Blayney (Wright et al, 1979).

Stratification, as assessed from influent and effluent temperature difference, was associated with increased faecal coliform survival (Wright et al, 1979).

4.3.2 Effect of Sunlight

Solar radiation has some inactivating effects on pathogens (Ohgaki et al, 1986; Bitton et al, 1979). Ohgaki et al (1986) did field experiments with submerged bottles in the oxidation pond and sunlight exposure experiment showed that coliphage could be inactivated by sunlight only near the water surface (less than 10 cm depth) in the oxidation pond.

Inactivation effect at greater depth is lower because of diminishing radiation intensity, especially ultra-violet rays, through water (Ohgaki et al, 1986).

Sarikaya and Saatci (1987) analysed the effect of solar radiation on the die-off of coliforms and they found out that a linear relationship exists between the die-off rate constant and the light intensity.

Direct inactivation of pathogenic organisms with sunlight is limited to the pond surface. The removal is mainly attributed to photosynthesis by algae which occurs in presence of sunlight. Ohgaki et al (1986) found that the degree of removal of coliphage in oxidation pond in test site (Bangkok, Thailand) with design retention time of 20 days was 90 %. This reduction was partly due to sunlight inactivation but largely due to photosynthesis of algae under sunlight.

4.3.3 Effect of Pond Depth

The die-off of bacteria in shallow ponds is faster than the die-off in deeper ponds. Sarikaya and Saatci (1987) used the coliform removal data reported by Polprasert et al (1983) for 0.86 m and 0.60 m deep pilot waste stabilization ponds to analyze the effect of pond depth. They concluded that bacterial die-off rate was faster in shallow than in deep ponds.

Further evidence of pond depth effect was obtained by Mara and Silva (1979) for a 1.0 m and 1.25 m deep maturation ponds. Their results shows that bacterial die-off rate is higher in shallow ponds.

5 METHODOLOGY

5.1 Pilot Plant Description

Pilot scale ponds were constructed at the University of Dar es Salaam main campus which lies approximately latitude 6 48'S and longitude 39 13'E. The ponds were located about 80 m above sea level. According to the data from the University of Dar es Salaam weather station, the mean monthly air temperature in Dar es Salaam varies between 23 C to 28 C with the mean value near 26 C. The temperature is very conductive for wastewater treatment which together with other advantages make the waste stabilization ponds an attractive choice.

The pilot plant consists of six trapezoidal shaped cells with the following features (Figure 10):

	Set A	Set B and C
Top width (m)	3.2	3.2
Top length (m)	3.2	3.2
Water depth (m)	1.5	1.0
Side slopes (V:H)	1:0.8	1:1.2
Freeboard (m)	0.15	0.15
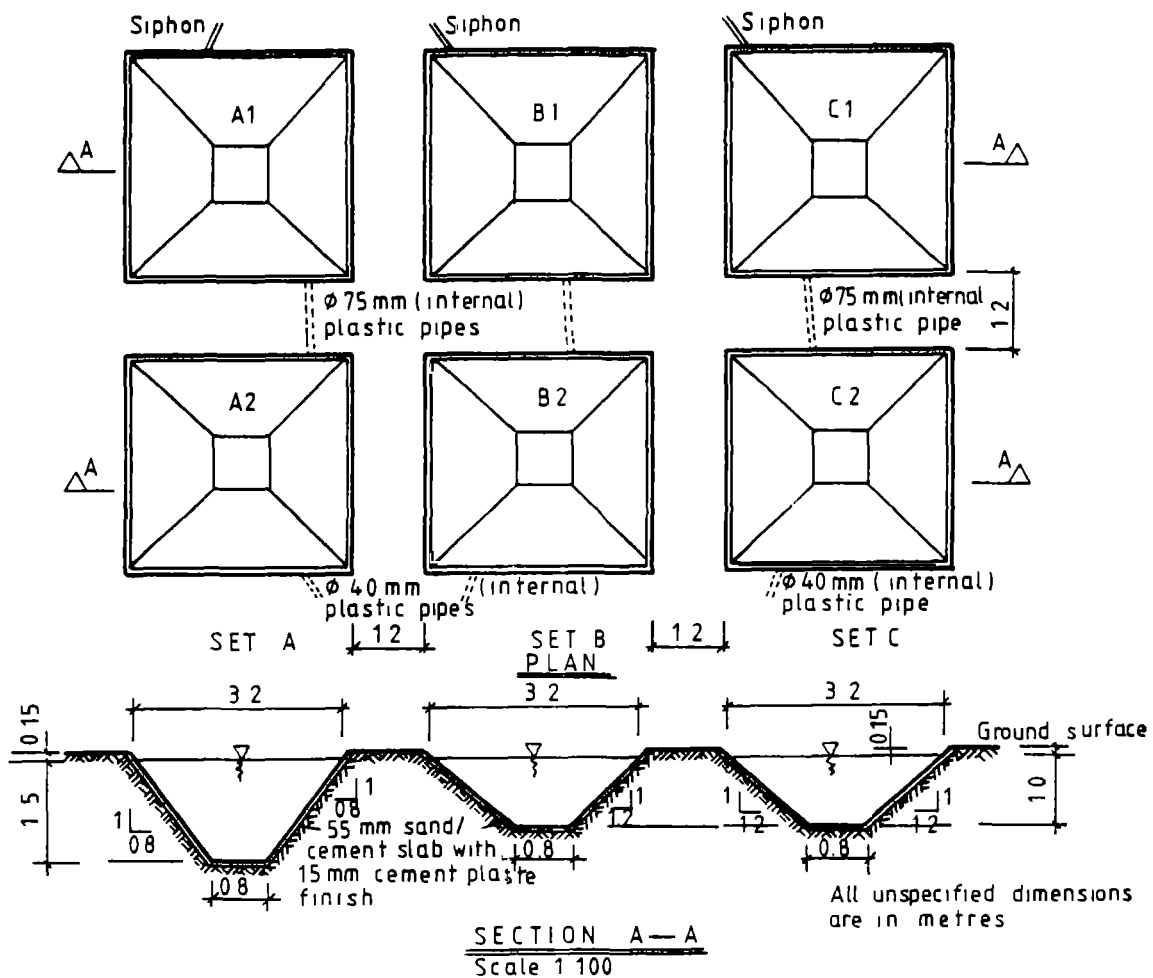
Figure 10. Pilot wastewater ponds constructed at the University of Dar es Salaam.

All cells were lined with 55 mm cement sand slabs. The slabs were plastered and fine cement paste finish applied afterwards to reduce chances of water leakage. Each set has two cells with similar dimensions interconnected by a submerged pipe of 75 mm internal diameter. Final effluent was discharged through 40 mm overflow pipes.

All three sets were operated in parallel during the entire study period. Daily additions of wastewater to each set were effected by means of a constant flow siphons discharging the contents at an average rate of 13.3 l/min from the nearest primary ponds receiving domestic wastewater. Influent flow rate was measured by a graduated cylinder and a stop-watch.

The hydraulic elements and wastewater flows are presented in Table 1.

Table 1. Hydraulic elements and wastewater flows.

Item	Set A (each cell)	Set B (each cell)	Set C (each cell)
Hydraulic elements:			
Basin volume (l)	6000	4000	4000
Surface area (m^2)	10.2	10.2	10.2
Water depth (m)	1.5	1.0	1.0
Wastewater:			
Average flow (l/d)	600	400	800
Detention time in each cell (d)	10	10	5
Number of cells in series	2	2	2

The theoretical hydraulic detention time was obtained by the expression:

$$\text{Detention time} = \frac{\text{Basin volume}}{\text{Rate of flow}}$$

All the the ponds were inoculated with algae from the nearest field scale maturation ponds and allowed to attain steady-state conditions for about fourty (40) days.

5.2 Sampling

Grab wastewater samples for bacteriological analysis were collected from the effluent of each cell of each series and siphon contents from primary pond in sterile widemouth bottles of about 300 ml at irregular time intervals. Grab samples for pH, dissolved oxygen, temperature and Biochemical Oxygen Demand (BOD) analyses were collected in clean plastic containers which were stoppered after collection of samples.

No effort was done in this work to use composite samples due to labour constraint. However, Moeller and Calkins (1980) found that the use of composite samples did not significantly alter the coliform density. Pearson et al (1987) suggested the use of grab samples for pH and faecal coliforms though other tests such as BOD should be based on 24 h composite samples. The sampling method used is therefore expected to produce reliable results.

Sample collection dates were randomly selected but all the collections were made between 8.30 am and 10.00 am.

5.3 Sample Examination

All laboratory examinations were carried out in accordance with standard methods as laid in the Standard Methods for Examination of Water (APHA, AWWA, WPCF; 1985). Dissolved oxygen, pH and temperature measurements were carried out in the field while faecal coliforms were selected as tests organisms in this study.

Samples were processed immediately after collection. Samples for faecal coliform count were preserved in the refrigerator for a period not exceeding 6 hours, while awaiting analysis.

The number of faecal coliforms in the samples were established using standard membrane filter procedure using two replicates in appropriate dilutions prepared in buffer dilution water. Dilution water was prepared from stock phosphate buffer solution, presterilized in the autoclave and allowed to cool at the room temperature. All the class ware used for faecal coliform analysis were sterilized in air oven at 170° C for two hours.

Appropriate dilutions were filtered through 0.45 μm sterile filter papers and inoculated into media prepared from M-FC broth dehydrate. Enumeration of faecal coliforms was done 24 hours after inocubation at 44° C.

Dissolved oxygen measurements were conducted with a dissolved oxygen meter (Delta Scientific model HI8424) was used to measure pH and temperature of the samples.

Both filtered and non-filtered BOD measurements were carried out. BOD samples were incubated at 20° C for five days after being prepared in appropriate dilutions.

Direct solar radiation was measured by Linke - Feussner pyrheliometer manufactured by Kipp and Zonen. Signals from pyrheliometer were fed into Kipp and Zonen electronic integrator provided with printing facilities and a timer and operated from a 240 volts, 50 Hz power source. Results were printed at 1 hour intervals. The sensor calibration factor used was 5 μV per watt/ m^2. Since the calibration factor for the pyrheliometer was 3.9 mV per cal/cm^2/min, all the printed results in WH/m^2 were divided by 13.0 to convert the energy to cal/cm^2/d.

5.4 Sunlight Exposure Experiment

The test comprises of two open mouthed aluminium cylindrical containers each of about 20 cm diameter. All containers were filled with 3000 ml of wastewater from the primary pond effluent. One container was left open and exposed to direct sunlight while the other was covered with thick paper box to cut out solar radiation.

pH values were monitored at intervals of one hour while four faecal coliform analyses were performed at 3 hours intervals.Data for solar intensity was automatically printed at one hour interval.

5.5 Submerged Bottles Experiments in Pond

About 600 ml wastewater samples from the effluent of pond cell A1 were incubated in situ above the water surface and at the depths of 15 cm and 100 cm below the water level in clear and opaque bottles. Samples for faecal coliform analysis were collected at irregular time intervals (but at least once in 2 days). Samples were collected at 10.00 am. on the sampling day.

6 RESULTS AND DISCUSSION

6.1 Sunlight Exposure Experiment

Figure 11 shows the faecal coliform survival against received solar intensity at the surface of the container and Table 2 shows the results of the sunlight exposure experiment.
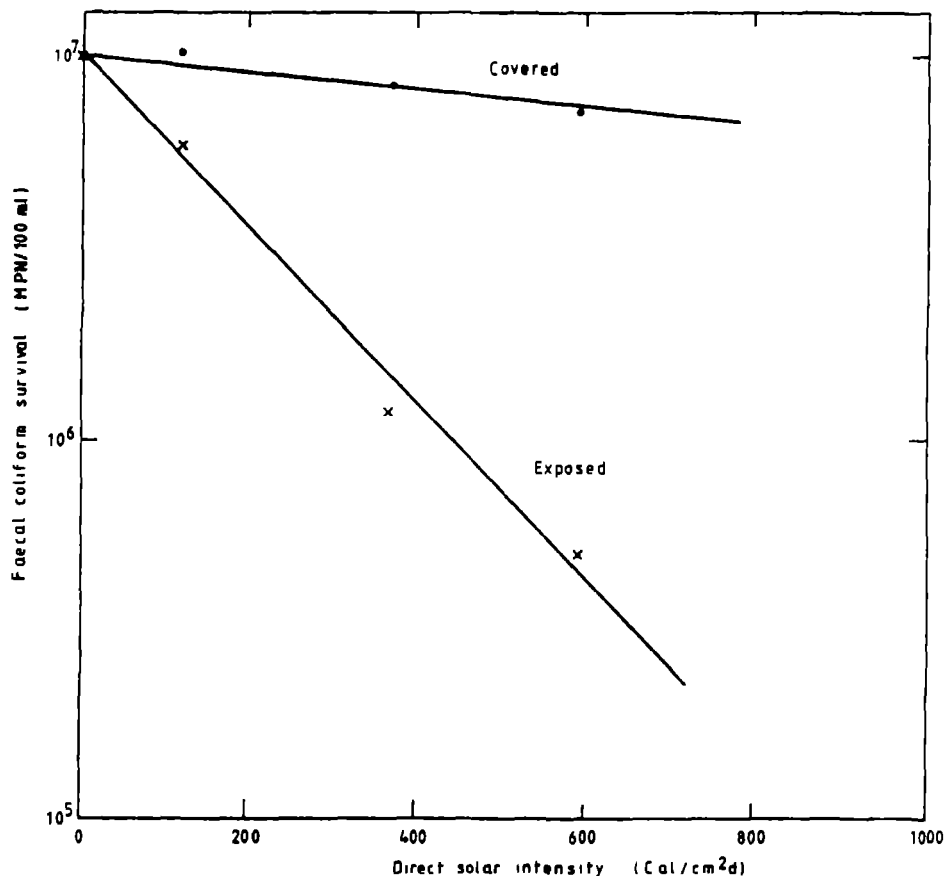
Figure 12. Faecal coliform survival as a function of received direct solar radiation at the surface of the containers.

Table 2. Results of Sunlight Exposure Experiment (30/12/1987).

Time	pH		Faecal coliforms MPN/100 ml (x 10^6)		Solar energy 3 h-total direct solar intensity (cal/cm^2)
(hr)	Exp-osed	Cove-red	Exposed	Covered	
7.00	8.0	8.0	10	10	0
8.00	8.2	8.1			
9.00	8.5	8.2			
10.00	8.6	8.2	5.8	10.2	123
11.00	8.9	8.2			
12.00	9.3	8.3			
13.00	9.5	8.3	1.2	8.4	369
14.00	9.6	8.2			
15.00	9.8	8.2			
16.00	9.7	8.3	0.5	7.2	588

Sunlight radiation does not directly affect the inactivation rate in dark conditions. However, inactivation rate as a function of sunlight intensity was used to facilitate quantitative comparison between covered and uncovered conditions.

Sunlight dose required to inactivate 90 % of the original faecal coliform number was used as a quantitative indicator of inactivation. The survival of faecal coliforms is expressed by: (Kondo, 1972, cited by Ohgaki et al, 1986).

$$S = 10^{-(R/Rgo)}$$

where: S= Survival of faecal coliforms (-)
R= Sunlight radiation received (cal/cm^2)
Rgo= Sunlight dose required to inactivate 90 % of the original value (cal/cm^2).

Rgo was found to be about 425 cal/cm^2 for the wastewater exposed to direct sunlight while for the covered case Rgo was 4680 cal/cm^2. The results show that the faecal coliform die-off is higher on exposure to direct solar radiation.

6.2 Submerged Bottle Experiments

The results of the findings are summarized in Table 3.

Table 3. Summary of Results of Submerged Bottle Experiment.

			Faecal coliform count (MPN x 10^6/100 ml)					
Date	Daily direct solar intensity (cal/cm^2)	Cumu-lative direct solar intensi ty(cal/cm^2)	Surface clear glass	Surface opaque glass	15 cm deep clear glass	15 cm deep opaque glass	1.0 m deep clear glass	1.0 m deep opaque glass
7/1/88	0	0	472	472	472	472	472	472
8/1/88	599	599	11.9	203	190			
9/1/88	245	844	9.8		66	200	160	
10/1/88	466	1310						
11/1/88	654	1964	0.246	28.7	22	78	82	110
12/1/88	244	2208			15		61	
13/1/88	297	2505	0.011	18				39
14/1/88	201	2706	0.007		7.8	35	49	

Figure 12 shows the variation of faecal coliforms with solar intensity at different incubation conditions.

Figure 12. Change of faecal coliform count with received direct solar radiation for samples clear and opaque glass bottle incubated at various depths in the pond.

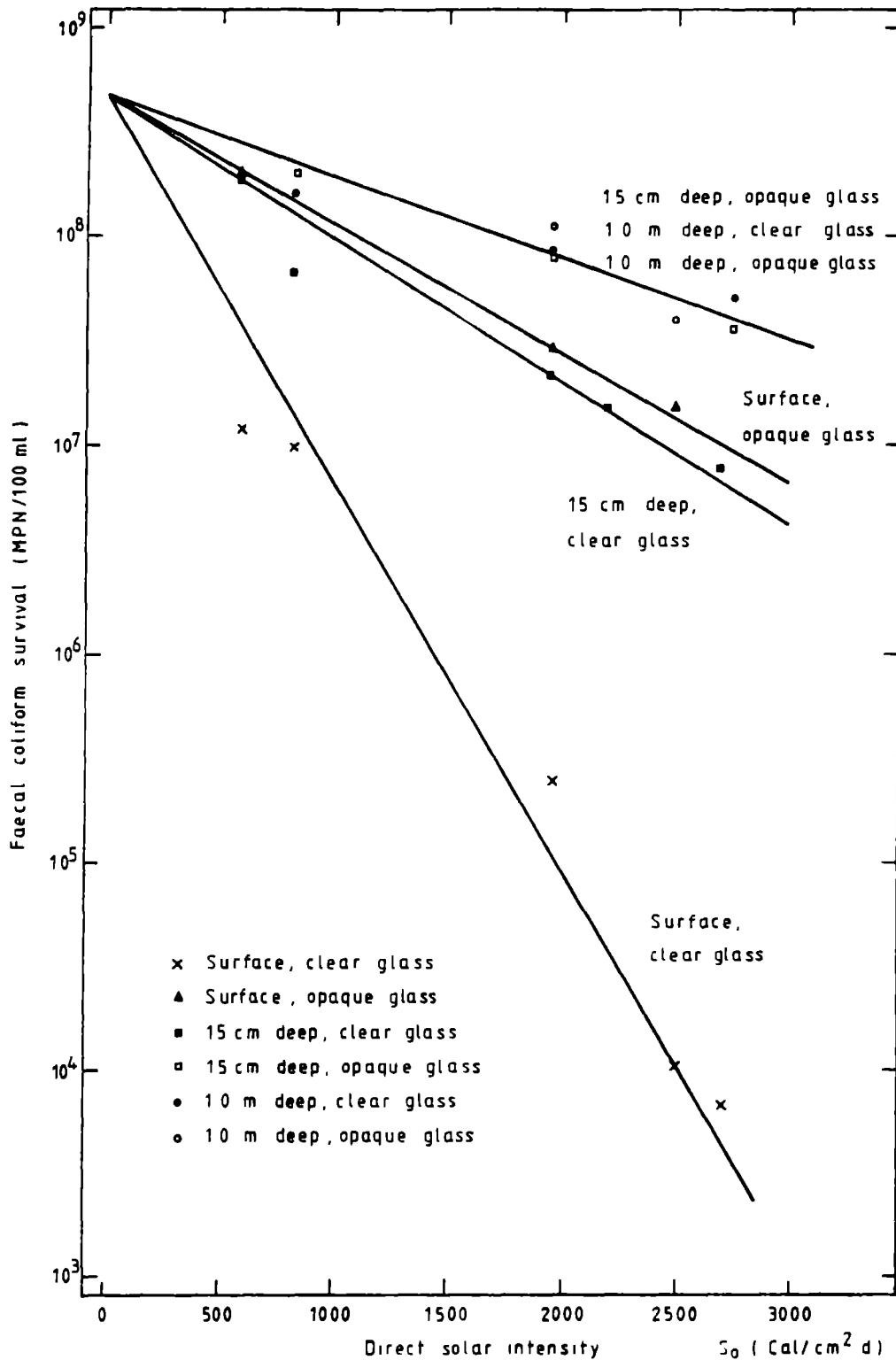The mortality of faecal coliforms in clear glass bottles was higher at the surface and decreased with increasing depth. Faecal coliform die-off was highest on the sunny days. Day 1 with about 600 cal/cm² solar intensity caused

the death rate of 97% and 64% for clear bottle samples at the surface and 15 cm depth, respectively. Faecal coliforms decreased by an average of only 48 percent per day for clear bottle at the surface and 32 percent per day for clear bottle 15 cm deep on day 5 with daily solar intensity of 244 cal/cm^2.

Mortality rate in opaque bottles was slightly higher for samples incubated at the surface, but samples below water (0.15 m and 1.00 m depth) had similar survival rates. Samples in opaque bottles at the surface had slightly higher die-off rates probably due to some light penetration through the brownish glass bottles (sample contents were visible). Algae disappearance was also observed for samples incubated at the surface within one day probably due to excessive heating (or "cooking") by direct solar radiation.

Time for 90 % faecal coliform die-off, T90, for samples exposed to sunlight at the water surface was about 21 hours. T90 values for 0.15 m and 1.0 m deep samples was 90 hours and 150 hours, respectively.

Figure 13 depicts the relationship between faecal coliform die-off rate and the depth of sample below the water surface. Sample data used were those from clear glass samples incubated at the surface, 15 cm and 1.0 m below water level. The die-off rate constant as calculated from Chick s law which is expressed as: (Gloyna, 1971; Sarikaya et al, 1987).

$$\frac{Ne}{Ni} = 10^{-k_1 t} = e^{-kt} \qquad (5)$$

where: Ni = Faecal coliforms in the influent (MPN/100 ml)
       Ne = Faecal coliforms in the effluent (MPN/100 ml)
       k, k_1 = Mortality rate constant (d^{-1})
       t = Hydraulic detention time (d)

Note: $k_1 = k/2.3$

Although only limited points were available for plotting the graph, the shape was predicted from the fact that solar intensity diminishes with pond depth. Calkins et al (1976) and Moeller and Calkins (1980) found that only about 5 % of ultraviolet solar radiation penetrates below 20 cm and about 1 % below 30 cm of the wastewater body. Moreover, the results of covered bottles had shown that the die-off rate does not vary with depth. It is therefore expected that the faecal coliform die-off rate will follow the shape of variation of solar radiation intensity with depth. From Figure 13 the faecal coliform die-off rate in the dark was found to be $k_1 = 0.16$ d^{-1}. The average die-off rate for a 1.0 m deep pond was predicted by taken the area on the left side of the curve and dividing by the depth of the pond in question. For a 1.0 m pond, the predicted faecal coliform mortality rate is 0.20 d^{-1}.
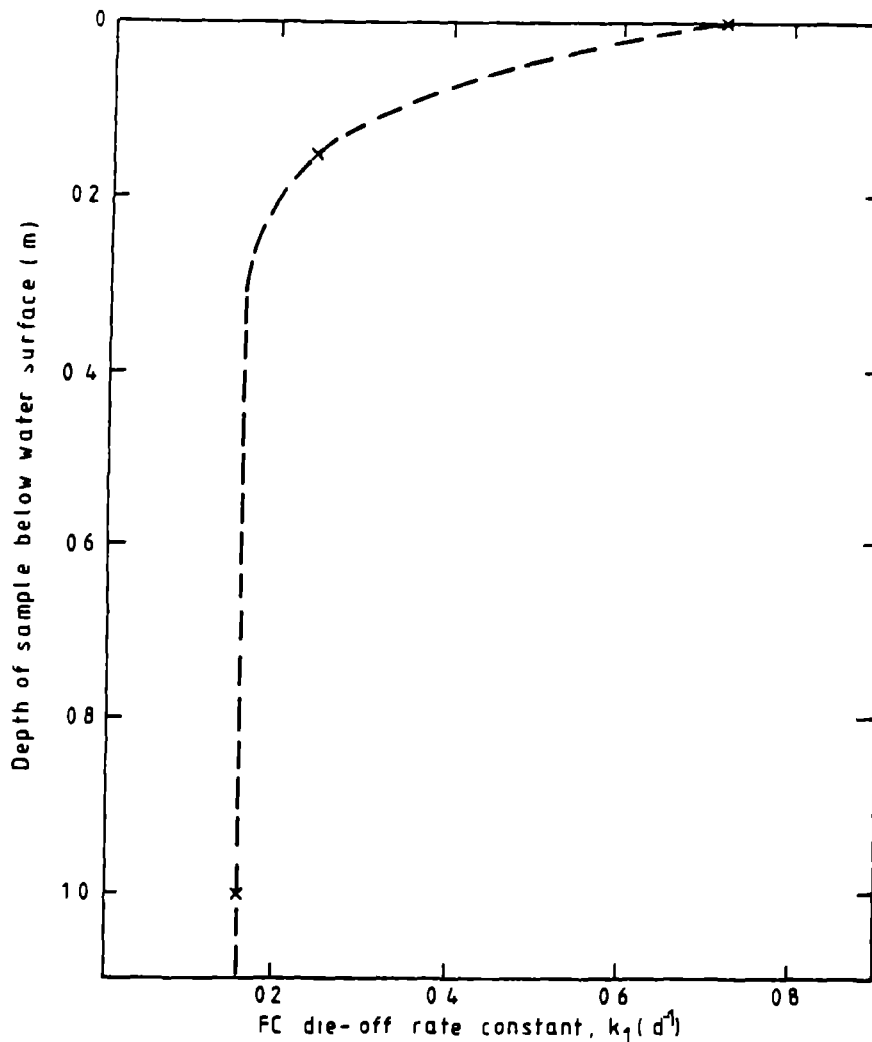
Figure   13.   Faecal coliforms die-off rate versus depth   of
               samples below the water surface.

Ohgaki   et   al (1986) did similar test using coliphages   as
test organisms and Toms et al (1975) using Escherichia Coli
and faecal streptococci as test organisms. The pattern   of
mortality   rates was similar although the die-off rate   was
different   as   it   can   be   seen   in   Tables   4   and   5.

Table   4 shows T90 values for different types of   organisms
while   Table 5 shows   sunlight   dose required to inactivate
90 % of organisms.

Table 4. T90 value for different organisms at different locations in the lagoon.

| Test organisms | Location in the lagoon | | | Source of information |
	Above the water sur- face (expo- se to dire- ct sunlight)	15 cm deep	1.0m deep	
Faecal coliforms	21 h	90 h	150 h	Results of this study
Escherichia Coli	19 h		131 to 225 h (dark values)	Toms et al (1975)
Faecal streptococci	11 h		73 to 112 h (dark values)	Toms et al (1975)

Table 5. Sunlight dose required to inactivate 90 % of initial value (cal/cm^2).

| Test orga- nisms | Test conditions | | | | Source of information |
| | Exposed to di- rect sunli- ght | In clear glass bottles | | In dark bottles | |
		10 to 15 cm	100 cm	15 to 100 cm	
Faecal coliforms	425 to 550	1700	2500	2500	Results of this study
Coliphages	80 to 420	1100	2600	1100 to 2600	Ohgaki (1986)

The summary of results in Tables 4 and 5 shows that the decay rate differs with the type of organisms and exposure conditions. The die-off rate is higher for samples incubated at the surface but decreases with depth for all types of organisms tested.

6.3 Pilot Plant Results and Discussion

6.3.1 Faecal Coliform Results and Discussion

6.3.1.1 Variation of Faecal Coliform Density with Time

The results of faecal coliform analysis carried out during the study period can be seen in appendix 1. Figure 14 shows the variation of influent and cell effluent faecal coliform concentration with time.
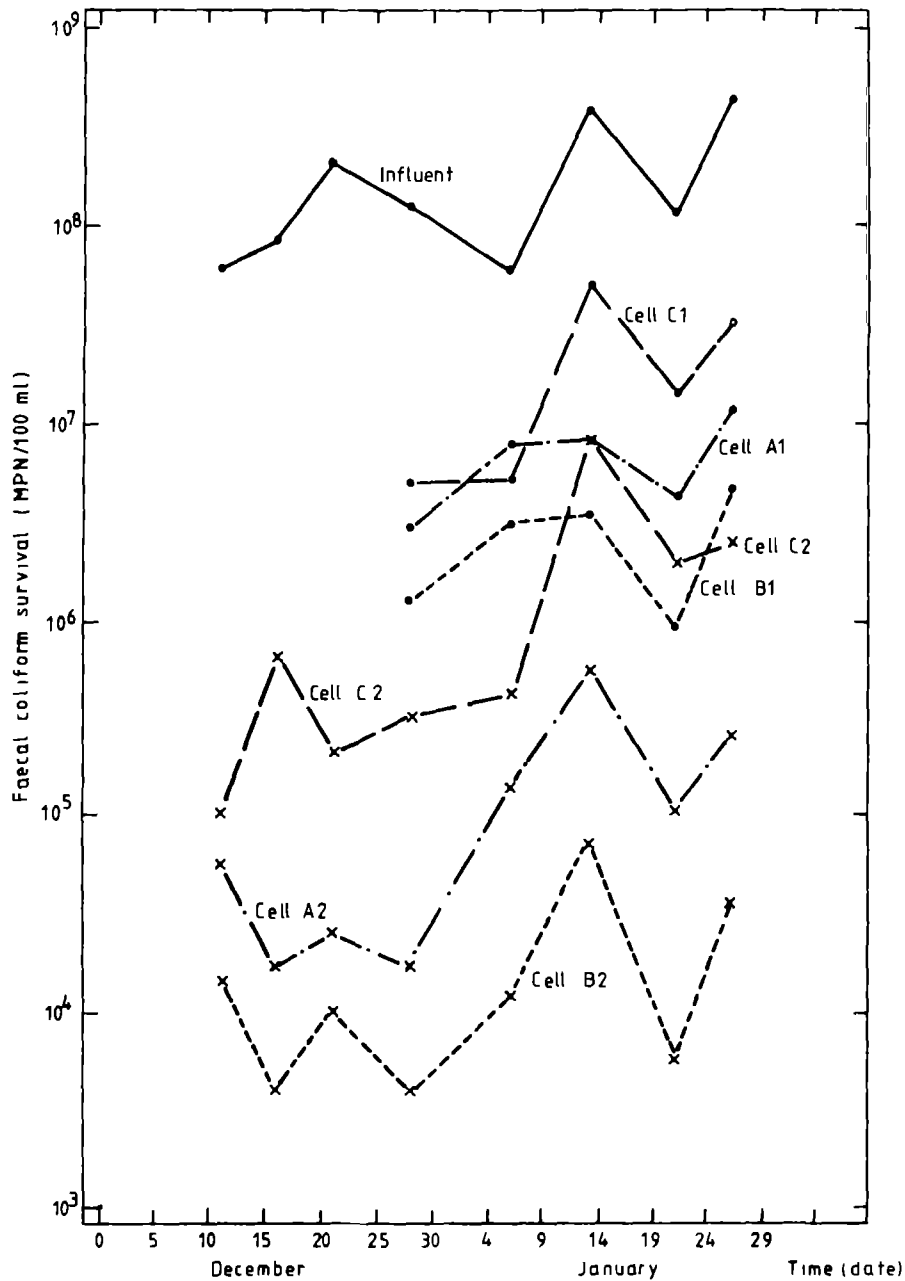
Figure 14. Variation of influent and cell effluent faecal coliforms with time.

As Figure 14 shows, the concentration of faecal coliforms in the ponds did not exhibit any distinct parallelism in December but did show a distinct parallelism in January. This means that an increase in the faecal coliform in the

effluent of subsequent ponds (and vice versa) in January in all ponds, but did not necessarily follow this pattern in December.

Pond A1 has shown a very steady condition with only very little variation in faecal coliform density. The change of faecal coliform density in all ponds were different from each other but in general set A ponds has shown less tendency of short circuiting. This may be due to its greater depth compared with other ponds.

6.3.1.2 Effect of Pond Depth on the Die-off Rate

Sarikaya and Saatci (1987) proposed the depth averaged bacterial die-off constant k in vertically mixed ponds as:

$$k = kd + \frac{ks \ So \ (1-e^{-KH})}{KH} \qquad (6)$$

where:

kd = bacterial die-off rate constant in the dark (d^{-1})

ks = constant for the light mortality term (d^{-1})

So = light intensity received at the pond surface (cal/cm^2d)

K = light attenuation coefficient (m^{-1})

H = pond depth (m)

The depth of maturation pond encountered in practise ranges from 0.9 to 1.5 m (Marais, 1963; Oraqui et al, 1986). Moeller and Calkins (1980) reported the light attenuation coefficient in tertiary ponds as 16 m^{-1}. The exponential term e^{-KH} would therefore range from 3.78 x 10^{-11} to 5.57 x 10^{-7}. Sarikaya et al (1987) reported K = 7.8 m^{-1} in secondary effluent from two stage trickling filters. Using their figure of K = 7.8 m^{-1} the exponential term e^{-KH} would range from 8.29 x 10^{-6} to 8.94 x 10^{-4}. Therefore the term e^{-KH} can be neglected with an error of less than 1 % when dealing with pond depth greater than 0.9 m.

Equation above may therefore be reduced to

$$k = kd + \frac{ks \ x \ So}{K \ x \ H} \qquad (7)$$

Using this relationship a graph of k against (So/H) was plotted (Figure 15). The line of best fit obtained by regression analysis was found to be:

$$k = 0.118 + 6.52 \ x \ 10^{-4} \ (\frac{So}{H}) \qquad (8)$$

when the faecal coliform die-off rate from Table 6 were plotted against (So/H). Solar radiation So is an average value from the previous sampling day to the sampling day in question. The die-off rate was calculated from Chick's law.
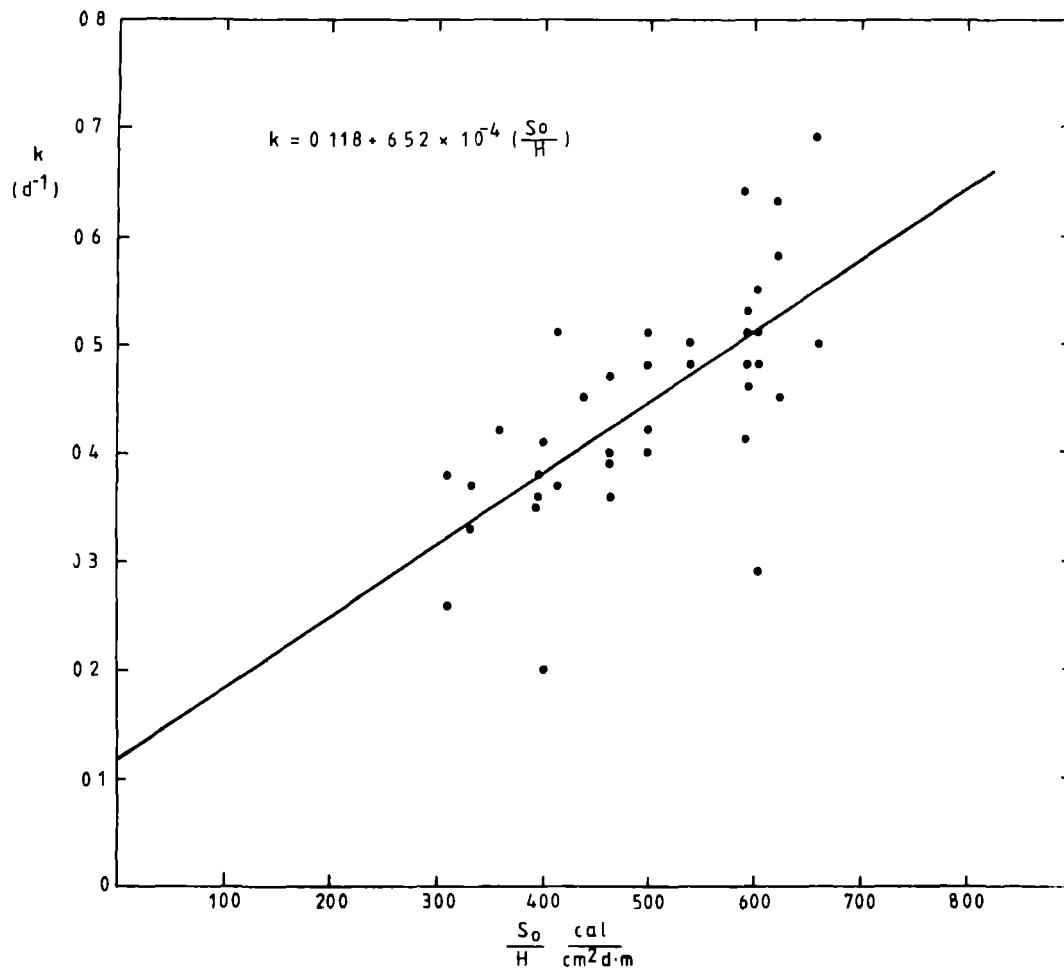
Figure 15. Faecal coliforms die-off rate constant k versus depth averaged solar intensity (So/H).

Table 6. Faecal Coliforms die-off rate constant versus average daily solar intensity.

| Date | Average daily solar intensity (cal/cm^2d) | Temperature of sample on the sampling day ($^{\circ}C$) | Die-off rate constant (d^{-1}) ||||||
			k_{a1}	k_{b1}	k_{c1}	k_{a2}	k_{b2}	k_{c2}
11/12/87	593	28.3				0.35	0.41	0.64
16/12/87	542	30.6				0.42	0.50	0.48
21/12/87	660	34.1				0.45	0.50	0.6
28/12/87	624	29.5	0.37	0.45	0.63	0.51	0.58	0.55
6/1/88	604	30.1	0.20	0.29	0.48	0.41	0.55	0.51
13/1/88	466	26.2	0.38	0.47	0.40	0.26	0.38	0.36
21/1/88	500	30.8	0.33	0.48	0.42	0.37	0.51	0.40
26/1/88	596	29.4	0.36	0.46	0.53	0.38	0.48	0.51

Note:
The values of 11/12/87 to 21/12/87 are in fact average values because effluent of first cells were not analysed.

Table 7 shows the kd values obtained for pilot waste stabilization ponds in Thailand, Saudi Arabia and Tanzania.

Table 7. kd values obtained by different researchers.

kd(d^{-1})	Temperature range ($^{\circ}C$)	Arithmetic mean temperature ($^{\circ}C$)	Source of information
0.093	28.6 to 32.6	30.4	Data from Polpraset et al, (1983) analysed by Sarikaya and Saatci (1987)
1.156	26 to 31	28.1	Sarikaya et al (1987)
0.118	26.2 to 34.1	29.9	This report (1988)

Abdulfattah (1987 cited by Sarikaya et al, 1987) reported that the term kd is affected by temperature. From Table 7 the temperature range of three different sets of pilot waste stabilization ponds is very similar, yet kd values varied significantly. Temperature may be one of the factors affecting kd, but kd is probably affected by other factors as well.

The rate constant in the dark, kd, is 0.118 meaning that faecal coliform die-off progresses even in the dark, the fact which is supported by figures 13 and 16. The effect of die-off rate constant in the dark is small in very shallow ponds. With the average daily solar intensity of about 550 cal/cm^2 in Dar es Salaam, the die-off rate constant k would be 0.36 d^{-1} (kd/k = 0.33) in 1.5 m deep pond and 0.84 d^{-1} (kd/K = 0.14) in 0.5 m deep pond. However, since the depth of maturation pond encountered in practise ranges from 0.9 m to 1.5 m, the contribution of kd in die-off is appreciable.

The ratio of (ks/K) obtained in the pilot scale waste stabilization ponds at the University of Dar es Salaam in Tanzania was 6.52 x 10^{-4} cm^2/cal. Data from Polprasert et al (1983) for oxidation ponds at the Asian Institute of Technology in Thailand gives ks/K = 6.44 x 10^{-4} cm^2/cal, (analysed by Sarikaya and Saatci, 1987) whereas Sarikaya et al (1987) reported ks/K = 6.72 x 10^{-4} cm^2/cal for pilot waste stabilization ponds at the King Abdulaziz University in Saudi Arabia. The closeness of these results confirms the fact that solar intensity has a significant relationship to the bacterial die-off constant.

Table 8 summarises the mean, standard deviation and confidence intervals of k values of each set of pilot scale ponds at the University of Dar es Salaam.

Table 8. The mean, standard deviation and confidence intervals of k values of each set of pilot scale ponds at the University of Dar es Salaam.

Item	Set A operated at detention time of 10 d and 1.5 m depth	Set B operated at detention time of 10 d and 1.0 m depth	Set C operated detention time of 5 d and 1.0 m depth
Number of samples analysed	13	13	13
Mean die-off rate constant, k (d^{-1})	0.37	0.47	0.51
Standard deviation σ_{n-1}	0.078	0.074	0.101
Confidence interval using student t-distribution at 95 % confidence interval	$0.32 \leq \bar{k} \leq 0.42$	$0.43 \leq \bar{k} \leq 0.51$	$0.44 \leq \bar{k} \leq 0.57$

The differences between the mean values of the die-off rate constants for different sets were also tested at 5 % significance level by applying student t-test. (See appendix 4). The statistical analysis shows that the first order die-off rate constant k is independent of pond hydraulic detention time, but varies significantly with pond depth.

6.3.1.3 Effect of Hydraulic Detention Time on the Mortality of Faecal Coliforms

Figures 16 and 17 show the relationship between faecal coliform survival with hydraulic detention time plotted on a semilogarithmic paper. The die-off rate of faecal coliforms was found to follow Chick's law very closely.

The mean values of the mortality rate constant in 1.0 m deep ponds was found to be $k_1 = 0.2$ d^{-1} ($k = 0.46d^{-1}$). Data used for analysis can be found in appendix 1 (from 28/12/87 to 26/1/88). The difference in the mean mortality rate constant of set B and set C ponds was tested at 5 % significance level using student t-distribution. It was found that the difference is insignificant.

Faecal coliform survival (MPN/100 ml)

10^9

10^8

10^7

10^6

10^5

10^4

10^3

28/12 /87	×	k_1 = 0 18
6/ 1/88	●	k_1 = 0 13
13/ 1/88	o	k = 0 19
21/ 1/88	▲	k_1 = 0 21
26/1/88	■	k_1 = 0 20
mean		k_1 = 0 18

0   5   10   15   20

Nominal detention time (d)
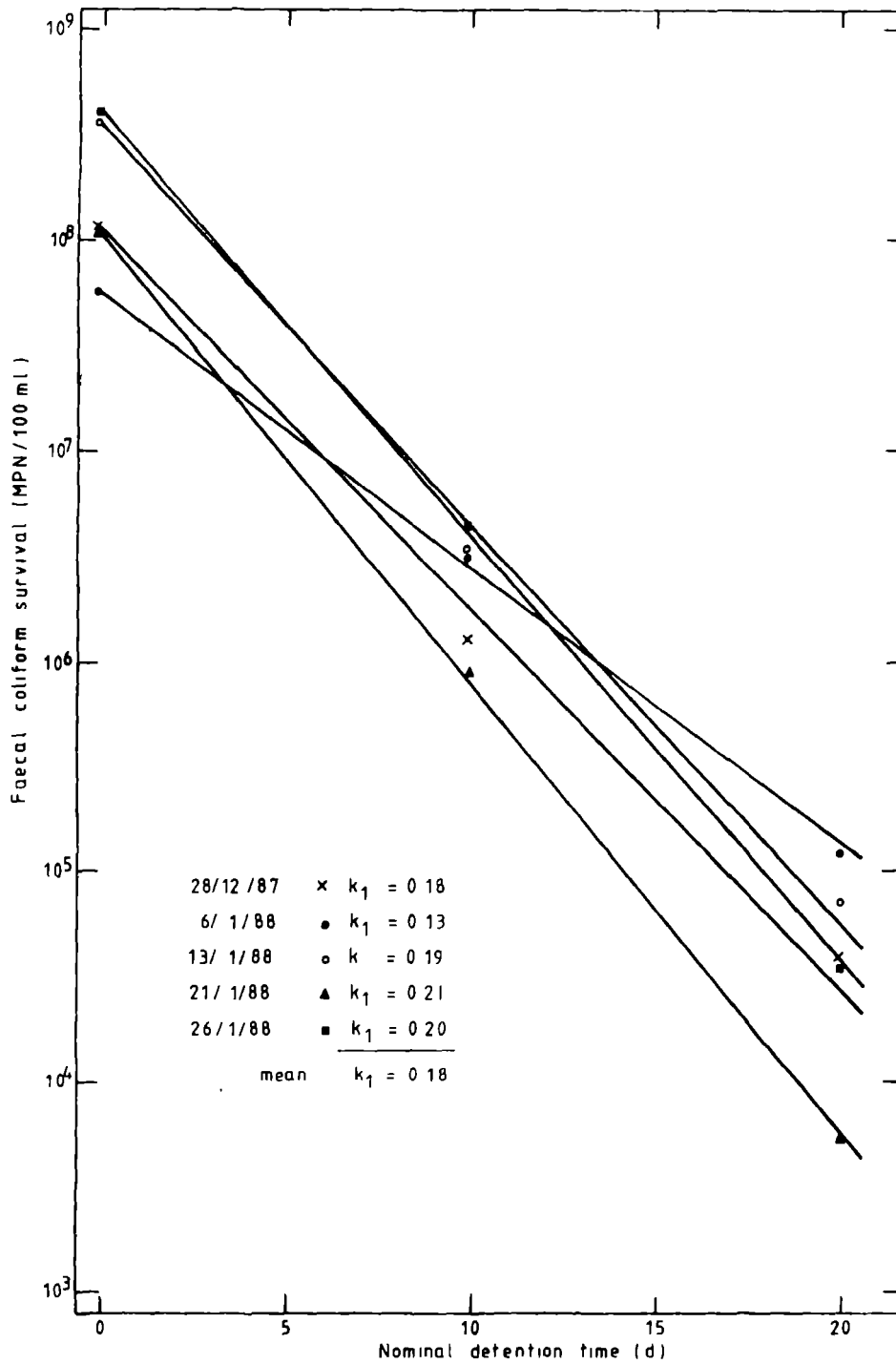
Figure 16. Faecal coliform survival versus nominal detention time in set B ponds.
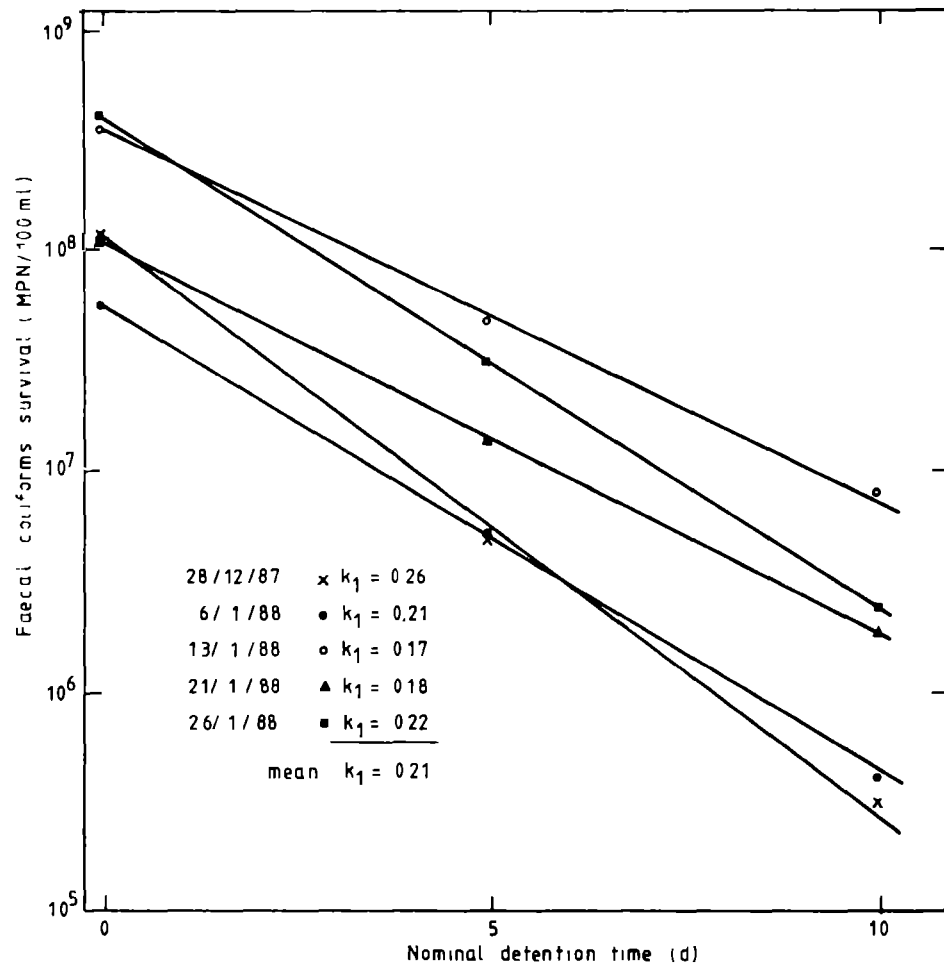
Figure 17. Faecal coliform survival versus nominal detention time in set C ponds.

Faecal coliforms were reduced by an average of 3.8 log scales in two ponds in series each with hydraulic detention time of 10 days and pond depth of 1.0 m. Ponds with similar dimensions, but with hydraulic detention time of 5 days in each cell (2 ponds in series) reduced faecal coliforms by an average of 2.3 log scales.

The mean overall percent reduction of faecal coliforms in set B ponds was 99.98 % whereas set C ponds reduced faecal coliforms by 99.50 %. Inspite of such impressive reductions the mean faecal coliform survival was 21 x 10^3/100 ml after purification in set B ponds and 692 x 10^3/100 ml after treatment in set C ponds. These densities are still too high necessitating further purification by either increasing hydraulic detention time or increasing number of ponds in series or both.

The overall percent reduction of both enteric and pathogenic bacteria reported in the literature should be taken with caution because it provides inadequate description of bacterial removal rates. The mortality rate constant should be used as a parameter indicating bacteria reduction efficiency instead of in conjunction with overall percent reduction.

6.3.2 Comparison of BOD and Faecal Coliform Results

Table 9 presents a summary of mean values of influent and cell effluent BOD_5 and percent reduction for filtered and non-filtered samples.

Table 9. Summary of mean values of influent and cell effluent BOD_5 and percent reduction for filtered and non-filtered samples.

Sample	Non-filtered BOD_5		Filtered BOD_5	
	mean (mg/l)	percent reduction	mean (mg/l)	percent reduction
Influent	341	–	220	–
Cell A1 effluent	187	45	81	63
Cell B1 effluent	164	52	89	59
Cell C1 effluent	213	38	102	54
Cell A2 effluent	151	56	62	72
Cell B2 effluent	148	57	70	68
Cell C2 effluent	139	59	89	59

The results indicate that unfiltered samples have relatively higher BOD_5 values than filtered samples. Relatively higher values of BOD_5 in unfiltered samples is due to the presence of algae in the samples and suspended solids. Varma et al (1963) found that if samples are incubated in standard conditions (dark incubation method), algae may die, and dead algae will consequently increase BOD. Bucksteeg (1987) found that algae in pond effluents increase the residual organic load. He found that about 100 μg chlorophyll-α represent about 5 mg BOD_5 in the average.

Although no quantitative support was established, the influent had relatively low algae concentration (based on visual inspection of wastewater) when compared to final pond cells of each set. Itis therefore thought that the low BOD_5 values of filtered samples (when compared with unfiltered samples) is partly contributed by suspended matter of organic nature.

The results have contradicting behaviours. While unfiltered BOD_5 results show that better removal efficiency is obtained in shallow ponds, filtered sample results indicate vice versa. The cause of the difference was not immediately established.

The percent BOD_5 removal is higher when wastewater is detained for longer period with an exception of cell C2 non-filtered sample results.

Because of the differences in pond depth, organic load applied to the ponds was expressed in terms of BOD per day per unit volume. The summary of mean BOD loading rates can be found in Table 10.

Table 10. Mean BOD loading rates of individual pond cells.

Pond cell	Organic loading rate based on unfiltered BOD samples (g/m^3/d)	Organic loading rate based on filtered BOD samples (g/m^3/d)
A1	34.1	22.0
B1	34.1	22.0
C1	68.2	44.0
A2	18.7	8.1
B2	16.4	8.9
C2	42.6	20.4

Organic loading based on filtered samples was considered for comparison with faecal coliform die-off rates. Table 11 summarises results of organic loading rates and faecal coliform die-off rate constant at various operational conditions.

Table 11. Faecal coliform die-off rate constant at different organic loading rates and operational condit

	Ponds operated at 10 days detention time and 1.5 m depth		Ponds operated at 10 days detention time and 1.0 m depth		Ponds operated at 5 days detention time and 1.0 m depth	
	Cell A1	Cell A2	Cell B1	Cell B2	Cell C1	Cell C2
Mean organic loading rate (filtered) g/m^3/d	22.0	8.1	22.0	8.9	44.0	20.4
Mean FC die-off rate constant *k(d^{-1})	0.33	0.39	0.43	0.50	0.49	0.47

* k values are average of five results for sets A, B and C taken from 28/12/1987 to 26/1/1988.

The mean values of faecal coliform die-off rate constant is higher in ponds with low organic loading rate . Although it is difficult to judge the effect of organic loading rate with only few tests it is doubtful whether organic organic loading rate has a big impact on the bacterial die-off rate.

Some researchers including Canter et al (1969) reported that pathogen removal efficiencies decreased with increasing loading rates. They suggested that at lower organic loading rates, there is probably limited nutrients

available and therefore organisms are unable to compete successfully. However, a substantial increase in organic loading rate only causes a slight decrease in bacterial removal efficiency.

On the other hand, Skerry and Parker (1979) reported that the bacterial die-off rates does not appear to be directly related to organic loading rates. The argument of Skerry and Parker seems to be valid in this study. Pond cells A1 and B1 were operated at the same volumetric organic rate, yet the die-off rate constant was much higher in cell B1. Cell C1 was operated at twice the organic loading rate of cell A1 but it was found that faecal coliform death rate was faster in cell C1. The operational conditions may therefore be regarded as the more significant factor than organic loading rates. At higher rates (between 20 and 44 $g/m^3/d$ in this study) the organic loading rate does not have any influence on the bacterial die-off when operation depth was kept constant.

It is doubtful whether competition for nutrients in this study may be the cause of bacterial reduction. McGrew and Mallette (1962) reported that some bacteria of intestinal origin could survive at concentration levels of organic matter less than 5 mg/l. BOD_5 was never less than 29 mgl for individual samples and never below 62 mg/l for the mean values, which suggests that enough organic material was available for the growth of bacteria.

6.3.3 pH Results

Figure 18 shows typical diurnal variation of pH at the surface of the last cells of each set. pH was observed to start rising at around 6.00 am in the morning and the highest values were reached during the late afternoon hours (around 15.00 hours). The pH values remained above the mean values for each cell until well after sunset. The maximum pH values were found to lag by about 2 hours when compared with maximum value of direct solar intensity.
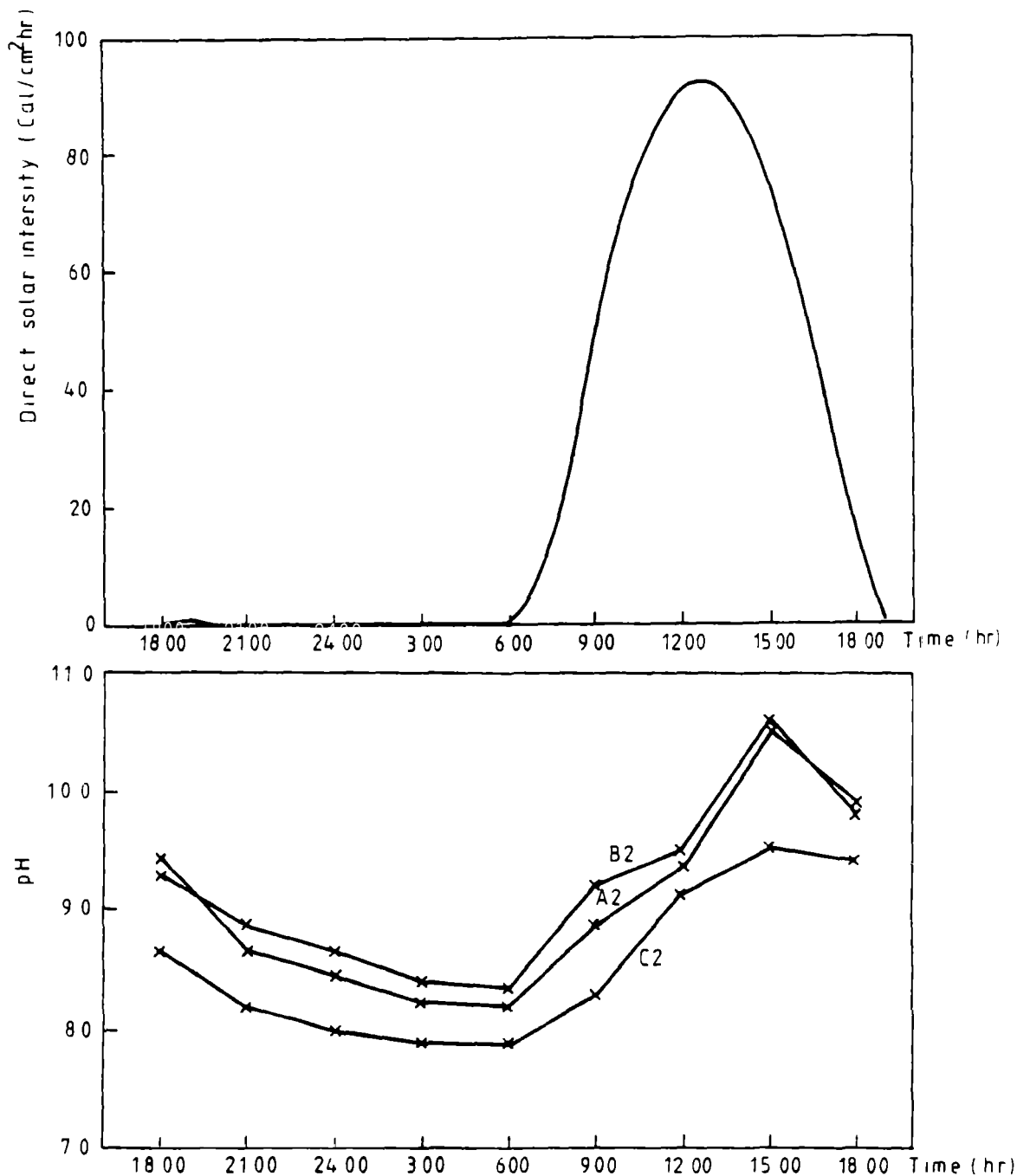
Figure 18. Typical diurnal variation of direct solar intensity (above) and pH (below). (Data can be seen in appendix 6.)

The diurnal fluctuation of pH is caused by the algal cells which consume the carbondioxide released by bacterial stabilization of organic matter, resulting in the dissociation of carbonic acid, consequently causing an increase in pH.

As Figure 18 shows the depth of pond had little effect on the variation of pH levels within the depth range tested, the fact which was also observed by Williford and Middlebrooks (1967).

An increase in light intensity is accompanied by an increase in pH since photosynthetic action of algae is dependent on light. Excess light during daylight hours allows algal activity to proceed unabated until close to sunset. After sunset, carbondioxide is no longer consumed by algae while at the same time bacterial production of carbondioxide continues, resulting in a decrease in pH levels as Figure 19 shows (data in appendix 7.)
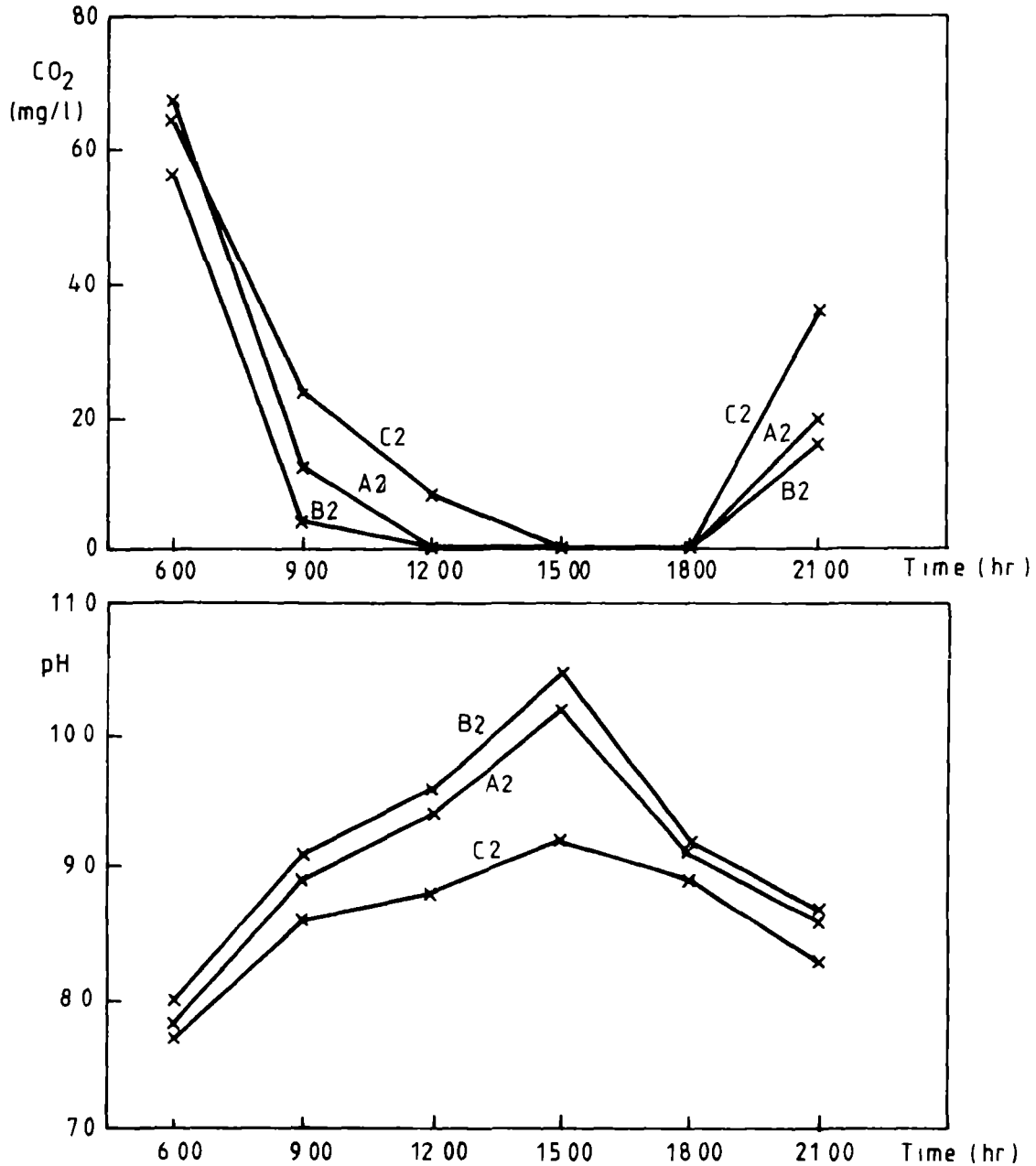
Figure 19. Typical diurnal variation of CO_2 (above) and pH (bottom) in cells A2, B2 and C2. (Sampled on 24/12/87.)

However, the pH levels were relatively higher in shallower ponds than deeper ponds. While depth does not show any significant variation of pH, the same was increased by almost one scale on increasing the detention time twofold (from 5 days in pond C2 to 10 days in pond B2).

pH levels of all the ponds are highest near the surface
during day hours and drops gradually towards the bottom of
each cell. The last cell of each set have recorded
relatively higher pH values at the same depth when compared
with first cells as shown by Figure 20 and appendix 5.
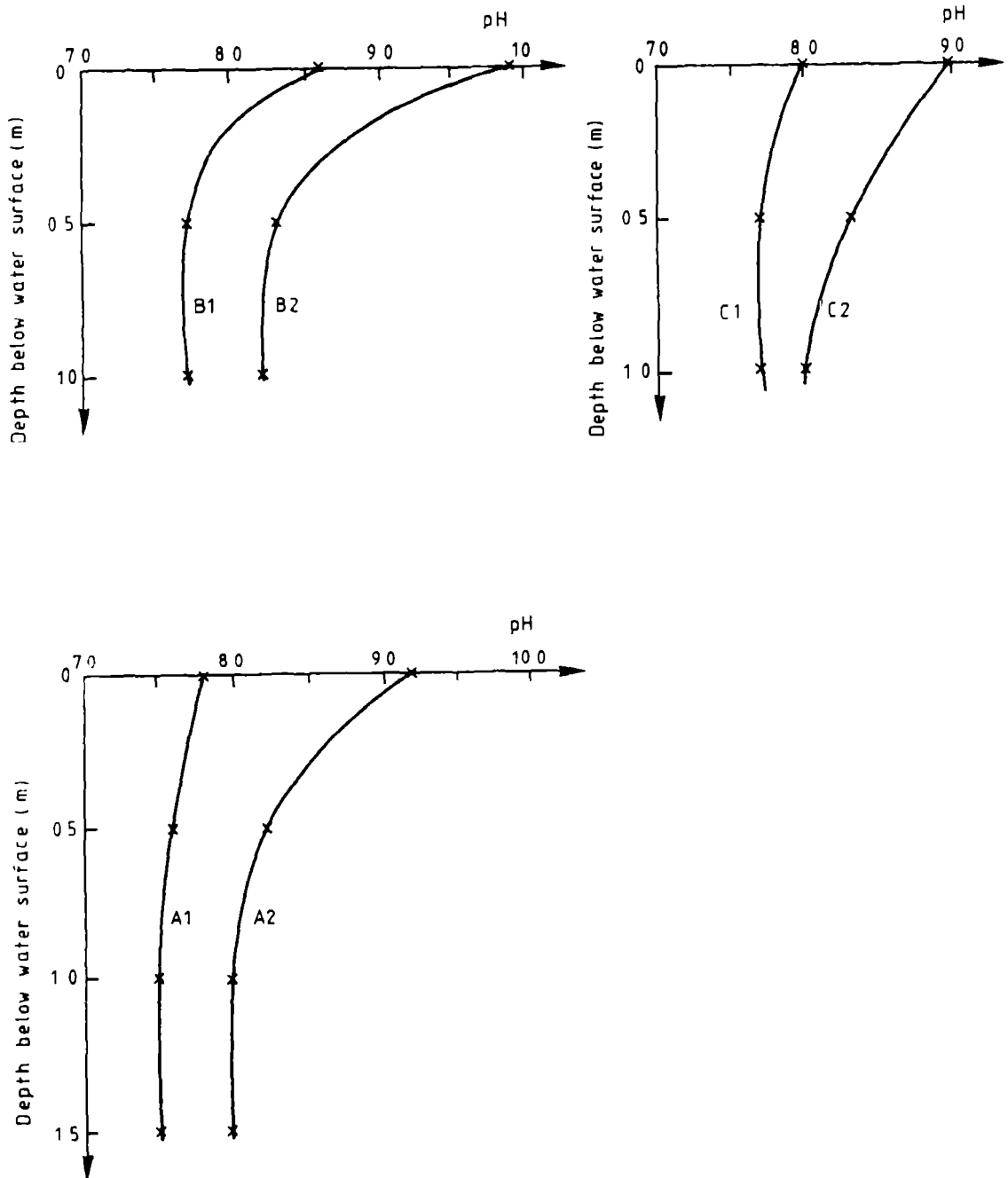
Figure 20. Typical variation of pH with pond depth in each
cell.

The pH values were higher in the last cells probably due to
low BOD loading per unit volume. The low organic loading
rate has two possible effects. Firstly, more light can
penetrate deeper into the pond resulting into higher algae
production which consumes carbon dioxide consequently
rising pH. Secondly, due to low organic rate, the
production of carbon dioxide is lower, meaning that pH is
expected to be higher in ponds with lower organic loading.
This phenomenon is confirmed by Figures 19 and 20. Below
0.5 m there is generally no change in pH values for all the
cells while the maximum values were obtained near the
surface.

Low pH values in deeper zones of all cells is a result of
diminishing sunlight intensity with depth. Algae grows near
the surface and therefore very little change in pH is
expected in deeper zones of the ponds, unless the pond
contents are effectively mixed by wind or any other means.

The rate of change of pH is higher in the last cells of
each set when compared to first cells as Table 12 shows.
The results also show that higher pH values are attained in
shallower ponds with longer retention period. Longer
detention time enhances growth of algae by reducing
possibilities of algae cell wash-out thus providing a
better chance of algae growth and therefore high pH values.
On the other hand since organic load in the last cells is
less than in the first cells, the carbondioxide content is
expected to be low in the last cells, carbondioxide is
therefore depleted faster in the last cells, resulting in
higher pH values.

Table 12. Change of pH with detention time of wastewater in
the lagoon system (average of values taken from 28/12/87 to
26/1/88).
(See appendix 1 for pH values of individual days.)

Sampling place	Set A, 1.5 m ponds		Set B, 1.0 m ponds		Set C, 1.0 m Ponds	
	Total detention time (d)	pH	Total detention time (d)	pH	Total detention time (d)	pH
Influent	0	7.3	0	7.3	0	7.3
first cell effluent	10	7.8	10	8.0	5	7.7
second cell effluent	20	8.9	20	9.2	10	8.5

6.3.4 Dissolved Oxygen Results

Table 13 shows the diurnal variation of dissolved oxygen concentration. Diurnal dissolved oxygen concentration followed customary patterns in all the ponds reaching supersaturation values near the pond surfaces in the afternoon, whereas oxygen levels dropped below 0.5 mg/l in all cells by midnight. As Table 13 shows, dissolved oxygen pattern follows solar radiation intensity very closely.

Table 13. Typical diurnal variation of dissolved oxygen concentration (from 18.00 pm on 15/12/87 to 18.00 pm on 16/12/87).

Date	Time (hr)	Solar intensity received at the pond surface in 3 hours period (cal/cm^2)	Dissolved oxygen concentration (mg/l)		
			Cell A2	Cell B2	Cell C2
15/12 /87	18.00	–	>15	>15	12.0
	21.00	1	2.2	1.9	0.4
	24.00	0	0.3	0.2	0.4
16/12 /87	3.00	0	0.3	0.2	0.3
	6.00	0	0.3	0.3	0.3
	9.00	77	>15	>15	10.0
	12.00	241	>15	>15	>15
	15.00	255	>15	>15	>15
	18.00	113	>15	>15	14.2

The basic phenomenon involved is that algae cells consume the carbondioxide released by bacterial stabilization of organic matter in the presence of light producing more algae cells and oxygen.

The primary pond effluent recorded very low dissolved oxygen level (always below 0.6 mg/l) and did not rise significantly even during the afternoon due to high organic load, necessitating bacterial degradation and therefore depleting dissolved oxygen in the process. A similar phenomenon has also been reported by Canter et al (1969) and Folkman et al (1973).

The general trend has shown that dissolved oxygen levels tends to rise towards the last ponds; the effluent of primary pond recorded the lowest values while last cell of each set recorded the highest values (See appendix 1.).

Very high dissolved oxygen concentration above saturation were observed in the last cell of each set probably due to relatively low BOD. Production of dissolved oxygen in first ponds is offset by rapid depletion of oxygen produced by organic material. Cell B1 has also recorded relatively high values when compared with cell A1 and C1 probably due to a combination of its shallow depth and long retention time. The ratio of solar intensity received on the pond surface

per volume of water in the pond is higher than that of cell A1 thus having a better oxygen production. Cell C1 has low values of dissolved oxygen due to premature algae cell washout as a result of short hydraulic retention time and due to high organic loading rate. Low BOD loading in cell C2 has enabled it to attain a substantial increase in dissolved oxygen concentration.

6.3.5 Comparison of Faecal Coliform Mortality Rates from Submerged Bottle Experiment and Pilot Scale Ponds

Table 14 summarises the faecal coliform die-off rate constant predicted from submerged bottle experiment and data from pilot scale waste stabilization ponds for the pond depth of 0.75 to 1.75 m.

Table 14. Faecal coliform die-off rate constant k_1 predicted from Figure 13, Equation 8 and Figures 16, 17 and 21.

Pond depth (m)	Submerged bottle experiment k_1 (d^{-1})	Pilot scale waste stabilization ponds	
		Equation 8 $k_1 = \dfrac{k}{2.3}$ (d^{-1})	Figure 16, 17 and 23 k_1 (d^{-1})
0.75	0.21	0.26	–
1.00	0.20	0.21	0.20
1.25	0.19	0.18	–
1.50	0.19	0.16	0.13
1.75	0.18	0.14	–

Note that:
(1)   The average daily direct solar intensity, So, in Dar es Salaam is about 550 $cal/cm^2/$ d.
(2)   Die-off rate constant from equation 8 , k, (from ln scale) was converted to k_1 (log 10 scale) by relationship $k_1 = k/2.3$

Three different methods used in the prediction of faecal coliforms die-off rate constant have very similar values. Prediction using results of submerged bottle experiment shows only a slight change in k_1 value whereas results of pilot scale shows significant changes. It was also observed that faecal coliform die-off rate decreases with increasing pond depth in all 3 cases.

The graphical solution of submerged bottle experiment was found to be within 20 % of the values obtained from equation 8 of pilot waste stabilization ponds for the pond depth range of 0.75 m to 1.50 m. Results of three different methods have come close to each other for the pond depth of 1.0 m.
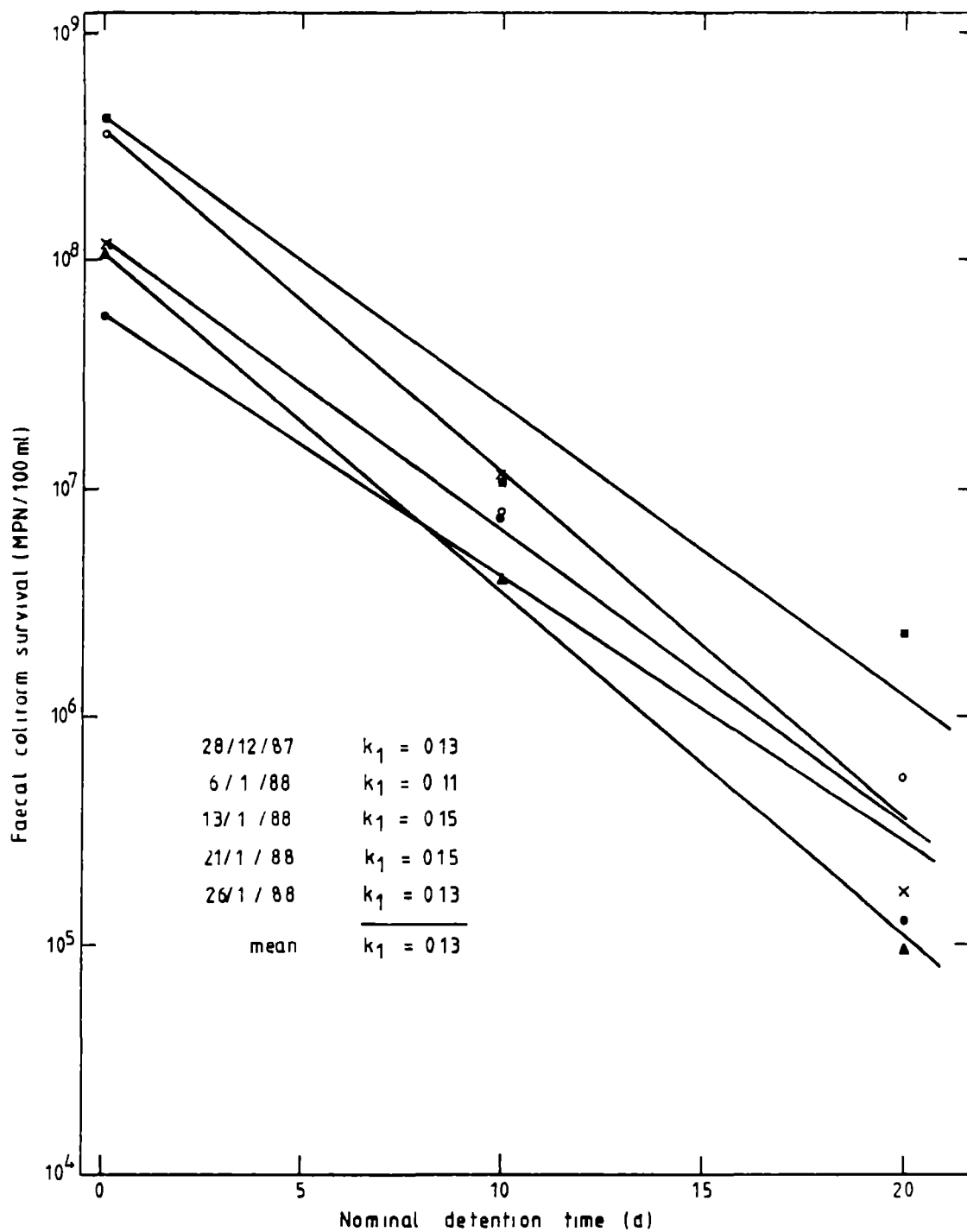
Figure 23. Faecal coliform survival versus nominal detention time in set A ponds.

7 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Due to complex ecosystem existing in waste stabilization ponds, it has been difficult to determine major influential factors contributing to bacterial die-off.

In this study, faecal coliform used as test organisms were found to die more rapidly near the water surface in the pond. The possible mechanisms of bacterial die-off are:

1) that the bacteria are killed due to inactivation power of ultraviolet radiation.

2) that the increase in direct sunlight intensity increases the activities of algae thereby increasing pH and dissolved oxygen in the water body consequently killing bacteria.

Both possibilities may exist in this study. In the cells A1, B1 and C1, pH was rather low, most often below 8.0. Dissolved oxygen concentration was similarly low, although values above saturation were recorded in January, 1988. Dissolved oxygen and pH are unlikely to cause bacterial die-off in the first cells.

Due to low organic loading rates in cells A2 and B2, resulting in deeper penetration of light and higher consumption of carbon dioxide, pH values above 10 have been recorded in the afternoon. Dissolved oxygen was very often above 15 mg/l by 9.00 am. Therefore dissolved oxygen and pH may contribute to bacterial die-off.

Bacterial die-off in the first cells is considered largely to be due to the effect of ultraviolet solar radiation together with other factors such as temperature. In the cells A2 and B2 the effect of solar ultraviolet radiation together with an additional effect of high pH levels above saturation value has increased further the die-off rate.

The effect of sunlight is however limited to within 30 cm of the upper layer of water body due to turbidity caused by algae and suspended solids in wastewater. Due to this reason the bacterial die-off caused by solar radiation only happens near the surface, the fact which was also confirmed by submerged bottle experiment.

In this study, the relationship between direct solar intensity, depth and faecal coliform die-off rate constant was found. The die-off rate (death per day) was found to be independent of hydraulic detention time but varied significantly with the pond depth (see appendix 4). The reader should not confuse between die-off rate and total reduction of bacteria after detention time t. Faecal coliform die-off rate was found to be higher in shallow ponds than deep ponds and increased with increasing solar intensity.

The faecal coliform die-off not only depends on pond depth and amount of solar intensity received at the pond surface but it also varies with the type of test organisms, detention time and other factors. In this study, it was observed that faecal coliforms disappearance is higher when wastewater is detained for longer period of time. It was also observed that faecal coliform disappearance followed Chick's law very closely. Faecal coliforms were reduced by an average of only 2.3 log scales when wastewater was detained in two ponds in series each with detention time of 5 days, whereas doubling detention time to 10 d. in each cell has reduced faecal coliforms by an average of 3.8 log scales.

7.2 Recommendations

1)   The overall percent reduction of bacteria in wastewater ponds is not enough to provide description of bacterial removal rates. For instance, faecal coliforms were reduced by 99.5 % in set C ponds. However, in spite of such impressive reduction the mean survival was 692 x 10PT3PT/100 ml. Whenever possible the mortality rate should be used as a parameter indicating bacterial removal efficiency either in conjunction with or instead of overall percent reduction.

2)   The coliform constant for the light mortality term, ks, has been found to be rather similar for tests done in different parts of the world. However, the coliform die-off rate constant in the dark, kd, was significantly different in the same tests. Some researchers have reported that kd varies with temperature. However, when the results of three different experimental sites in different parts of the world with similar temperature were compared, kd values were found to be significantly different. Therefore, factors influencing kd should be investigated in future.

3)   The results obtained in this study were based on the pilot scale experimental ponds. There is a need to confirm these results with longer observation period in large scale ponds in operation.

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APPENDICES

Appendix 1 Data collected from pilot ponds.

Date	Sample	Dissolved Oxygen (mg/l)	Temperature (°C)	pH	Unfiltered BOD_5 (mg/l)	Filtered BOD_5 (mg/l)	Faecal coliform count (MPN/100 ml)
11/12/87	Influent	0.2	28.5	7.3	280	142	59×10^6
	Cell A2 effluent	10.8	28.7	8.7	130	73	54×10^3
	Cell B2 effluent	13.9	28.3	8.8	140	76	15×10^3
	Cell C2 effluent	8.3	28.0	8.4	70	64	97×10^3
16/12/87	Influent	0.3	30.2	7.2	312	244	80×10^6
	Cell A2 effluent	>15	30.8	8.9	160	96	17×10^3
	Cell B2 effluent	>15	30.4	9.2	184	117	4×10^3
	Cell C2 effluent	10.0	30.5	8.3	107	104	640×10^3
21/12/87	Influent	0.3	34.0	7.5	330	279	200×10^6
	Cell A2 effluent	>15	34.2	9.0	180	81	24×10^3
	Cell B2 effluent	>15	34.0	9.2	194	70	10×10^3
	Cell C2 effluent	14.9	34.0	8.6	152	132	200×10^3
28/12/87	Influent	0.3	29.8	7.3	340	240	117×10^6
	Cell A1 effluent	1.3	29.5	7.6	148	90	2.9×10^6
	Cell B1 effluent	3.3	29.5	7.7	154	128	1.25×10^6
	Cell C1 effluent	0.2	29.5	7.5	274	116	4.9×10^6
	Cell A2 effluent	9.6	29.4	8.2	147	36	16.8×10^3
	Cell B2 effluent	11.2	29.5	8.9	153	105	3.94×10^3
	Cell C2 effluent	6.6	29.4	8.4	192	84	320×10^3
6/1/88	Influent	0.6	30.6	7.1	232	183	56.5×10^6
	Cell A1 effluent	3.3	29.4	7.7	175	84	7.57×10^6
	Cell B1 effluent	11.0	30.5	8.0	105	90	3.05×10^6
	Cell C1 effluent	2.9	30.4	7.5	196	132	5.1×10^6
	Cell A2 effluent	>15	30.3	9.1	163	53	130×10^3
	Cell B2 effluent	>15	30.2	9.3	67	57	12×10^3
	Cell C2 effluent	14.4	29.8	8.4	145	105	400×10^3

13/1/88	Influent	0.5	26.2	7.1	341	185	355×10^6
	Cell A1 effluent	9.3	26.2	7.4	153	50	7.88×10^6
	Cell B1 effluent	10.1	26.2	7.4	158	60	3.3×10^6
	Cell C1 effluent	8.2	26.3	7.4	192	71	47.1×10^6
	Cell A2 effluent	>15	26.3	8.2	134	29	560×10^3
	Cell B2 effluent	>15	26.3	8.4	147	38	71×10^3
	Cell C2 effluent	>15	26.3	7.6	147	59	7.77×10^6
21/1/88	Influent	0.6	31.3	7.3	471	240	110×10^6
	Cell A1 effluent	>15	30.8	8.6	217	100	4×10^6
	Cell B1 effluent	>15	31.7	9.1	180	70	0.9×10^6
	Cell C1 effluent	12.0	31.7	8.7	153	87	13.5×10^6
	Cell A2 effluent	>15	30.5	9.9	147	68	98×10^3
	Cell B2 effluent	>15	30.2	10.2	153	44	5.5×10^3
	Cell C2 effluent	>15	32.0	9.4	158	61	1.8×10^6
26/1/88	Influent	0.4	31.6	7.7	420	244	420×10^6
	Cell A1 effluent	8.2	30.8	7.8	244	82	11×10^6
	Cell B1 effluent	10.4	30.8	8.0	223	89	4.3×10^6
	Cell C1 effluent	7.1	30.8	7.6	249	103	30×10^6
	Cell A2 effluent	>15	30.7	8.9	149	60	240×10^6
	Cell B2 effluent	>15	30.7	9.1	145	55	35×10^3
	Cell C2 effluent	>15	30.8	8.9	138	105	2.37×10^6

Appendix 2B Meteorological data from January 1988.

Date	Direct Solar radiation (Cal/cm^2/d)	Air Temperature (°C)			Rainfall (mm)	Evaporation
		Max	Min	Mean		
1/1	550	32.4	25.4	28.9	0	1.0
2/1	539	32.2	24.5	28.4	0	0.3
3/1	529	31.7	25.4	28.6	1.3	0
4/1	648	32.0	24.5	28.3	7.1	0
5/1	666	32.5	26.0	29.3	0	0.5
6/1	667	33.2	26.5	29.9	0	0.5
7/1	675	33.2	25.0	29.1	0	0.8
8/1	220	29.5	25.3	27.4	0.6	0
9/1	454	31.8	24.0	27.9	8.1	0
10/1	633	31.3	24.1	27.7	0	0.5
11/1	277	30.4	24.6	27.5	0	0.3
12/1	338	29.7	24.0	26.9	5.4	0
13/1	159	28.8	24.0	26.4	4.2	*
14/1	485	30.5	23.5	27.0	45.2	*
15/1	633	31.8	25.3	28.6	3.1	*
16/1	531	32.2	25.6	28.9	0	*
17/1	562	32.3	24.7	28.5	1.8	*
18/1	569	32.2	26.4	29.3	0	*
19/1	590	32.3	25.7	29.0	0	*
20/1	474	31.5	24.5	28.0	1.2	*
21/1	601	32.0	25.0	28.5	0	*
22/1	599	32.5	25.5	29.0	0	*
23/1	577	31.7	*		0	*
24/1	602	31.8	*		2.5	*
25/1	598	32.0	*		0	0.7
26/1	532	32.4	25.8	29.1	0	1.5
27/1	*	31.7	23.5	27.6	22.1	0
28/1	*	30.5	26.2	28.4	0	0.4
29/1	553	30.8	26.7	28.8	0	0.5
30/1	573	32.3	26.4	29.4	0	0.7
31/1	518	*	26.8		0	*

* Measurements were not taken.

Appendix 3 Hourly direct solar radiation intensity received at the pond surface as recorded by KIPP and ZONEN solar integrator on specific dates.

Date	7.00	8.00	9.00	10.00	11.00	12.00	13.00	14.00	15.00	16.00	17.00	18.00	19.00
16/12/87	7.2	21.8	47.6	68.6	81.7	90.4	92.2	87.3	75.7	58.9	37.9	15.9	1.3
24/12/87	1.9	15.1	27.2	36.6	66.5	85.5	92.3	87.9	77.2	60.9	40.9	19.0	2.3
30/12/87	2.4	18.0	44.3	61.1	71.5	84.7	89.5	86.5	74.2	58.5	37.3	16.9	1.5
7/1/88	2.2	18.3	42.3	53.3	81.2	89.6	92.5	88.8	78.0	62.1	42.6	20.8	2.8
8/1/88	0.7	2.4	4.5	33.0	17.8	20.9	43.5	17.0	25.0	29.6	19.0	6.0	1.0
9/1/88	3.2	20.7	26.7	15.8	41.1	34.8	81.6	88.3	77.2	62.1	5.3	0.7	0.4
10/1/88	2.7	4.3	7.9	59.4	73.4	92.5	94.9	90.6	80.2	64.2	40.6	20.2	1.8
11/1/88	1.6	16.3	26.2	51.6	41.9	47.1	31.2	12.9	12.2	15.7	13.1	6.5	0.9
12/1/88	0.7	2.5	7.4	51.9	39.2	52.5	69.5	18.7	19.6	40.6	23.9	10.0	1.5
13/1/88	1.1	9.8	7.9	2.9	0.1	2.2	0.0	14.8	41.7	36.7	31.2	8.8	2.0
14/1/88	4.4	5.8	16.3	36.6	70.3	90.6	79.5	33.8	62.3	40.3	32.9	14.2	4.0

Appendix 4 Testing variation of faecal coliform die-off rate constant with pond depth and with hydraulic detention time at 5 % significance level using student t-distribution.

Experimental values of all sets can be obtained in appendix 1. The mean and standard deviation values of A, B and C was summarised in table 8.

$$to = \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2}} \times \frac{\bar{k}_1 - \bar{k}_2}{\sqrt{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}} \quad -eq.A1$$

where: n is the sample size of the set

S is the standard deviation

$\bar{k}$ is the mean die-off rate constant

(a) **Testing effect of detention time on the mean k values (set B and set C).**

To test the hypothesis that $\bar{k}_b = \bar{k}_c$ using statistical values from table 8 and equation A1, to = 0.83. The critical value obtained from student t-distribution tables using $n_b + n_c - 2 = 23$ is $C_1 = -2.07$ and $C_2 = 2.07$ since $C_1 <$ to $< C_2$, then the hypothesis that $\bar{k}_b = \bar{k}_c$ is valid. The mean output is statistically the same and therefore the faecal coliform die-off rate constant is independent of detention time in this test.

(b) **Testing effect of pond depth on the mean k values (set A and set B).**

To test the hypothesis that $\bar{k}_a = \bar{k}_b$ using statistical values in table 2 equation A1, to was found to be 3.35. Using student t-distribution tables with $n_a + n_b - 2 = 24$ degrees of freedom, the critical values were found to be $C_1 = -2.06$ and $C_2 = +2.06$. The hypothesis that $\bar{k}_a = \bar{k}_b$ is rejected since t_o was found to be outside the range. The mean faecal coliform die-off rate constant therefore significantly varies with pond depth.

Appendix 5 Typical variation of temperature, pH and
dissolved oxygen with pond depth in each cell.

(All measurement taken on 22/12/87 from 9.15 am. to 10.15
am.)

Pond cell	Depth below water surface (m)	Temperature (°C)	pH	Dissolved oxygen (mg/l)
A1	0	27.7	7.8	5.7
	0.5	27.9	7.5	0.7
	1.0	28.0	7.7	0.6
	1.5	27.8	7.6	0.6
A1	0	32.5	9.2	>15
	0.5	28.4	8.2	2.1
	1.0	28.0	8.1	1.0
	1.5	27.9	8.0	1.2
B1	0	32.0	8.6	14.0
	0.5	27.7	7.7	0.7
	1.0	27.6	7.7	0.7
B2	0	33.0	9.9	>15
	0.5	27.7	8.3	2.3
	1.0	27.8	8.2	2.2
C1	0	32.4	8.0	4.0
	0.5	28.4	7.7	1.3
	1.0	27.8	7.7	0.4
C2	0	32.6	9.0	>15
	0.5	28.5	8.3	9.0
	1.0	28.2	8.0	3.6

Appendix 6 Typical diurnal variation of temperature, pH and dissolved oxygen at the pond surface with time.

(Measurements taken from 18.00 hr on 15/12/87 to 18.00 hr on 16/12/87.)

Pond cell	Date	Time (hr)	pH	Dissolved oxygen (mg/l)	Temperature (°C)
	15/12	18.00	8.6	9.8	31.8
		21.00	7.8	0.7	29.0
		24.00	7.7	0.2	27.9
A1	16/12	3.00	7.6	0.1	27.2
		6.00	7.5	0.1	26.8
		9.00	8.0	3.1	30.6
		12.00	8.3	5.9	34.6
		15.00	8.8	8.4	35.7
		18.00	8.6	8.0	31.3
	15/12	18.00	9.5	>15	31.7
		21.00	8.7	2.2	29.1
		24.00	8.5	0.3	28.0
A2	16/12	3.00	8.3	0.3	27.2
		6.00	8.2	0.3	26.8
		9.00	8.9	>15	30.8
		12.00	9.4	>15	34.7
		15.00	10.5	>15	35.9
		18.00	9.9	>15	31.4
	15/12	18.00	8.5	12.1	31.5
		21.00	8.0	0.9	28.7
		24.00	7.8	0.2	27.7
B1	16/12	3.00	7.7	0.1	26.7
		6.00	7.6	0.1	26.4
		9.00	8.2	3.8	30.7
		12.00	8.5	6.9	34.6
		15.00	9.1	9.1	36.0
		18.00	8.7	9.0	31.8
	15/12	18.00	9.3	>15	31.3
		21.00	8.9	1.9	28.6
		24.00	8.6	0.2	27.4
B2	16/12	3.00	8.4	0.2	26.6
		6.00	8.3	0.3	26.2
		9.00	9.2	>15	30.4
		12.00	9.5	>15	34.7
		15.00	10.6	>15	36.0
		18.00	9.8	>15	31.9

	15/12	18.00	7.8	8.1	31.4
		21.00	7.4	0.3	28.7
		24.00	7.3	0.1	27.5
C1	16/12	3.00	7.3	0	26.6
		6.00	7.2	0	26.1
		9.00	7.6	1.1	29.4
		12.00	8.3	4.5	34.5
		15.00	8.7	6.9	36.1
		18.00	7.9	3.9	31.6

	15/12	18.00	8.6	12.0	31.6
		21.00	8.2	0.4	28.7
		24.00	8.0	0.4	27.6
C2	16/12	3.00	7.9	0.3	26.8
		6.00	7.9	0.3	26.5
		9.00	8.3	10.0	29.5
		12.00	9.2	>15	34.4
		15.00	9.5	>15	35.9
		18.00	8.9	14.2	31.2

Appendix 7 Typical diurnal variation of pH and carbondioxide concentration at the pond surface (samples taken on 24/12/87).

Sampling place	Sampling time (hr)	pH	CO_2 (mg/l)
Cell A2 surface	6.00	7.8	68
	9.00	8.9	12
	12.00	9.4	0
	15.00	10.2	0
	18.00	9.1	0
	21.00	8.6	20
Cell B2 surface	6.00	8.0	56
	9.00	9.1	4
	12.00	9.6	0
	15.00	10.5	0
	18.00	9.2	0
	21.00	8.7	16
Cell C2 surface	6.00	7.7	64
	9.00	8.6	24
	12.00	8.8	8
	15.00	9.4	0
	18.00	8.9	0
	21.00	8.3	36