THE DYNAMICS OF ALGAL GROWTH, SUBSTRATE CONSUMPTION, OXYGEN PRODUCTION/CONSUMPTION IN BATCH BENCH-SCALE COMPLETELY MIXED AQUATIC ECOSYSTEMS.

BY

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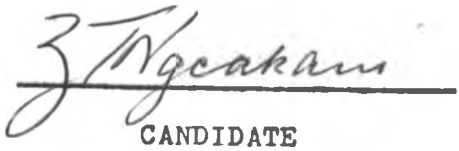
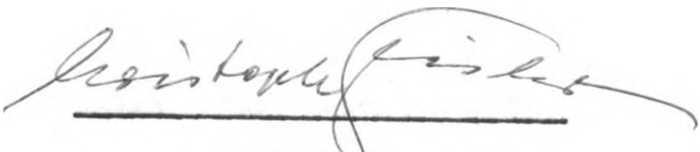
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.



CANDIDATE

This thesis has been submitted for examination with my approval as University Supervisor.



SUPERVISOR

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NOTATION

The following symbols and abbreviations are employed. Symbols not included herein have been defined as they appear in the text. Some symbols have different meaning in different sections and they have been clearly defined.

General Symbols

ALK	Alkalinity as Calcium Carbonate (mg/l)
ATP	Adenosine Triphosphate
BOD	Biochemical Oxygen demand (mg/l)
Chlor.	Chlorophyll concentration (mg/l)
COD	Chemical Oxygen demand (mg/l)
CODF	Chemical Oxygen demand of the filtered samples (mg/l)
CODU	Chemical Oxygen demand of the unfiltered samples (mg/l)
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen concentration (mg/l)
EOP	Embakasi Oxidation Pond liquor
EQ.CO_2	Equilibrium Carbon Dioxide concentration (mg/l)
G.M.	Growth medium (i.e. mixture of Embakasi oxidation pond liquor or bacterial-algal seed prepared in the laboratory and synthetic sewage)
MPN	Most Probable Number(s)
PARAM	Parameter(s)
R^+	Resistance factors
TTC	Triphenyl Tetrazolium chloride
TF	Tetrazolium formazan

Temp^{o}C	Temperature
WHO	World Health Organisation
% Sat.	Percent saturation.

SUMMARY

Ecosystems composed of algae, bacteria, and other biota occur widely in lakes, rivers, impoundments, estuaries and waste stabilisation ponds. They enhance under favourable conditions, the self-purification capacity of the water bodies in which they occur, by degrading and assimilating organic and nutrient inputs. Algae and bacteria perform critical functions, which may be beneficial or harmful to water quality, in several areas of water resources management and water pollution control. A proper understanding of the complex and interrelated physical, chemical, biological and environmental factors affecting the algal photosynthetic oxygenation of the water bodies and the bacterial degradation of organic inputs is necessary for the development of a picture of the biological reactor environment as a predictable functional ecosystem. A study of the dynamics of algal growth, substrate consumption, oxygen production/consumption was made in model, batch, completely mixed reactors.

Two types of batch reactors designed to operate as completely mixed closed systems were used during the study. An environment characterised by constant light intensity, similar mixing conditions and reasonably constant temperatures was chosen for the study. The growth reactors were artificially illuminated. Nine experiments were performed using two types of synthetic sewage as growth mediums. All the experiments maintained aerobic conditions except experiment six. Operation of the biomass growth reactors was monitored by analysing samples for dissolved oxygen, chemical oxygen demand, chlorophyll,

bacterial numbers and carbon dioxide. Measurements of hydrogen concentration, temperature and microscopic examinations for identification of algae species in the samples were carried out.

The behaviour of the studied completely mixed biomass growth reactors showed that the relationships between aerobic bacterial growth, substrate consumption, algal growth, oxygen production/consumption may be characterised as coupled and inter-dependent in nature. The reaction sequences of aerobic bacterial growth, substrate-consumption-oxygen consumption on the one hand and algal growth - oxygen production/consumption on the other hand were linked and the intensity of each response was influenced by that of the preceding component of the system. Symbiotic relationships were established but were affected by changing bacterial and algal active phases brought about by food and nutrient availability, as well as by prey-predator interactions.

INTRODUCTION

Ecosystems composed of algae in association with bacteria and other biota are found widely distributed in the aquatic environment. These systems occur in water bodies such as lakes, rivers, impoundments, estuaries, oceans etc. In these aquatic environments, under natural conditions, they are an important part of the biological food chain and perform beneficial functions such as the production, assimilation and recycling of organic matter and nutrients, as well as enhance the purification capacity of the water bodies in which they occur. Such water resources systems may thus serve as giant reactors for the biological cycle in which bacteria oxidise or mineralise organic matter to liberate nutrients and algae reduce minerals to support other life. However, under certain conditions, activities of man may upset the beneficial balance existing amongst algae, bacteria, and other biota and give rise to environmental problems such as eutrophication of lakes and undermine the natural self purification capacities of rivers, impoundments, estuaries etc. Such an imbalance, in general, may be manifested by excessive growths of algae and/or bacteria, deoxygenation of the water bodies, fish-kills etc, resulting in the impairment of the water quality and the utility of the water resource.

The solution of many problems associated with waste water management and treatment, river pollution, eutrophication of lakes, self-purification of impoundments and reservoirs etc, require a proper understanding of the role played by algae and bacteria in the natural purification

processes taking place in these aquatic environments. The above mentioned ecosystems have also found application as engineered biological treatment systems for stabilisation of domestic, industrial and agricultural waste-waters and are exemplified by waste stabilisation ponds. In essence, the waste stabilisation pond simulates the overall purifying activity of the natural environment in a single process and under more concentrated and, to a degree, regulated conditions.

The aquatic environments of lakes, rivers impoundments, waste stabilisation ponds etc are different from each other. For example, the aquatic environment of a flowing river is vastly different from that of an impounded body of water. However, factors and interrelationships affecting the growth of algae, bacteria and other biota, factors affecting production, assimilation and recycling of organic matter and nutrients in these water bodies are basically the same (Rawson, 1939), and therefore, under certain conditions, the behaviour of one water body may be comparable to the behaviour of another water body.

1.1 WASTE STABILISATION PONDS

Ponds have been used for centuries to store and treat animal and house-hold wastes; however, it is only within the last twenty-five years that specific design criteria have been developed in terms of volumetric requirements, organic loading rates and detention periods. Practical experience

with stabilisation ponds has established their suitability for use under all types of environmental conditions. Pond systems are used under cold, temperate and tropical conditions. A world Health Organisation (WHO) world-wide survey showed that waste stabilisation ponds are in use in at least thirty-nine countries spread over the Americas, Europe, Africa, the Middle East, Asia and the Western Pacific. (Gloyna, 1971).

Today stabilisation ponds play an important role in water pollution abatement and water resources management programmes of developing countries and constitute an important barrier against the hazards of water-borne diseases.

1.1.1 DEFINITION OF WASTE STABILISATION PONDS

A waste stabilisation pond can be defined as a shallow man-made basin utilising natural processes, under partially controlled conditions, for the oxidation of organic matter and the reduction and elimination of pathogenic organisms in waste-waters.

Stabilisation ponds are on the first step of the ladder of control of biological degradation processes. At the top, are processes such as activated sludge, thermophilic completely mixed anaerobic digestion, trickling filters etc, in which the environmental and physical factors influencing the process are almost completely controlled. At the bottom are stabilisation ponds, in which the environment is virtually beyond the control of the designer. Normally only the physical parameters such as depth and loading can be controlled to a degree. As wide fluctuations in the environment are natural,

the pond design must be such that the organic degradation and pathogens reducing processes will operate satisfactorily within a range of expected environmental conditions. Extreme conditions beyond the limits provided for in the design will occasionally and invariably occur, and the pond will show signs of over-loading or fail temporarily. The designer, after a study of the physical, biochemical and environmental factors which influence pond behaviour, can, within the economic means available, the treatment objectives envisaged and the engineering constraints imposed, only endeavour to provide those conditions which will beneficially influence the behaviour of the pond such that it is of maximum usefulness to the communities it serves.

1.1.2 CLASSIFICATION OF WASTE STABILISATION PONDS

There are several types of waste stabilisation ponds, the nomenclature of which is varied and sometimes confusing. Terms used in literature include sewage lagoons, oxidation ponds, redox ponds, maturation ponds, facultative lagoons, anaerobic lagoons, aerobic lagoons and mechanically assisted ponds. For our purposes, the term waste stabilisation ponds will be used to describe any pond or pond system designed for biological waste treatment.

Waste stabilisation ponds can be divided into the following types (see fig. 1.1).

1.1.2.1 ANAEROBIC PRETREATMENT UNITS

Anaerobic pretreatment units are ponds in which the

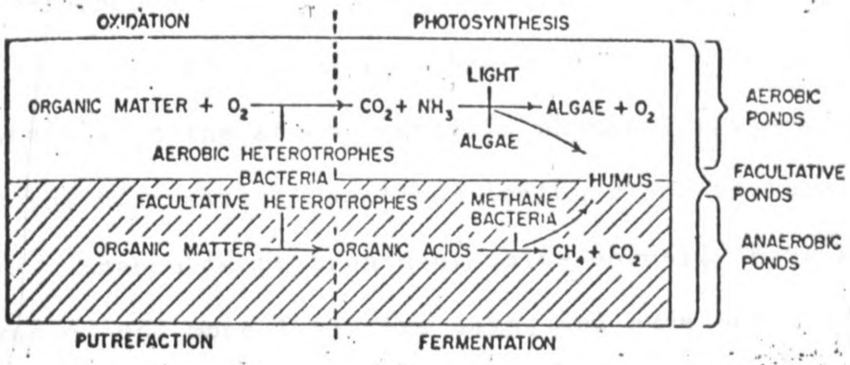


FIG. I.I BIOLOGICAL ACTIVITIES AND RELATED CLASSIFICATIONS FOR WASTE STABILISATION PONDS (AFTER OSWALD, 1963)

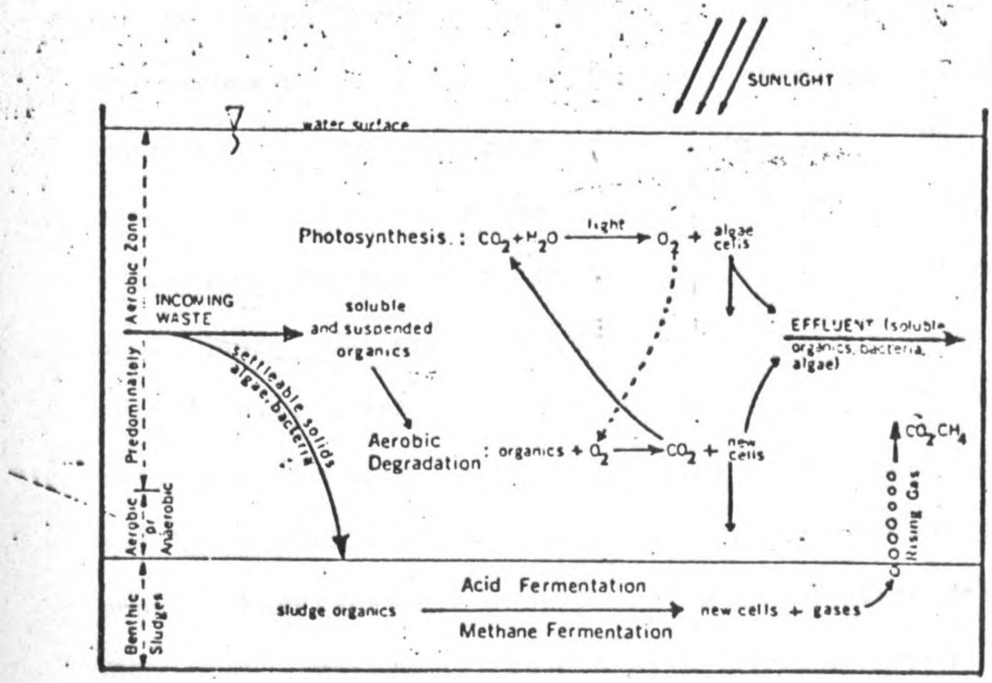


FIG. I.2. ECOLOGICAL CHARACTERISATION OF AN OXIDATION POND (AFTER HENDRICKS AND POTE, 1974)

whole pond content is anaerobic, that is, the pond mass is devoid of dissolved oxygen. Included amongst these are septic tanks, aqua-privies and pretreatment tanks. They differ from anaerobic ponds only in that an artificial cover is provided to shield the anaerobic mass from contact with the atmosphere; the physical environment and the biochemical reactions taking place in them is the same. Anaerobic pretreatment units normally have retention times of not more than five days, and have one characteristic in common; and that is, their effluent requires further treatment to render it suitable for discharge to receiving water courses.

1.1.2.2 FACULTATIVE PONDS

Facultative ponds are ponds in which the pond liquid content is aerobically-anaerobically degraded and the bottom sludge layer anaerobically stabilised. The bulk of the oxygen necessary for aerobic oxidation processes is provided by algal photosynthesis. Thus oxygen is only generated to the depth the radiation of the sun can penetrate. Distribution of the oxygen throughout the pond liquid is largely influenced by physico-environmental factors e.g. thermal stratification, wind conditions, baffles etc, which inhibit or promote mixing of the pond liquid and the distribution of the algal mass throughout the photic zone. Therefore as a result of the alternating aerobic and anaerobic environment, facultative organisms predominate in the liquid mass of the pond.

A well defined sludge-layer is found on the bottom

of a facultative pond. This sludge layer is brought about by settlement of suspended organic and inorganic material under the quiescent conditions normally obtaining in the liquid mass above. The internal mass of the sludge layer is permanently anaerobic and the majority of the organisms in this layer are probably obligate anaerobes. The sludge layer exerts a significant influence on the behaviour of the pond, (Oswald, 1964; Marais, 1966) and is reported to account for the destruction of thirty percent of the influent 5 day Biochemical oxygen demand (Marais, 1970).

Facultative ponds are by far the most common type of waste stabilisation ponds in the world and are often called oxidation ponds.

1.1.2.3 HIGH RATE AEROBIC PONDS

High rate aerobic ponds are waste stabilisation ponds which operate on the principle of minimum depth with maximal algal production. In these ponds, the depth of the liquid is limited to 0.30 - 0.46 m and the retention times from 1 - 3 days. The shallow depths permit sunlight radiation to penetrate throughout the mass of the liquid. The entire contents, including the settled material are periodically mixed by mechanical means. High concentrations of algae develop and their photosynthetic action produces oxygen in quantities exceeding the applied BOD of the waste (Oswald et al, 1957). The nutrient in the organic pollution is substantially converted to algal cell material which must then be separated from the liquid if the organic content of

the effluent is to be reduced.

The high rate pond process is essentially a carbon conversion process (Marais, 1970) and it requires close control. This latter requirement makes it unsuitable for small communities with inadequate technological facilities.

1.1.2.4 MATURATION PONDS

Maturation ponds are used to treat effluents from conventional sewage facilities and as the last stages in a series of waste stabilisation ponds. They serve to "polish" or "mature" the effluent by reducing settleable solids and faecal organisms; Biochemical oxygen demand is reduced to a small extent only. The success of maturation ponds as a buffer against environmental bacterial pollution is such that Stander and Meiring (1962) states:-

> "although a faecal E. Coli count of nil per 100ml cannot always be obtained in maturation ponds, the degree of safety (as indicated by faecal E. Coli count) that can be obtained is comparable with that obtainable in practise where sand filtered effluent is chlorinated".

Maturation pond effluent has been used to produce water for supplementing domestic supplies (Cillie et al, 1966). The reclamation plant relies on algal activity in the maturation ponds to reduce ammonia (NH_3 - N) concentrations in the intake water to acceptable levels for subsequent break-point chlorination of the product water. The ponds remove more than 90% of the influent ammonia (NH_3 - N) under normal summer conditions (van der Post et al, 1973).

Generally, maturation ponds modify the effluent in such a way that no fungus and filamentous growth develop in the receiving water course, even if there is little or no stream flow.

1.1.2.5 MECHANICALLY-ASSISTED PONDS

Mechanically assisted ponds are ponds in which mechanical means are employed to achieve intrapond or inter pond recirculation in order to provide adequate reaeration of the pond contents (Wannstrom, 1955), or help to destroy thermal stratification in the pond thereby inducing better mixing conditions (Abbott, 1962; Marais, 1970).

In intrapond recirculation, the liquid is discharged to a furrow or over packed stones and gravitates back to the pond; whereas in interpond recirculation the liquid is recycled from a secondary pond in a series to the primary pond. However, its main objective is to reduce the load on the primary pond by "washing out" some of the organic load to the secondary pond. In systems where the primary pond is anaerobic, interpond recirculation is principally used to reduce or completely suppress odour development (Abbott, 1962; van Eck, 1965).

1.1.2.6 AERATED PONDS

Some consider aerated lagoons as not strictly falling under waste stabilisation ponds. However, the theory of facultative ponds can be applied to aerated lagoons with little modification.

For stabilisation of organic matter in aerated lagoons, the oxygen required is virtually all supplied by surface mechanical or bubble aeration. During operation, all the settleable solids in the pond are kept in suspension if the input of energy during aeration is sufficiently high. Under these conditions, algal growth is absent or greatly reduced, due to the violent agitation of the pond liquid and high turbidity. High oxygen concentrations (up to 6 mg/l) may be attained in the pond, so that the oxygen transfer rate, which is proportional to the difference between the actual and saturated oxygen concentrations per unit energy input, is relatively low. The input energy is therefore inefficiently utilised for this purpose. On the otherhand, reducing the energy input reduces the oxygen concentration in the pond, but increases the transfer rate per unit energy input. However, the agitation may now be insufficient to keep all the settleable solids in suspension and a sludge layer is formed on the bottom of the pond. This layer decomposes anaerobically as in the facultative pond.

In aerated ponds, algal oxygenation of the pond is completely dispensed with and the pond becomes permanently dependent on oxygen introduced by artificial means. Although the rate of introduction of oxygen is now controlled, this benefit is achieved at the cost of increased control.

The various types of ponds listed above provide the designer with the armamentarium from which to build an intergrated pond system capable of fulfilling treatment objectives within the economic means and technological

development of the community to be served.

1.1.3 POND SYSTEMS LAY-OUT

Waste stabilisation pond systems may comprise one facultative pond only or a combination of several types of ponds either in series operation and/or parallel operation; with or without interpond or intrapond recirculation. For example, facultative, anaerobic, aerated etc ponds may be designed to operate singly or in parallel as shown in figure 1.3. If a higher degree of treatment is desired, a maturation pond might be added beyond the facultative pond.

The choice of pond systems lay-out is governed by many factors, included amongst which are:-

1. Types of wastes and their characteristics i.e. domestic, industrial, strength, organic and inorganic content, amount etc.
2. Environmental and climatic conditions i.e. Geology, hydrology and meteorology of the region; tropical, temperate or actic regions; arid or wet regions etc.
3. Treatment objectives i.e. effluent standards to be met etc.
4. Resources management objectives
5. Aesthetic considerations - odour problems etc.
6. Public Health considerations (insect problems, disease transmission problems)
7. Local zoning restrictions, economic and engineering considerations.

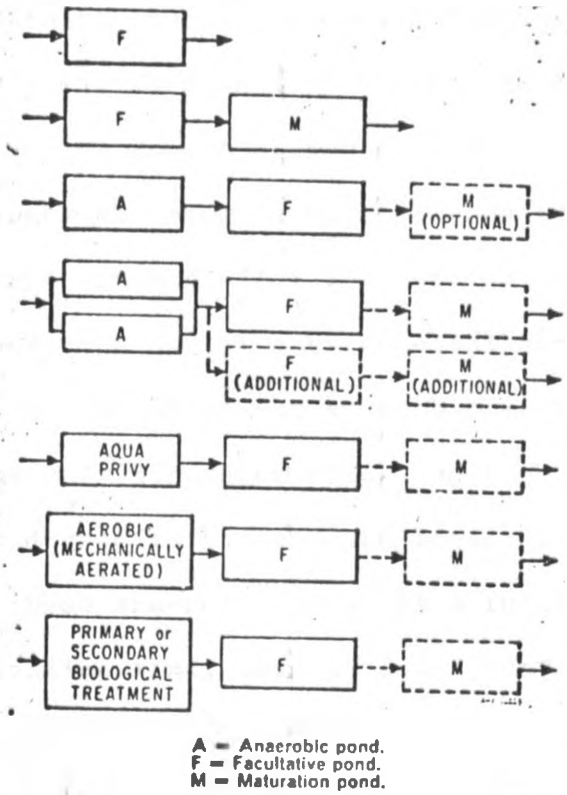


FIG. I.3 TYPICAL WASTE STABILISATION POND S SYSTEMS. (GLOYNA, 1971)

growing interest and importance for developing countries the anaerobic-aerobic pond system.

1.1.3.1 ANAEROBIC-AEROBIC POND SYSTEMS

Anaerobic-aerobic pond systems have been used to treat estic, industrial and municipal wastes (Parker et al, 1959 lo, 1960; Abbott, 1962; Coerver, 1964; Van Eck, 1965). anaerobic pond, used in this manner, is employed to uce the greatest amount of waste in as small a space as sible, thereby allowing low-cost lagoon treatment to be d where large areas of flat land are not available. In erobic ponds, the strict environmental control usually sidered to be essential for anaerobic treatment is not sible. The only parameter which can be controlled to ignificant degree is the organic loading. The BOD uction in these anaerobic ponds is a function of:-

1. Retention time, usually 2 to 5 days
2. Temperature
3. Quantity of sludge
4. Waste characteristics

ur problems arising from the formation of sulphides in anaerobic ponds can be solved by recirculation from the obic ponds, into which the anaerobic ponds discharge, the rate of 13% to 40% of the influent flow.

The fermentation rate is greatly affected by tempe- ure which causes a build-up of sludge in winter and a rease in summer. For pond temperatures above 20°C, reduction in 5-day BOD is reported as being of a higher er than that obtained in a Dortmund sedimentation tank

receiving raw sewage (45.5% reduction -van Eck, I965) At temperatures above 25°C removals of over 85% 5-day BOD have been recorded (van Eck, I965). During winter the efficiency of BOD removal decreases on occassion to zero and a thick scum forms providing a layer for fly breeding. Anaerobic ponds are reported to be efficient reducers of coliform bacteria. Ten fold decreases in faecal coliform numbers in two parallel anaerobic ponds, having retention times of 4 days each, were observed at Nakuru, Kenya (Mara I972). Reductions of faecal coliform numbers of 72-80% have also been reported (Van Eck et al I966). Field tests in Texas, have also shown that the use of anaerobic ponds significantly improves the die-off of faecal bacteria (Davis et al, I972).

The use of anaerobic-aerobic systems (Septic tanks, aqua privies discharging into oxidation ponds) in Zambia is said to be more than a method of partial stabilisation. It is a vital factor in the provision of a system of sanitation for small communities (Marais, I966). This is so because in general:-

I. Conventional water-borne systems with conventio treatment are expensive to install, maintain an operate.

2. Soakaways fail to function in areas of unsuitab soil conditions and high water tables.

3. Aqua-privies fail to function satisfactorily wh the water-seal is not maintained by daily manua

4. Pit latrines have a short life and in high density housing areas, space for relocation is limited. Difficulties are experienced in sinking holes in hard laterite, in rocky areas and where the water table is high.
5. Bucket systems require supervision of a high order, if standards of health are to be maintained and labour is difficult to recruit and retain.

The system evolved in Zambia incorporates the use of the aqua-privy or septic tank for each house-hold or group of house-holds and the discharge of the tank effluent into oxidation ponds. The tanks retain all the inorganic solids and large indigestible organic solids. Anaerobic fermentation changes the digestible organic material to a finely dispersed state. The self-cleansing velocity for this effluent in a sewer can be reduced to 0.30 m/s and the lower sewer gradients thus possible virtually eliminate the need for pumping where the land is flat. This system ensures that the water seal in the aqua-privies is automatically maintained as all the household wastes are discharged into the tank and also the need for soakaways is dispensed with.

Anaerobic-aerobic lagoon systems have a promising future for incorporation into low-cost pollution abatement programmes especially in developing countries for treatment of domestic, industrial and municipal waste waters. The High BOD removals attained in anaerobic ponds enables considerable economies of land to be achieved in the design

of facultative ponds receiving settled sewage; eliminates problems of rising sludge in the coupled aerobic pond and greatly enhances the removal of faecal bacteria.

1.1.3.2 FACULTATIVE - AEROBIC SYSTEMS

The term facultative-aerobic systems is used for convenience only because these systems may describe advance treatment systems in which ponds are used for tertiary treatment or pond systems in which facultative ponds are followed by maturation ponds. The incorporation of stabilisation ponds into conventional treatment systems may serve the following purposes:-

1. to improve the effluent quality of the treatment facilities so that it conforms with the stringent standards set by regulatory bodies.
2. to relieve overloaded treatment works thereby increasing their capacity.
3. to modify the quality of the effluent by reducing the ammonia (NH_3-N) concentrations and the microbial quality in cases where part of the effluent is used to supplement drinking water (Van der Post 1973).

1.1.4 POND PROCESS DESIGN

1.1.4.1 ANAEROBIC PRETREATMENT UNITS

Anaerobic pretreatment units have a high volumetric 5-day BOD loading (greater than 100 gms/m^3/day); and are

best suited for wastes with strength in excess of 500 mg 5-day BOD in climates where temperatures are above 15°C. Odour release is negligible in ponds receiving domestic sewage at a volumetric loading less than 400 gms/m^3/days (Meiring et al, 1968).

1. Volumetric loading L_v is given by

$$L_v = L_i \, Q/V \qquad (1.4)$$

where, L_v = Volumetric loading, gms/m^3/day

L_i = Influent BOD of wastes, gms/m^3

Q = Daily flow, m^3/day

V =, Pond volume, m^3

2. Therefore time t, is given by

$$t = \frac{V}{Q} \text{ days} \qquad (1.5)$$

3. Therefore substituting for Q/V in equation (1.4) we have

$$L = L_i/t, \text{ gms/m}^3\text{/day} \qquad (1.6)$$

The design of the anaerobic pond is on the basis of the retention time that gives the required BOD removal. Mara (1973) suggested the following design values based on actual results obtained from anaerobic, ponds in India, South Africa, Australia and Southern United States of America and are valid for temperatures 20-25°C.

50% reduction after one day

60% reduction after two and half days

70% reduction after five days.

Once the retention time is chosen then the mid-depth area is given by:-

$$A = \frac{Qt}{D} \qquad (1.7)$$

where D = depth of the pond m.

Recommended depths are from 2 - 4 m. The depth is not too important a criteria for design in anaerobic lagoons except as it may affect heat loss. It is generally agreed that it is desirable to keep the waste at as high a temperature as possible and where lagoons are being constructed in cold climates this can be a very important factor. Generally the deeper the lagoon the better, consistent with economics.

1.1.4.2 FACULTATIVE PONDS

There are basically four approaches to the design of facultative ponds:-

(a) Gloyna's empirical procedure

(b) Procedures based on equating BOD removal with solar radiation

(c) Procedures based on the assumption that BOD removal follows first order kinetics.

(d) AIT empirical procedure

(a) Hermann and Gloyna (1958) on the basis of results from a series of model oxidation ponds established that the retention time for the series, to give a fixed reduction of 5-day BOD of 80-90% at the pond optimum temperature

($35^{o}C$) was 3.5 days. At any other temperature T, the retention time R_T was given by:-

$$\frac{R_T}{R_{35}} = \theta^{(35-T)} \qquad (1.8)$$

where R_{35} = Retention time for 90% reduction at $35^{o}C$

R_T = Retention time for 90% reduction at $T^{o}C$

θ = Arrhenius constant equal to 1.072

Then the design procedure is as follows:-

1. The minimum mean monthly temperature for the year is substituted for T as this gives the largest pond area.
2. The influent average 5-day BOD in U.S.A. is taken as approximately 200 mg/l, hence 90% reduction gives an effluent quality approximately 20 mg/l during the coldest month. Then in order to keep the effluent value approximately constant for any influent 5-day BOD value P, the retention time is adjusted in the ratio

$$t = P/200 \qquad (1.9)$$

Then the mid-depth area is

$$A = \frac{Qt}{D} \qquad (1.10)$$

where D = depth of facultative pond (1.0 - 1.5 m)

A = mid-depth area, m^2

Q = waste flow m^3/day

t = Retention time, adjusted, days

Different values of R_{35} have been reported

3.5 days (Hermann and Gloyna, 1958)

7.5 days (Marais, 1966)

7 days (Huang and Gloyna, 1968)

Gloyna (1971) recommends a value R_{35} = 7 days θ = 1.085 and that the 5-day BOD value P in equation (1.9) should be understood as the ultimate BOD i.e. 1.5 times the 5-day BOD if the influent is raw sewage. The 5-day BOD value may be used if the influent is settled sewage.

The disadvantages of this procedure which really preclude its use are:-

(a) the uncertainty of the value R_{35}; this is critical since, for any combination of waste flow, waste 5-day BOD and temperature it controls the size of the lagoon.

(b) the ratio P/200 limits the application of the procedure to those wastes whose 5-day BOD is a "proximal deviation" from 200 mg/l (Gloyna 1968).

(d) There is no justification for using the ultimate BOD in some cases and the 5-day BOD in others.

(b) Procedures based on solar energy equate the daily BOD removal in a lagoon with daily production of oxygen by the lagoon algae; which is in turn based on the intensity of solar radiation on the lagoon (Oswald and Gotaas, 1957;

Jayangondar et al, 1970). The procedures developed are not satisfactory since they imply that all the oxygen produced by the algae is available for stabilisation of the influent waste and the solar energy constants developed in the theory vary from place to place.

(c) The procedure that has been used in Tropical Africa and Southern Africa is based on first order kinetics. Marais and Shaw (1961), noting that Gloyna's emperical procedure implies that during the summer the 5-day BOD value of the effluent should be much less than in the winter and observing the lack of variation of effluent 5-day BOD, proposed a kinetic model based on first order kinetics in a continuously stirred reactor; with the reaction constant independent of temperature.

The following assumptions were made:-

1. Complete and instantaneous mixing of influent with pond contents, hence effluent BOD equals pond BOD.
2. Degradation is according to first order reaction with the degradation constant independent of temperature and retention time.
3. No pollution losses due to seepage.
4. No settlement of influent BOD as sludge.

Let L_i = Influent 5-day BOD, mg/l

L_e = Pond or effluent 5-day BOD, mg/l

Q_i, Q_e = Influent and effluent flows per day, m^3/day

V = Volume of pond, m^3

R_i , R_e = Influent and effluent retention times, in days and defined as V/Q_i and V/Q_e respectively.

K = first order degradation constant, $days^{-1}$

Then the degradation process is defined by the following differential equation.

change in pond BOD = Influent BOD - degradation - Effluent pond BOD

$$\frac{dL}{dt} = \frac{L_i Q_i}{V} - KL - \frac{LQ_e}{V} \qquad (1.11)$$

$$= \frac{L_i Q_i}{V} - L\left(K + \frac{Q_e}{V}\right)$$

since $R_i = \frac{V}{Q_i}$ and $R_e = \frac{V}{Q_e}$

Then $$\frac{dL}{dt} = \frac{L_i}{R_i} - \left(K + \frac{1}{R_e}\right) \qquad (1.12)$$

Under equilibrium conditions with L_1, R_i and R_e constant

$$\frac{dL}{dt} = 0 \quad \text{and}$$

$$L = \frac{L_i}{KR_i + \frac{R_i}{R_e}} \qquad (1.13)$$

If seepage and evaporation losses are neglicted $R_e = R_i = R$ and equation (1.13) reduces to:-

$$L = \frac{L_i}{KR + 1} \qquad (1.14)$$

The ratio R_i / R_e ranges from zero to one depending on liquid losses but can be taken as equal to one with little error.

In short retention ponds $R_i/R_e = 1$, losses small.
In long retention ponds $KR_i >>> R_i/R_e$, influence of R_i / R_e small.

The maximum value of effluent 5-day BOD consistent with the maintenance of predominantly aerobic conditions was found by Marais and Shaw (1961) to be related to the lagoon depth

$$L_e = \frac{1000}{2D + 8} \qquad (I.15)$$

For purposes of design the numerator in equation was reduced to 750 (Marais and Shaw, 1961)
600 (Meiring et al, 1968)
700 (Marais, 1970)

For design purposes the value K, which was found to be 0.23 day^{-1} for Southern Africa, was reduced to 0.17 day^{-1}.

Therefore the design procedure is to:-

1. Determine R from equation 1.14 using a modification of equation 1.14 and K = 0.17 day^{-1}
2. Calculate the mid-depth area from

$$A = \frac{QR}{D}, \quad D = 1 - 1.5 \text{ m} \qquad (1.16)$$

Marais (1966) modified equation 1.13 by permitting K to vary with temperature. The equation recommended was

$$K_T = K_{35}(1.085)^{35-T} \qquad (1.17)$$

The value of K at $35^{\circ}C$ was 1.2 day^{-1}

The values of K as given by equation (1.17) are rather too high. Mara (1973) suggested a rather more conservative equation for design purposes and proposed

$$K_T = 0.30(1.05)^{T-20} \qquad (1.18)$$

taking the effluent 5-day BOD as a fixed value of 60 mg/l. Marais (1970) developed the theory further incorporating the effect of the sludge layer.

1.1.4.3 MATURATION PONDS

The design of maturation ponds is based on retention time so as to produce an acceptable effluent. For most purposes a 5-day BOD of less than 25 mg/l and a faecal coliform count below 5000 per 100 ml.

Marais and Shaw (1961) found that in order to achieve a 5-day BOD of less than 25 mg/l it was necessary to provide two maturation ponds in series, each designed on a retention time of 7 days.

Then the mid-depth area is given by

$$A = \frac{Qt}{D} \qquad (1.18)$$

where A = mid-depth area, m^2

Q = influent flow m^3/day

D = depth m (usually 1.5m)

The detention time of 7 days assumes that the influent to the first lagoon i.e. the effluent from the facultative lagoon, has a 5-day BOD of 75-50 mg/l.

A kinetic model for the reduction of faecal bacteria in stabilisation ponds was presented by Marais and Shaw (1961). The theory was based on first-order kinetics with a constant value for the specific death rate. In 1966 and 1970 and 1974 Marais extended it to incorporate the effect of anaerobic conditions and temperature on the specific death rate. Assuming instantaneous and complete mixing conditions and that the reduction of bacteria takes place according to Chick's Law.

$$\frac{dN}{dt} = -KN \qquad (1.19)$$

where t = time in days

N = concentration of faecal organisms per unit volume.

K = decay constant dependent on temperature (day^{-1})

and postulating a relationship between K and temperature to be

$$K_T = K_{20}\,\theta^{T-20} \qquad (1.20)$$

in which K_T and K_{20} = decay constant at T^oC and 20^oC respectively: θ = constant (K_{20} = 2.6 and = 1.19)

He found that the reduction of bacteria in a pond can be described by the equation for a single pond

$$N = \frac{N_o}{KR + 1} \qquad (1.21)$$

and

$$N_n = \frac{N_o}{(KR + 1)^n} \qquad (1.22)$$

for an n series of ponds.

where R = retention time based on influent flow.

In plug-flow conditions the effluent quality is given by

$$N = N_o e^{-Kt} \qquad (1.23)$$

Per unit of retention time the plug flow is the most efficient, the series pond intermediate, and the single pond the least efficient. The efficiency of the series increases as the number of ponds increase for a fixed retention time. From practical considerations, up to 90% reduction a single pond is normally adequate; up to 99% two ponds in series, up to 99.9% three ponds in series etc. Plug flow conditions are difficult to achieve so that the series system is the most practical (Marais 1974)

1.1.5 PUBLIC HEALTH ASPECTS

In the past decade, research work has been carried out directed at determining the ability of waste treatment systems - including the algal-bacterial treatment system to remove or inactivate enteric viruses (Christie 1967; Coetzee

et al 1965; Potten, 1972; Slanetz et al, 1970; Sobsey et al 1973).

On the whole, it has been found that these systems are able to reduce enteric bacteria extensively. The mechanisms in operation and causative agents of removal and inactivation have also been postulated and these range from unfavourable environmental conditions, settlement, adsorptive processes on suspended matter, anti-bacterial agents such as ultra-violet light from the sun, to extracellular toxins produced by the algae. There is, however, a growing body of literature and research, that indicates that as a result of the increasing use of drug therapy in the medical profession as well as in animal husbandry, the incidence of drug resistant bacteria may pose a potential environmental pollution problem and constitute a serious health hazard (Grabow et al 1973, 1974)

A current prominent type of resistance is mediated by resistance (R) factors (extrachromosomal nucleic acid elements) which may cause high level resistance to drugs. The R factor confer resistance to anti-bacterial agents like drugs (Davies et al, 1972), phages (Takano et al, 1968), mercury, nickel, and cobalt (Smith 1967, Summers et al, 1972), colicines (Siccardi, 1969) etc. R factors usually mediate resistance to high concentrations of as many as eight drugs, simultaneously (Watanabe 1963, 1971; Mikhael et al, 1972). One organism may carry varying numbers of the same or a combination of different R factors which may result in

both increased resistance and a wider spectrum of resistance (Farrar et al, 1972; Van Rensburg, 1972).

Thus R factors are transmissible among gram-negative bacteria such as enterobacteria including E.Coli and the pathogens salmonella typhi, shigella dysenteriae and other organisms like pseudomonas aeruginosa, aeromonas species and vibrio cholerae (Aoki et al, 1971; Bryan et al, 1972; Yokota et al, 1972). Ingestion of bacteria carrying R factors (R^+ bacteria) may result in the transfer of these factors to the normal intestinal flora. These organisms then act as reservoirs of resistance factors which they may transfer to sensitive bacteria involved in disease and thereby reduce the efficiency of anti-bacterial therapy (Richmond 1972; Watanabe 1971). Experiments carried out on some maturation ponds indicate an enrichment of the ponds with drug resistant coliforms (Grabow et al, 1973).

The health hazard posed by R factors is not restricted to drug resistance. They may enhance the infectivity and virulence of pathogens such as salmonella typhi and shigella species (Gangarosa et al, 1972, Thomas et al, 1972). Since there is increasing use of drug therapy in developing countries and most of the population still uses, for drinking purposes, untreated water, sometimes drawing their water not far from the outfalls of treatment plants, this phenomenon of drug resistance and survival poses a potential health hazard.

1.1.6 POND SYSTEMS ADVANTAGES

In the years since the first ponds for treatment of wastes were put into operation the application of the pond concept to a wide variety of waste treatment problems has occured. When the role of ponds in waste-treatment is considered, perhaps the most valuable contribution is found in the opportunity afforded to small communities, and to developing countries in general, to acquire a water carried sewage disposal system which provides generally excellent treatment. There appears to be no upper limit to the size of a community that can be served by ponds, except as economically limited by land costs. The size of any pond has finite limits, but with split flows and parallel operation, the largest of cities could use ponds.

Economically, the pond system is still the best in treatment facilities and costs per capita do not rise as sharply, as is the case with other treatment alternatives, with decreasing numbers of population served.

Simplicity of operation and ease of construction are the outstanding virtues of the pond method of treatment. Maintenance of dikes to preserve water-holding capacity of the ponds and eliminate mosquito breeding is a necessary item in operation along with seasonal draw-downs in colder regions to permit complete holding during winter months.

Waste stabilisation ponds form an effective means of pathogen removal and can better withstand hydraulic and organic shock loadings than other methods of sewage treatment. They can be used to remove certain toxic compounds present

in industrial waste. The algae formed are a potential source of protein which can be exploited by fish farming in maturation ponds. Lastly the land which is being used by stabilisation ponds could be easily reclaimed and used for other purposes should it become necessary.

I.2 OTHER WATER BODIES

As stated above (Section I.I) waste stabilisation ponds are engineered waste treatment systems that utilise the self purification processes that occur in natural water bodies. In the waste stabilisation pond the purification processes are purposely intensified in order to accomplish, in a brief period of time and in a small space, the changes normally brought about in nature only in extended periods of time and over long distances of travel or wide areas of dispersion.

Water is transferred to the earths atmosphere through evapo-transpiration of moisture from land surfaces, water surfaces, terrestrial and emergent aquatic plants. Precipitation as rain and snow returns the water to the land and sea. Overland and subsurface flows through lakes, and rivers to the sea complete the hydrological cycle.

Water during its passage over and through the ground from the clouds to the sea, is subjected to natural pollution and to cultural pollution by the manifold uses to which it is put by man. Nature, however, provides many and varied but closely interrelated and mutually dependent forces of purification. These forces rid water of most pollutants.

The proper husbanding of our water resources demands a more enlightened knowledge and understanding of the natural process of self purification of water bodies such as, lakes, rivers, impoundments and estuaries and their capacities.

1.2.1 LAKES

Lakes are natural or man-made inland basins containing large volumes of standing water. According to Fair et al, 1971, lakes are essentially closed communities in which foodstuffs are maintained or accumulated by circulating through the various trophic levels. They may be regarded as such because inflows and outflows are small relative to the size of the lake and hydraulic retention times are long. Although the concept of a lake as a closed community must be applied with care, it does permit the classification of these bodies of water according to their total productivity. The productivity levels of lakes are generally classified as oligotrophic, eutrophic and dystrophic.

Oligotrophic lakes are normally deep. They have a large and cold hypolimnion which contains little organic matter in suspension or on the bottom. Dissolved oxygen is found at all depths throughout the year. The plankton are quantitatively restricted. Algal blooms are rare, although algal species many.

Eutrophic lakes are often shallow, sometimes deep. Shallow lakes contain little, if any, cold water. There is much organic matter in suspension and on the bottom. Deep

stratified lakes that have become eutrophied contain little or no dissolved oxygen in the hypolimnion. The plankton is quantitatively abundant but varies in quality. Algal blooms are common.

Dystrophic lakes are usually shallow with abundant organic matter in suspension and on the bottom. In stratified dystrophic lakes dissolved oxygen is generally absent from deep waters. The plankton varies in composition and is usually low in numbers of species and in biomasses. Concentrations of calcium, phosphorus and nitrogen are small; concentrations of humic materials are large. Algal blooms are infrequent.

In the course of time natural and cultural pollution changes oligotrophic lakes into eutrophic lakes and eutrophic lakes eventually into dystrophic bodies of water.

When pollutants are discharged into a lake, a succession of changes in water quality takes place. If the pollutants are emptied into a lake, through an outfall, the pattern of changes occuring in the immediate vicinity of the outfall and away from it, vary in time and space, influenced by such factors as the nature of the currents that exist about the outfall, the season of the year and other factors which will be discussed in chapter two.

I.2.2 IMPOUNDMENTS

Impoundments are man-made basins containing large volumes of water. Their main function is to stabilise the flow of water either by regulating a varying supply in a natural stream or

by satisfying a varying demand of the ultimate consumers. Use of water from impoundments may be for irrigation purposes, generation of hydroelectric power, potable water supply, flood control etc. Like lakes impoundments may be considered as being essentially closed communities (Fair et al, 1971).

Impoundments begin with a level of biological productivity that depends on the nature of the area flooded and the degree of preparation in advance of its flooding. The self-purification capacity of the impounded stretch of a river is expected to be higher than before the impoundment was built (Imhoff, 1965). The retention of water enables sedimentation and provides longer times for biological oxidation of pollutants. This is advantageous for water quality improvement, unless there is an oxygen deficiency due to a lower reaeration rate (Krenkel et al, 1965). However, algal production might create problems for the evaluation of the self purification capacity of impoundments. In impoundments with longer retention times than one month the importance of autochthonous (i.e. generated from within the system) organic input increases in comparison with the inflow organic matter (Hrbacek, 1965). The production of new organic matter may even surpass the decomposition resulting in an effluent with higher concentrations of organics. Self-purification processes taking place in the impoundments are discussed in Chapter two.

1.2.3 RIVERS

Rivers are natural surface streams of water of considerable volume and permanent or seasonal flow. Rivers vary radically in configuration, cross-sectional shape, depth, channel bed and hydraulic gradient. Two broad classifications are encountered in the natural hydrologic setting - namely, the pool and riffle type, and the regular gradient type.

The smaller tributaries and headwater streams, traversing the more rugged terrain, descend in irregular gradients consisting of a series of deep, quiet pools interspersed with steep, shallow reaches of riffles, rapids, or sharp falls. In contrast, the larger main channel rivers that transverse the lower portion of the drainage area descend gradually in regular, flatter gradients without exposed riffles or rapids. In the computation of waste assimilation capacity of rivers, these changes in channel characteristics must be taken into account reach by reach along the course of the waterway.

In the quantitative evaluation of stream self purification three relevant channel parameters are the occupied channel volume, surface area, and the effective depth, reach by reach. From these are derived two additional vital factors - time of passage and mean velocity, reach by reach. All five factors are related to stream runoff and at any specific runoff regime are determined from the channel cross-section soundings. The time of passage curve affords a method of determining the mean velocity of flow at any location or reach along the course of the stream. These

calculations permit the investigations of the potentialities of sludge deposit and scour in any reach along the river. Factors influencing self-purification in rivers are dissolved oxygen, nature of the organic matter, biological forces, toxic substances, physical characteristics of the stream, dilution, weather conditions, sedimentation and sludge deposits, temperature etc. These will be discussed briefly in chapter two.

1.2.4 ESTUARIES

The complexity of estuaries has led to several approaches to evaluation of self purification. These can be roughly grouped into five schools (Velz, 1970).

(a) tidal prism

(b) mathematical model

(c) Experimental model

(d) Statistical

(e) Rational

The early work of engineers centered on the belief that the waters of the tidal prism are completely available for dilution and that wastes are flushed to the sea on each ebb tide, not to be returned on the following flood. However, except in unusual situations where sources of pollution are located near an estuary outlet to the ocean and where there are strong lateral ocean currents, this theory does not hold. It is now established that in the majority of polluted estuaries wastes carried toward the sea on the ebb tide

actually return on the flood tide, oscillating back and forth with but slow net movement toward the sea.

Divergence among schools currently centers on different approaches to determining mixing or diffusion of wastes, accumulation patterns and mean retention time within the estuary.

Bowden, (1963) has defined an estuary as "a partially enclosed body of water which has an influx of fresh water at one end and which is in free communication with the sea at the opposite end." As a result of tidal action, the water in an estuary moves upstream with the incoming (flood) tide and downstream when the ebb tide flows out. In addition, there is a downstream flow of fresh water from the upper portions of the river. The water in the estuary thus consists of a mixture of fresh water and sea-water in proportions varying from one point to another and dependent on the state of the tide. Although the above definition may appear to be reasonably clear, it is by no means easy to say exactly where the tidal part of a river ends and the non-tidal part begins. Moreover, the position is complicated by the fact that the height of a tide may depend upon various man-made locks and on the phases of the moon.

In the current state of knowledge the use of the rational approach holds some promise. In this approach, the complex problem is broken down into rational parts and primary relevant factors are applied to each part.

Fundamental principles of self-purification developed for application to inland waters are applied to estuaries. It is only necessary to consider modifications associated with tidal translation and seawater intrusion.

2. THEORY

2.1 WASTE STABILISATION POND THEORY

In discussing waste stabilisation pond theory, attention will be focussed on the behaviour of the oxidation or the facultative pond. On a practical basis, oxidation ponds involve the use of outdoor ponds into which raw sewage or partially treated wastes are introduced; and the application of design characteristics which will bring about the growth of algae in quantities sufficient to meet or exceed the oxygen requirements of the wastes under treatment. Sunlight energy is absorbed by the pond algae which, through photosynthesis release molecular oxygen into the pond liquid. This oxygen is used by aerobic sewage bacteria in decomposing organic matter from the wastes newly introduced into the pond and from feed-back of sludges accumulated in the pond, as a result of settlement of suspended organic matter. During oxidation of organic matter, its basic molecular components such as carbon dioxide, ammonia and phosphates are released into the liquid and become available for use by algae. The cycle continues as long as light, energy and nutrients are available.

In order to make analyses of each of the factors acting on the pond a simplified picture of the pond behaviour is presented in fig. (2.1) below. It depicts the main biochemical process features of the oxidation pond system, under steady static conditions. One of the main features of waste water treatment is the destruction of the energy

in the influent. Figure 2.1 depicts the magnitude of the flows of energy into and out of each component process by the width of the flow path. The measure of the energy is taken in terms of the 5-day BOD value, (Marais, 1970)

2.1.1 POND DYNAMICS

2.1.1.1 POND BOD

When a waste flow is discharged to a facultative pond, part of the organic load enters the liquid body of the pond and the remainder settles on the bottom of the pond to form a sludge layer. In the sludge, degradation action is anaerobic; in the pond liquid it is mostly aerobic and facultative and at times anaerobic.

The products of fermentation in the sludge layer are gases, principally methane, carbon dioxide and nitrogen as well as soluble products such as ammonia and other complex organic compounds. The anaerobic character of facultative oxidation pond sediments has been shown by the continuously negative redox potential measurements (Brockett et al, 1972), the identification of methane in the gas evolved from oxidation ponds and the isolation of methane bacteria by Darby (1972). The gases escape to the air, except for a small fraction absorbed by the supernatant liquid. The soluble products are diffused or mixed into the supernant liquid by the escaping gases. The gas methane, has a high energy content (or equivalent 5-day BOD content). Its loss to the atmosphere therefore,

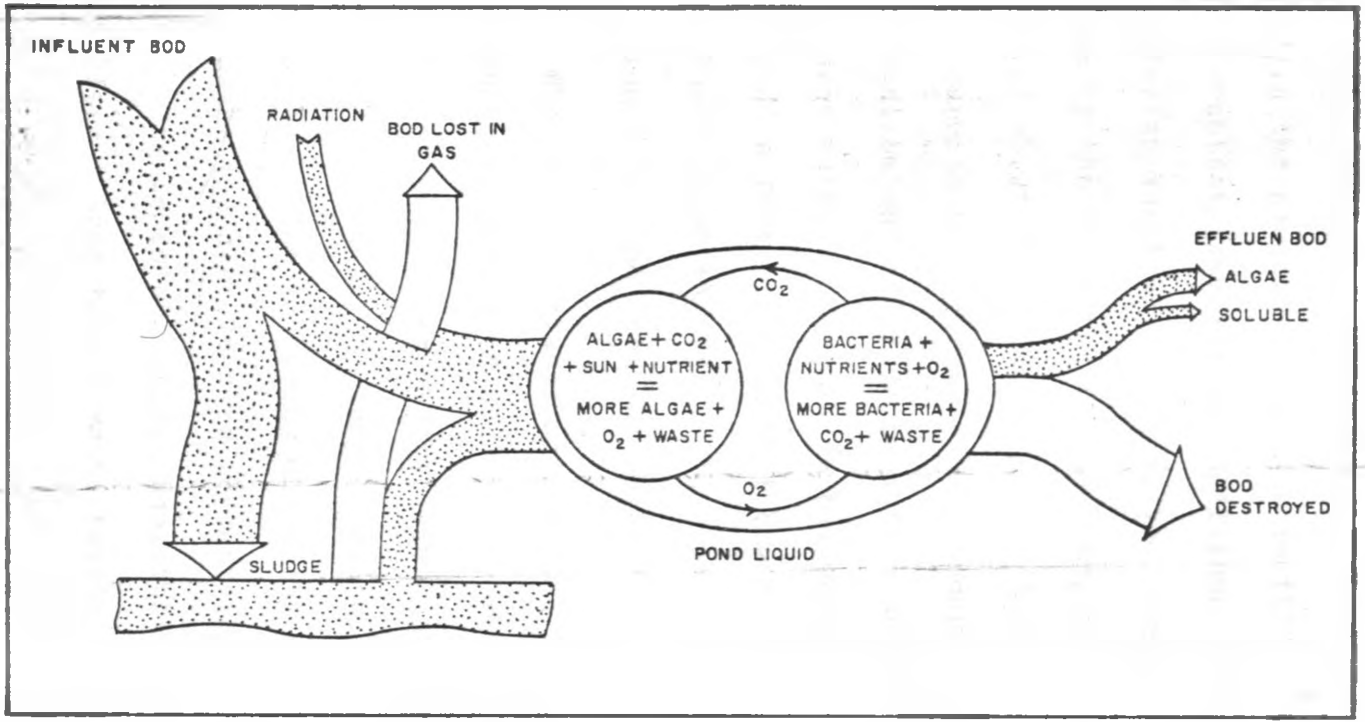


FIG. 2.1. ENERGY FLOWS IN OXIDATION POND DEGRADATION PROCESSES (MARAIS, 1970)

reduces the organic load on the whole system. Oswald (1968) has shown that up to 70% of the applied BOD and 60% of the applied nitrogen is lost from facultative ponds in the gaseous form. Marais (1970) reports that under constant temperature conditions, where steady state equilibrium has been attained, the energy lost to the system by the discharge of gases appears to be of the order of about 30% of the influent 5-day BOD. Brockett 1976) reports that volatile acid production, methane fermentation and sulphide reduction showed significant increases with increase in depth; methane production increased a factor of 2.8 by increasing the depth 100 cm. The sludge layer therefore plays an important role in removing pollution from the waste water.

When a waste stabilisation pond is put into operation, the sludge is deposited at a faster rate than the rate at which it can be removed by fermentation, consequently there is a build-up of sludge. As the sludge builds up, fermentation also increases until the mass deposited per day equals the mass lost by fermentation and an equilibrium sludge mass is established. Equilibrium may be attained only after a long time (two to twenty years) depending on the rate of fermentation which in turn depends on temperature.

The input to the pond liquid is the non-settling fraction from the influent and the feed-back from the sludge fermentation. If oxygen is present in the supernatant, aerobic degradation takes place. If the

pond liquid is mixed, the reaction approaches that of a continuously stirred reactor. Provided environmental conditions (especially temperature) remain constant, an equilibrium BOD value is established in the pond and this is the BOD measured in the effluent. This equilibrium value depends on:-

1. Influent and effluent flows
2. Influent BOD
3. Settlement of influent BOD or sludge
4. Liquid and sludge degradation rates
5. BOD feed-back from the sludge
6. Mixing conditions
7. Algal growth

The degradation rates in the pond liquid and the sludge layer are both temperature dependent and in general, change exponentially with the temperature. The rate of change of the sludge and pond liquid degradation processes differ markedly.

With cyclic changes in temperature it would be expected that the BOD in the pond changes correspondingly. In winter, because of low temperatures and therefore low degradation rates the BOD in the pond should be high. In summer, because of the high rates of degradation the BOD should be low. However, the sludge layer considerably moderates this fluctuation. During winter, little degradation takes place in the sludge and the sludge mass builds up virtually at the rate of deposition;

hence there is little or no feed-back to the pond liquid. The BOD in the pond is thus determined by the magnitude of the non-settling fraction of the influent BOD and the pond liquid degradation rate. During summer, when the degradation rate in the pond liquid is high, a low BOD value in the pond is expected. However, the high rate of fermentation in the sludge accumulated during the winter, off-loads significant quantities of sludge fermentation products to the pond liquid; which raises the pond BOD value. The nett result is that BOD variation in the pond is generally damped.

2.1.1.2 OXYGEN REQUIREMENT

The pond oxygen demand is equal to the product of the ultimate BOD and the degradation rate in the pond. As the pond process rate changes considerably with temperature, the oxygen demand changes accordingly. For the same BOD in the pond, the oxygen demand per day may be five times or more in summer than in winter. Hence oxygen requirements cannot be determined by inspecting BOD values only.

The oxygen required for aerobic degradation comes almost solely from photosynthetic activities of the algae. Although the algae, by supplying oxygen are essential to maintain aerobic conditions, this benefit is at the cost of increasing the pollution load for they incorporate solar energy into the chemical compounds of photosynthesis. They also exert a considerable respiration oxygen demand which

may completely mask the oxygen demand of the bacteria.

Oxygen production by algae in waste stabilisation ponds is a function of a number of factors. These include:-

1. Concentrations and type of algae
2. The intensity, spectral distribution and distribution over the hours of the day of sunlight (Moon 1940; Luebbers 1966).
3. Temperature
4. Mixing etc.

In studies on algal-bacterial systems Luebbers et al (1966) concluded that oxygen production is almost a linear function of the concentration of the algae and other biota. Deviations from linearity was postulated as caused by mutual shading of the algae as the concentrations increase. The production of oxygen was found to be markedly affected by temperature. The rate being low at 10°C increasing up to 20°C and the maximum rate of oxygen production being above 35°C. Further it is affected by both the diurnal cycle and periodicity. The maximum net rate of oxygen production being obtained with a periodicity of 55 to 60 per cent. Gotaas et al (1957) found the maximum oxygen production for an equal light-dark cycle to be 0.5 periodicity.

The visible portion of sunlight is the energy source of photosynthetic oxygenation. Data on the amount of available light energy together with data on the quantity of algal growths are used to predict the amount of oxygen that

may be produced through light energy fixation by algal cells. The amount of energy associated with oxygen liberation by algae is in the case of waste water grown algae like chlorella, scenedesmus and similar organisms 3.68 cal/mg of oxygen produced, (Oswald 1963). Also the efficiency of light energy conversion by algae grown in waste water has been found by Oswald and Gotaas (1957) seldom to exceed 10 or 12%. Because the probable value of the amount of available light energy may be obtained from Oswald and Gotaas solar radiation table, and photosynthetic efficiences assumed, the quantity of oxygen that will be produced for a given efficiency may be calculated.

2.1.1.3 ALGAL GROWTH

Algal growth is influenced by complex interaction of physical and biochemical factors. Of the physical factors, which affect the growth, concentration and types of algae in oxidation ponds, mixing, temperature and radiation are very important. Their relative importance change under different environmental conditions.

Mixing is a critical factor for all environmental conditions. Algal growth will be affected, irrespective of temperature, if poor mixing conditions obtain in the pond. In tropical and sub-tropical regions, temperature and radiation are normally not critical; but in the cold temperate and sub-arctic regions they may become critical during the winter. Under perfect mixing conditions, there

is equalisation of temperature and concentrations of oxygen and nutrients throughout the body of the pond, and non-motile planktonic algae are transported cyclically into the photic zone. During long periods of stratification (poor mixing conditions), the non-motile algae below the thermocline are unable to enter the photic zone and die-off from lack of light; those above the thermocline may settle and sink through the thermocline and die as a result of the diurnal rise in temperature causing a drop in the density and viscosity of the water. The non-motile algae, if trapped for lengthy periods in the top layers by stratification may also be adversely affected by the high radiation. In general, continuous stratification is characterised by a marked decrease in the concentration of non-motile algae. The motile forms, on the otherhand, migrate and concentrate at those depths of the pond where conditions are most equitable for their existence. They thus form a layer which may, by shielding the lower depths of the pond from the warming effect of radiation, promote the formation of the thermocline at this depth. From experimental work on stratified 0.6 m deep ponds during the summer in the U.S.A. Hartley et al (1968) established that, the species Euglena Rostifera avoid intensities of light in excess of 75 candles per square foot, and migrate to that depth of the pond where the intensity is less than this value; in the range of temperatures observed in the ponds i.e. 21-30°C the migration did not appear to be influenced by temperature. It was also observed that when the algae were flooded with

light of high intensity they encysted. The same pattern of behaviour has been observed in the Matero North Pond, Lusaka, Zambia, for other species of Euglena such as E. Polymorpha. Dangeard, E. Viridia Ehrenberg, and E acus E. acus Ehrenberg. (Marais 1966). In general, the following tendencies appear to be substantiated under tropical to warm-temperate climates:- with adequate mixing the non-motile algae proliferate and they achieve high densities in oxidation ponds; with long periods of stratification the motile algae predominate but their concentrations are much smaller than the concentrations attained during mixing periods.

Besides physical conditions, biochemical factors also exert a significant influence on the algal growth characteristics, algal concentrations and algal species succession in oxidation ponds. It has been shown that the rate of growth of algae is regulated by the availability of cell protoplasm building blocks such as carbon dioxide; phosphorus, ammonia and other micro-nutrients.

The relative chemical composition of living organisms demonstrates the importance of carbon as a nutrient. Carbon accounts for 50-77 per cent of the dry weight of algae and 44-55 per cent of the dry weight of bacteria. In the oxidation pond algae fix and transform inorganic forms of carbon (CO_2), from the pond liquid, into organic compound. This carbon dioxide is principally supplied by the bacterial oxidation of organic matter in the wastes and fermentation processes in the sludge. Essential to the functioning of this carbon cycle are phosphorus,

nitrogen,,sulphur and other micro-nutrients which are required by the algae.

Any change in the algal concentrations per unit volume of pond liquid is the net result of various rate reactions, some of which increase and some decrease the algae concentrations. Conceptually it can be stated that:-

change in algal concentration per unit volume	=	Algal input rate	-	Algal decay rate	+	Algal Growth rate	- Algal Sedimentation rate
	-	Algal decay rate	-	Algal Predation rate	+	Algal Resuspension rate of sedimented cells	
	+	Suspension rate of benthic algae.					

A constant algal concentration results when the positive factors balance the negative factors. In the case of a decline in the algal concentration the rates of increase are retarded or the rates of decrease accelerated. The most important positive factor in ponds is the algal growth rate. When the growth rate is lowered, a decline in the algal concentration would be expected. Similarly, an increased decay rate, predation rate, or sedimentation rate could result in reduced algal concentrations.

2.2 SELF PURIFICATION THEORY IN OTHER WATER BODIES

When a single heavy charge of organic matter is poured into a clean body of water, the physical quality of

the water is degraded. The water becomes turbid and sunlight is shut out of the depths. Green plants, which by photo-synthesis remove carbon dioxide from the water and release oxygen to it, die off. As decomposition intensifies, a shift to chemical degradation is biologically induced. Saprophytic organisms increase in number until they match the food supply. The multiplying organisms are derived inpart from the inflow and from the receiving waters. Other saprophytes enter with the run-off from agricultural lands etc. The intensity of their activity is measured by the concentration of the biochemical oxygen demand. The oxygen resources of the water are drawn upon heavily and biological degradation becomes evident in terms of the number, variety and organisation of the living organisms that persist or make their appearance. Nitrogen, carbon, sulphur, phosphorus and other important nutritional elements run through their natural cycles. Sequences of microbic populations breakdown, (1) waste matters that have been added, (2) natural polluting substances already within or otherwise entering the water and (3) food made available by the destruction of green plants and other organisms intolerant to pollution. The links of a food chain are forged from available nutrients by the growth and environmental adaptiveness of sequences of organisms.

Suspended matter in the discharge is carried along or removed to the bottom by sedimentation, depending on the hydrography of the receiving water. Benthal deposits are laid down in varying thicknesses. Their decomposition differs from that in the supernatant waters. In the presence of oxygen

dissolved in the overlying waters, benthal decomposition varies with depth of deposit from aerobic to largely anaerobic conditions.

The rates of growth of the different organisms, the species variety are influenced by the characteristics of the discharge, (chemical, physical, biological), hydrodynamic and environmental factors. In the course of time and distance, the energy values of a single charge of polluting substances are used up. The BOD is decreased. The rate of absorption of oxygen from the atmosphere, which at first lagged behind the rate of oxygen utilisation, falls in step with it and eventually outruns it. The water becomes clear. Green plants flourish in the sunlight and release oxygen to the water during photosynthesis. Other higher aquatic organisms, which are sensitive to pollution reappear and thrive. The waters are thus returned to normal purity. An example of observed changes in terms of some of the generally useful parameters of pollution and natural purification is illustrated in Fig. 2.2.

Degrees of pollution and natural purifications can be measured physically, chemically and biologically. Depending on the nature of the polluting substances and the uses the receiving body of water is to serve, measurements may be made of turbidity, colour, odour, nitrogen in its various forms, phosphorus; BOD organic matter, DO, other gases. In addition, analyses may be made for mineral substances of many kinds, bacteria, algae, protozoa etc. The composition of larger aquatic flora and fauna including

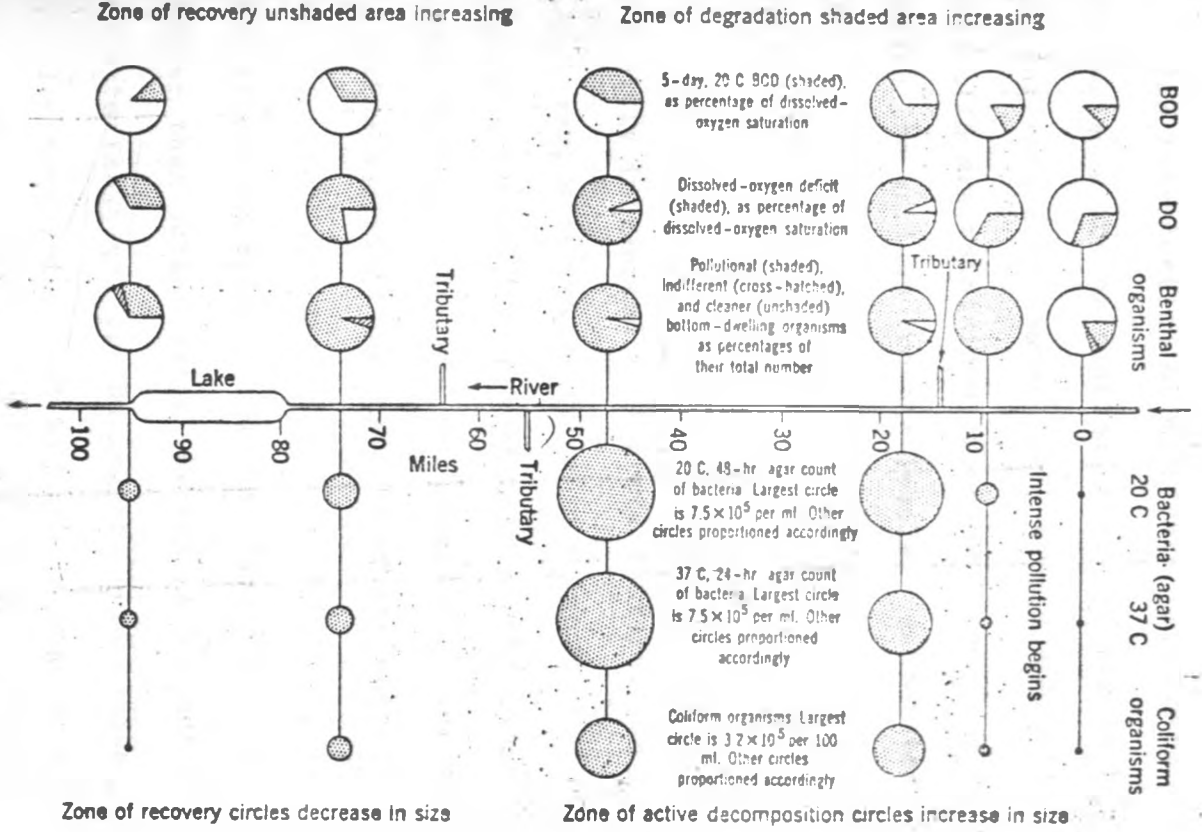


FIG 2.2 - POLLUTION AND SELF - PURIFICATION OF A LARGE STREAM.

(from a survey by the U.S. Public Health Service of the Mississippi River)

Fair et al 1971.

that of the bottom may be determined.

2.2.1 LAKES AND IMPOUNDMENTS

The introduction of nutrients into lakes and impoundments sets in motion a unique chain of events because standing waters are essentially closed communities in which foodstuffs are maintained or accumulated by circulating through the various trophic levels. Associated physical, chemical and biological conditions determine the various nutrient levels.

The response of lakes and impoundments to pollution is greatly influenced by thermal factors. In all sufficiently deep lakes, storage reservoirs and reservoirs formed by damming a stream, thermal stratification occurs during the summer months. This has the effect of lowering the pollution assimilation capacity of the water by reducing the dissolved oxygen resources (Isaak P.G., 1953). Thermal stratification depends upon the fact that warmer water is lighter than cooler water (except at $4^{\circ}C$ and below) and thus tends to float on it. During the cold season, the water is at a low temperature but with the coming of spring there is a rise in the temperature. The water, at this time, tends to become divided into an upper layer of least dense warm circulating water (The epilimnion and a deep heavier cold region (Hypolimnion). There is an intermediate region (the thermocline) where there is a maximum rate of decrease of temperature (see fig 2.5). The layers usually remain separate for the rest of the summer, although they can be

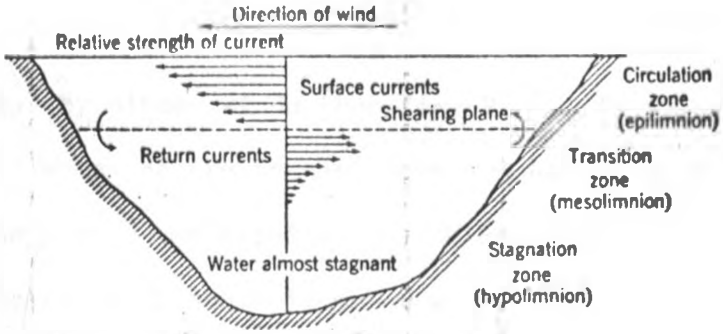


FIG. 2. 3
Direction and relative horizontal velocity of wind-induced currents in a lake or reservoir (idealized). (After G. C. Whipple, G. M. Fair, and M. C. Whipple, *Microscopy of Drinking Water*, 4th ed., Wiley, New York, 1948.)

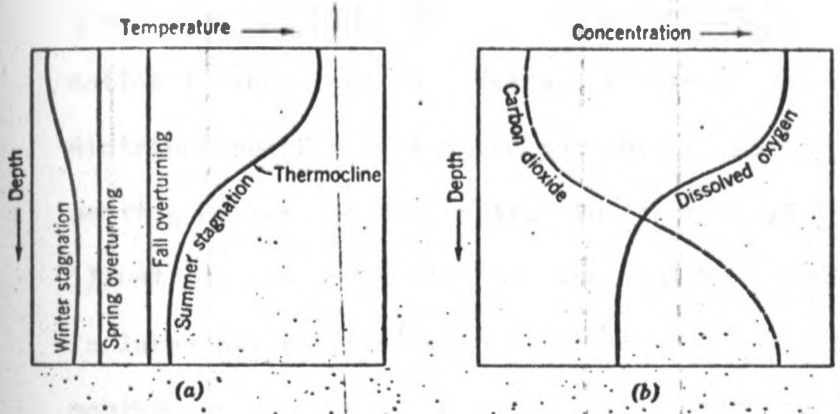


FIG 2. 4
Vertical gradients of temperature and water quality in lakes, reservoirs, and other bodies of water (idealized). (a) Characteristic thermal gradients; (b) oxygen and carbon dioxide gradients during summer stagnation.

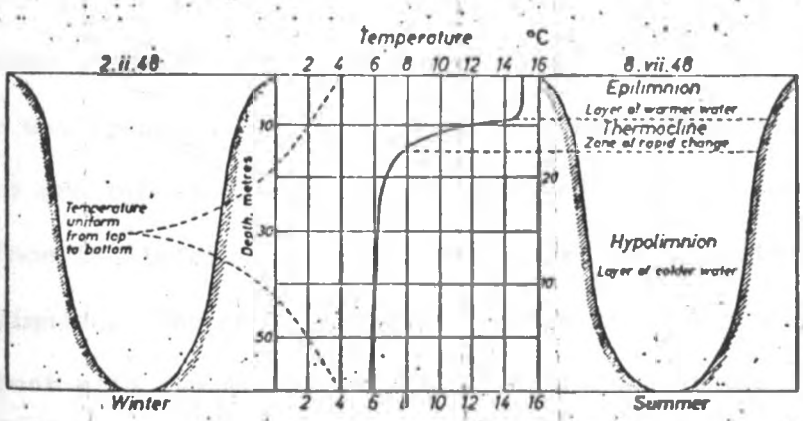


FIG 2. 5 **Thermal stratification of lake. Temperature of Windermere at different depths on 2 February 1948 and 8 July 1948. From data supplied by Dr C. H. Mortimer. [By courtesy of *Collins, London*]**

disturbed by strong winds (see fig. 2.3). In the autumn, the epilimnion starts to cool down and by winter it mixes completely with the hypolimnion. Wind action and turbulence keep the water of the epilimnion well mixed and consequently well aerated. As a rule, plant growth takes place only in the epilimnion since sunlight does not penetrate sufficiently to the hypolimnion. The interplay of temperature, density, and wind during the different seasons of the year produces a sequence of characteristic patterns of thermal stratification in lakes and reservoirs. Figure 2.3 shows the vertical distribution of wind-induced currents in an idealised cross-section of lake or reservoir. In figures 2.4 (a) and (b) is illustrated water quality and temperature gradients of waters in lakes and reservoirs that are ice-covered during the winter months.

Because molecular diffusion is relatively slow, the thermal gradients of lakes and similar water bodies are likewise gradients in the concentration of dissolved gases. Through the water surface radiant energy is absorbed by the lake water, oxygen is taken in and carbon dioxide and other gases are released. The oxygen absorbed at the water surface is distributed by the water circulating within the epilimnion. The gases of decomposition are released by contact with the air overlying the surface. Within the thermocline, there is a sharp drop in dissolved oxygen and a rise in the concentration of gases of decomposition. Below the thermocline, the concentration of dissolved oxygen is often zero, that of gases of decomposition, a maximum,

(Fig. 2.4,b). The degree of undersaturation of dissolved oxygen and the degree of supersaturation of gases of decomposition in the bottom water depend on the intensity and duration of the processes of decomposition. Decomposition is a function of inherent water quality and the organic matter content of the bottom deposits.

Thermal stratification controls the mass seasonal movements in otherwise quiescent bodies of water. As a result it produces gradients in water quality that are images of the thermal gradient itself. There is therefore, a vertical, seasonal variation in water quality within a reservoir or lake as well as a seasonal variation in water temperature. During the overturns, the quality of the water is the same due to complete mixing. The vertical mixing may carry resting algal cells from the dark depths into which they have settled, upwards to the surface, where they can flourish. At the same time food substances too, are made available within the upper strata and the overturns may be accompanied or followed by sudden, heavy growths or pulses of algae and other micro-organisms.

2.2.2 RIVERS

Self purification of rivers, which leads to the eventual elimination of the organic pollution is dependent to a large extent on biochemical reactions brought about by the activities of micro-organisms (especially bacteria). These micro-organisms, given sufficient dissolved oxygen utilise the organic matter as food and break down complex compounds to simple end products. Other factors, such as

dilution, sedimentation and sunlight play an important part in the self purification of streams. Self purification is a complicated process and each stream has its own specific capacity for purifying itself. This capacity can only be properly evaluated after an extensive chemical, physical, hydrological and biological survey.

Of importance to the self purification of streams is the dissolved oxygen content of the water. The uptake of oxygen in polluted waters is the result of increased metabolic activity of the inhabiting organisms such as bacteria, protozoa etc. The oxygenation of river water is a result of reaeration from the atmosphere, dilution with clean well-aerated waters and photosynthesis. The Combined influence of deoxygenation and oxygenation in a polluted stream causes progressive changes in the dissolved oxygen content of the stream, and creates a characteristic profile of the dissolved oxygen deficit along the path of water movement. The general mathematical properties of the sag curve, which underlie engineering calculation of the permissible pollutional loading of receiving waters, have been formulated by Streeter and Phelps, 1925. In these studies, photosynthetic oxygenation was not taken into account. However, it is now recognised that daily variation in dissolved oxygen in natural streams is a significant factor. These fluctuations are attributed to the activity of photosynthetic organisms, attached to the stream bottom. They have to be taken into account when characterising the dissolved oxygen in rivers.

Such physical characteristics as the velocity of the

stream current, the depth and cross-section of the stream, the character of the stream bed are all important factors which have a marked effect on the self-purification rate. Thus a shallow fast flowing stream will purify itself in a much shorter time than a stream which is deep and sluggish. Temperature, toxicity, weather conditions, dilution, sedimentation and sludge deposits all play an important role in the purification processes taking place in the river.

2.2.3 ESTUARIES

The mixing and movement of water in a polluted estuary are very complicated and have an important bearing on the dissolved oxygen concentrations at different points and depths. The dispersion of the organic discharge throughout the estuarine waters is dependent on tidal motion. Fresh water from the river tends to move the sea-water towards the open sea whilst, on the other hand, tidal action carries the salt sea-water upstream. This causes variations in salinity, dissolved oxygen etc.

The physical characteristics of the particular estuary e.g. shape, depth etc) determines the extent and character of mixing. In vertically mixed unstratified estuaries (Southgate, 1958), the vertical mixing is so complete that there is little difference in the salinity and dissolved oxygen level of the surface and bottom layers. In highly stratified estuaries, however, the difference in composition between surface and bottom layers is very considerable.

A heavy pollution load, due to discharges of sewage, causes a fall in the dissolved oxygen concentration in an estuary as a result of increased metabolic activity. When the oxygen concentration reaches 5-10 per cent of saturation the combined oxygen in nitrate is used by bacteria. The dissolved oxygen in the waters remains constant in the meantime. After the exhaustion of the combined oxygen in nitrate, the remaining dissolved oxygen is used up and anaerobic conditions set in. When nitrate and dissolved oxygen have been exhausted, sulphide is formed by the reduction of sulphate (always present in sea-water) by sulphate reducing bacteria. As long as DO and nitrate is present no sulphide is produced. The formation of sulphide results in the evolution of offensive H_2S. In order to avoid this nuisance it is imperative that the concentration of available oxygen in an estuary water not be permitted to fall to zero at any point.

Apart from the DO originally present in the fresh water of the upper river and in the sea-water, the main sources of useful oxygen in an estuary are the oxygen of the air, combined oxygen in hitrate and to a smaller extent, oxygen produced by photosynthetic activities of algae (Gameson et al, 1956).

Re-aeration from the air is clearly a very important factor. The rate at which oxygen enters an estuary from the air is much lower than in an inland stream. Wind has a pronounced effect on the exchange coefficient. During

gales the coefficient nearly doubles and so increases the dissolved oxygen level as well as cause sulphide to disappear from part of the anaerobic zone. Wind also creates local currents and thus assists in the dispersion of the sewage.

The water in estuaries varies in salinity from time to time owing to tidal action local currents, wind, rainfall, fresh water flow etc. The fauna that will persist in these waters are only those species that can easily adapt themselves to these salinity fluctuations.

In conclusion, self-purification and reaeration in estuaries are impeded because the pollution load moves backwards and forwards with the tidal cycle. Much depends upon the physical characteristics of the particular estuary. Gross pollution in an estuary acts as a barrier to the passage of migratory fish (salmon, trout and eels) descending to the sea or returning to fresh water.

2.3 BIOLOGICAL PARAMETERS

Current biological control or monitoring tests used in waste stabilisation ponds treatment systems and other water bodies may be divided into three categories:-

1. Unit operation efficiency e.g. BOD reduction and COD reduction
2. Biological population density e.g. microscopic counts, chlorophyll etc.

3. Biological oxidative capacities e.g. rate of BOD exertion as indicated by the first order rate constant k_1.

Measurement of microbial biomass, microbial activity and unit operation efficiency is of considerable concern to water pollution specialists and to waste treatment engineers. These parameters are used to determine the biomass and photosynthetic capacity of algae, and the degradation of the organic matter in water bodies. Improved understanding and application of these parameters can be beneficial in the evaluation, design and optimal operation of biological oxidation processes, such as occur in the waste stabilisation pond.

2.3.1 BOD AND COD

The common and most widely used measure of the efficiency of waste stabilisation pond systems is BOD removal. Application of the BOD test to lagoon effluent appears to be somewhat unwarranted since during large portions of the year these effluents contain considerable amounts of viable algae. Although the respiratory requirements of these algae will be included in the BOD values, the bacterial stabilisation of algal protoplasm will not occur in five days. In fact, Golterman (1964), found that algal cells remained alive for periods of up to five days, even under intense ultra-violet light. He also noted that autolysis of these algae must precede bacterial utilisation of algal protoplasm.

Another measure of the strength of organic wastes

is the COD exerted by these materials. The COD of lagoon effluent will include the oxygen demand of both biologically available and inert organic materials, regardless of their physical state.

The appropriateness of 5-day BOD reduction or COD reduction as treatment efficiency parameters has been discussed at length in the literature, (Ballinger et al 1962; Symons J.M. et al 1960; Davis E.M. 1971; King et al 1966). The time required to run the standard BOD test presents the most obvious difficulty. In addition BOD measurements on the treatment unit, and effluent only approximate unit efficiency because of the different K_1 values for BOD exertion of influent and effluent and the flow detention time within the unit. The COD test circumvents some of the disadvantages of the standard BOD test, but itself is considered unacceptable by many who question the relationship between material oxidisable under strong acid and high temperature conditions and material oxidisable by micro-organisms under physiological circumstances. The 5-day BOD and COD tests are of value in the measurement of gross unit efficiency as well as in design. They are also useful in research studies, although inadequate in studies requiring close monitoring of treatment unit activity.

2.3.2 PLANKTON BIOMASS

Common phytoplankton biomass parameters include chlorophyll, suspended solids, packed cell volume and microscopic counts. The biomass parameters most often used are chlorophyll concentration and microscopic counts

(in numbers or areal counts, ml^{-1}). Many other parameters have been studied and used to estimate plankton biomass; including turbidity, particulate organic carbon and nitrogen, packed cell volume, dry weight, protein, DNA and ATP. None of these parameters is ideal, although some are better than others. Particulate organic carbon and nitrogen, dry weight, turbidity and packed cell volume are non-specific parameters which suffer from the inclusion of non-viable organic detritus or suspended mineral matter. These methods are sometimes too insensitive and their precision and accuracy is often low. Other methods such as microscopic counts are theoretically more attractive but are tedious and time consuming. Cellular biochemistry offers a number of possible alternatives to the non-specific parameters. To be considered good biomass measures, cellular constituents should meet four criteria:-

1. Their concentration should be relatively constant from species to species and proportional to traditional measures of biomass such as dry weight or organic carbon.
2. The constituent should be relatively constant under normal environmental conditions.
3. The constituent should have a short survival time upon cell death.
4. The constituent should have a sensitive relatively simple and specific analytical method.

Possible candidates include DNA, ATP and chlorophyll. The merits and deficiences of these parameters have been discussed in detail elsewhere (Browne 1971, Strickland 1965).

DNA is present in all cells as genetic material and its cellular concentration is relatively constant and proportional to dry weight. The use of DNA as a biomass parameter was investigated by Genetelli (1967) for activated sludge and by Holm-Hansen et al (1968) for marine waters. The analytival method for DNA is involved and time consuming; more seriously DNA has a long survival time, after cell death, causing erroneously high biomass estimates (Holm - Hansen, 1969).

The protein content of well nourished cells is relatively constant, but nutrient deficiencies (especially nitrogen) lead to widely fluctuating protein levels. Use of protein as a biomass parameter also suffers from analytical deficiencies. The methods are relatively non-selective and include denatured proteins and peptides which survive long after cell death.

ATP is a constituent of all organisms. Its concentration on a dry weight basis has been shown to be relatively constant for bacteria (Patterson et al 1970, D'Eustachio et al 1968) and laboratory cultures of algae (Holm-Hansen, 1970). ATP has an extremely short survival time upon cell death, unless steps are taken immediately to inactivate cellular ATP-ases (ATP hydrolysing enzymes) and extract the ATP from the cells. The analytical procedure for ATP is highly sensitive and is based on the luminescent firefly reaction. ATP is required for the light producing reaction between luciferin and luciferase, and if all other conditions are constant the amount of emitted light is proportional to the ATP added. ATP was first applied to biomass measurements in marine environments Holm-Hansen and Booth (1966).

Comparison of ATP levels with other common biomass measures for plankton has been conducted by Brezonik et al (1975). Since ATP is a constituent of all organisms, problems arise when applied for measurement of algal biomass in heterogeneous communities. These problems relate to separation of the algal mass from the bacteria and protozoa occuring in the liquid mass of the pond.

Chlorophyll is doubtless the most common chemical measure of biomass. It has the advantage of being specific for photosynthetic organisms and is not associated with non-living material. The content of chlorophyll per cell varies among species and as a function of cell age, nutritional status and light conditions. Spoehr and Milner (1949) measured extremes of 0.1 and 6 per cent for chlorophyll on a dry weight basis but Round (1967) reported that chlorophyll is usually between 0.5 and 1.5 per cent of the dry weight. Chlorophyll is determined by extraction from filtered cells with acetone or methanol and spectrophotometric or fluorometric measurement. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve complete extraction of the pigments it is usually necessary to disrupt the cells mechanically with a grinder, blender or sonic disintegrator or by freezing, before extraction with acetone. Chlorophyll has the advantage that it permits sample storage, if frozen, for as long as 30 days (when immediate pigment analysis is not possible Grzenda et al 1960). The technique, however, suffers from the fact that inactive chlorophyll and degra-

dation products are determined along with the active chlorophyll of living organisms (Gloonchenko et al 1972). The concentrations of chlorophyll and degradation products can, however, be distinguished by relatively simple spectrophotometric techniques but this is not commonly practised. Chlorophyll can also be determined by direct flourescence measurements on intact cells and this technique is especially suited for continuous ship-bound monitoring. The method is attractive because of its simplicity, but standardisation is a serious problem. Flourescence intensity per unit chlorophyll varies widely among species (Lorenzen 1969) and use of the method for mixed populations in water bodies would require frequent calibration with the standard extraction technique. A number of researchers have used chlorophyll measurements with success and have found excellent relationships between phytoplankton standing crop, chlorophyll and photosynthesis (Bailey 1967, 1970, Strickland, 1960); as well as characterised(Tunzi et al 1974), Plankton populations by pigment analysis (Richards et al 1952).

2.3.3 BACTERIAL BIOMASS

Measurement of bacterial biomass and in particular bacterial activity as related to the biological degradation of organic matter, could be an additional and useful tool in the proper design, optimal operation and control of waste stabilisation ponds. Furthermore, use of simple methods permitting serial measurements of this parameter

in waste stabilisation ponds would be an invaluable asset in research studies. Up to date there exists no simple method for determining the oxidative capacity of a waste stabilisation pond system at any given time.

A biological activity parameter that has been used and continues to be used in the design of waste stabilisation ponds is the first order rate constant or K_1 value of BOD exertion. Equation 2.1 is the standard first order expression commonly used to describe the kinetics of BOD exertion.

$$y = L(1 - e^{-k_1 t}) \qquad (2.1)$$

where L = Ultimate first stage BOD of the sewage

y = Oxygen demand exerted in time t

K_1 = Kinetic rate constant day $^{-1}$

The k_1 value is estimated through a series of BOD tests (1 to 20 days). For domestic sewage K_1 (base e) normally falls within the range 0.16 to 0.70 day^{-1} (Fair and Geyer 1954). Variation in K_1 is the result of two major factors, i.e. the nature of the organic material being degraded and the oxidative ability of the organisms present to utilise this organic material (Sawyer and McCarty, 1967). Aside from the difficulty in distinguishing between the two factors, there is little reason to expect biological activity in long term, dilute and quiescent BOD tests (as measured by K_1) to reflect bacterial activity in more concentrated, though diffuse waste stabilisation ponds

and other water bodies subjected to ever changing physical, biochemical and environmental conditions. Thus k_1 cannot be a precise measure of waste stabilisation pond oxidative capacity and because its determination is time consuming it is impractical to employ as an operational and control parameter.

Biological activity can also be assessed by enumerating the populations of bacteria present in the water bodies. Recommended methods include membrane filtration and multiple tube fermentation - MPN technique (WHO, 1971). Mara (1972) successfully used the agal dip slides for estimating bacterial numbers in polluted waters. This method gives counts to the nearest order of magnitude in the range 10^4 - 10^9 cells $(100\ ml)^{-1}$. The method is simple to use and convenient for serial estimates of bacterial numbers.

Cellular biochemistry presents a number of possibilities for measurement of activity and biomass in water bodies. One such possibility is the determination of dehydrogenase activity. All major cellular reactions are mediated by enzymes. Enzymes of major interest for waste treatment purpose are those catalysing the oxidation reduction reactions related to substrate utilisation. These enzymes are classed generally as oxido-reductases, and the major representatives of this class are the dehydrogenases enzymes (Mahler et al 1966). The latter catalyse the oxidation of substrate and intermediate metabolites through transfer to hydrogen atoms (or electrons) from the reactants to cyclic intermediate acceptors (Mandelstam et al 1968). Bacterial cells may exist in either of two

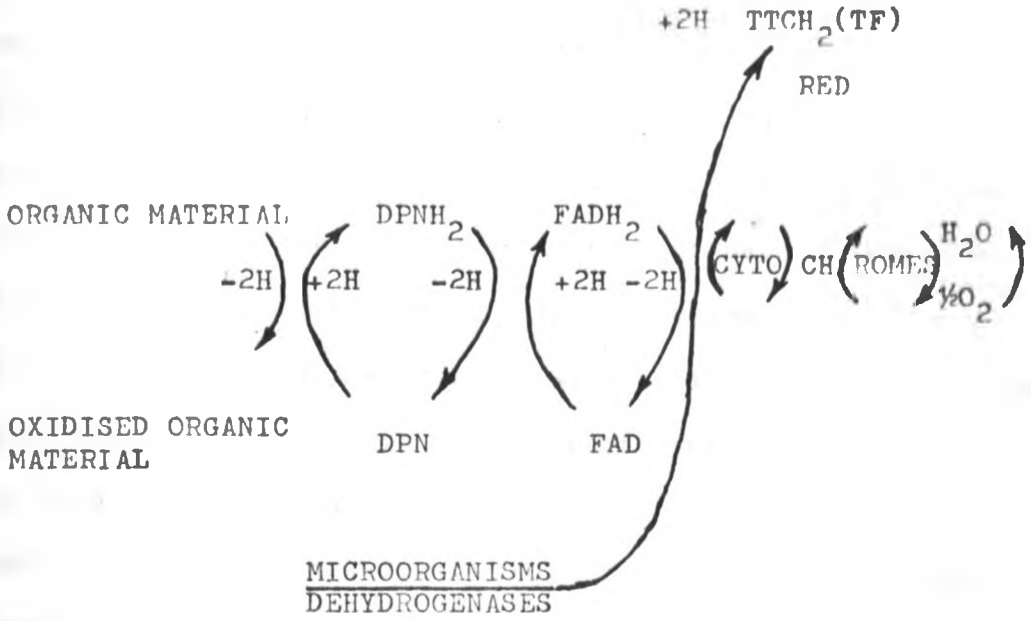
metabolic states i.e. in the presence of utilisable substrate or in the absence of substrate. The latter stage is termed endogenous respiration and the biomass metabolises storage products previously synthesised from exogenous substrate. Basal (endogenous) metabolic levels are indicative of the microbial population density while the metabolic activity in the presence of substrate represents the oxidative activity of the biomass.

The dehydrogenase activity test has been applied to activated sludge by a number of workers (Lennard et al 1964; Buckstoeg W. 1966; Ford et al 1966; Patterson et al 1969; Shik et al 1969; Klapwijk et al 1974; Ryssov-Nielsen, 1975) and has been found valuable both as a check for microbial activity and as a research tool in observing the various biochemical processes in activated sludge units.

In the simplest type of enzyme catalysed oxidation reaction, only one electron carrier is interposed between the substrate and molecular oxygen. In this oxidase reaction, the electron carrier is coupled to oxygen and no other substances. However, the aerobic dehydrogenase enzymes can pass electrons to certain reducible dyestuffs as well as to oxygen. In biochemical metabolic pathways organic compounds are broken down through a series of dehydrogenations.

The activity of the various dehydrogenases is therefore a good measurement of biochemical activity. These enzymes can easily be measured by using a tetrazolium salt (T.T.C.) as the hydrogen acceptor (Altman 1972). This couples the oxidation of the substrate to the reduction of

the colourless salt Triphenyl tetrazolium chloride (TTC). The intensity of the red colour, characteristic of the reduced form Triphenyl tetrazolium formazan (TF) is taken as a measure of dehydrogenase activity. The transfer mechanism is illustrated in the figure below.



TTC (COLOURLESS)

TRANSFER MECHANISMS

(Ford et al 1966)

These dehydrogenase enzymes catalyse the removal of hydrogen atoms from the organic substrate and most of these enzymes having associated coenzymes which serve as temporary acceptors of the transfer hydrogen as shown above. Each dehydrogenase is usually quite specific not only to its organic substrate but also to the coenzyme that it requires (Stanier et al 1963). The dehydrogenase activity in the activated sludge is assumed to be intracellular, as the centrifuged filtrate has been found to be incapable of reducing TTC (Lennard et al, 1964).

3. OBJECTIVE OF PRESENT STUDY

Algae and bacteria are important constituents of aquatic ecosystems and play significant roles in the production, assimilation and recycling of organic matter in water bodies. The mutually beneficial relationship that exist between bacteria and algae serves to reinforce other self-purifications forces such as sedimentation, chemical precipitation etc, in cleansing water bodies of pollutants. This relationship is characterised by bacterial oxidation of organic matter with the release of carbon dioxide into the waters. The carbon dioxide is used by algae together with sunlight energy and other micronutrients for growth and the photosynthetic oxygenation of the waters. The dissolved oxygen is in turn required by bacteria for the break-down of organic matter and synthesis reactions. Man has adapted algal-bacterial ecosystems to effect treatment and nutrient stripping of waste-waters in the waste stabilisation pond.

Studies of pollution control and water resources management have stressed the importance of dissolved oxygen in the self-purification processes that take place in water bodies. In the aquatic environments of lakes, impoundments, rivers and estuaries, it is best and desirable that oxidation of organic matter by bacteria and other organisms be accomplished under aerobic conditions. Only aerobic conditions guarantee nuisance free degradation processes, provides conditions not harmful to higher aquatic organisms useful to man. In

addition aerobic conditions have a beneficial influence in the production of water of a quality suitable for the manifold uses of water. Fair and Geyer (1971) have found a positive correlation between the water quality and dissolved oxygen concentration gradient in reservoirs. In situations where anaerobic degradation occurs in the depths of water bodies it is desirable that the top layers be aerobic.

Atmospheric reaeration and algal photosynthetic oxygenation are two processes that contribute to the establishment of aerobic conditions in aquatic environments. The magnitude of the individual contributions of atmospheric reaeration and algal photosynthesis to the oxygen resources of the water bodies varies with environmental, physical and biological conditions. In waste stabilisation ponds, shallow standing waters, shallow and slow flowing reaches of rivers, non-tidal sections of estuaries with little salinity, algal photosynthesis may be the dominant oxygenating factor. In swift flowing rivers, wind blown lakes, tidal estuaries, and wind blown impoundments and waste stabilisation ponds atmospheric reaeration may be the principal factor.

3.1 THEORY

Waste stabilisation ponds are designed to effect reduction of organic load as measured by the BOD or COD of the waste waters, elimination of pathogenic organisms and stripping of nutrients such as phosphorus and nitrogen from the waste waters.

These treatment objectives are achieved in waste stabilisation pond by application of a biological process involving the bacterial oxidation of organic wastes coupled with photosynthesis by algae to supply the necessary oxygen and strip nutrients. In other water bodies they may enhance the self-purification capacity of these bodies.

The relationship between the bacteria and algae has been characterised as symbiotic. (Humenik, 1971). It may be depicted as shown below and as shown in figure 3.1.

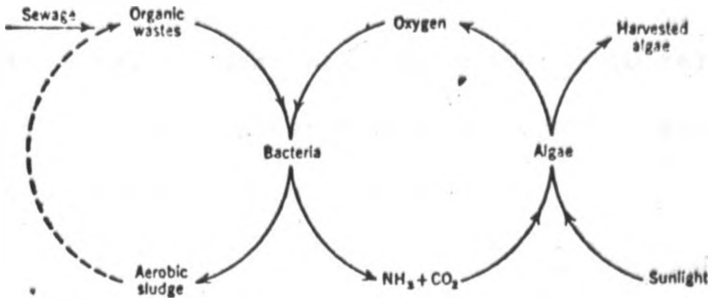
$$\text{Organics} + \text{Bacteria} + O_2 \xrightarrow{\text{Nutrients}} \text{new cells} + NH_3 + CO_2 + \text{Energy}$$

and

$$CO_2 + \text{Nutrients} + \text{Algae} \xrightarrow{\text{Light}} \text{New Cells} + O_2 + H_2O$$

Optimum conditions for bacterial degradation of organic matter, growth of algae, production and utilisation of oxygen and carbon dioxide are governed by complex interactions of physical, biochemical and environmental factors. Included amongst these are:-

1. Bacterial age, density, species variety, prey-predator interrelationships.
2. Types and concentrations of the predominant algal species, prey-predator interrelationship.
3. The nature, composition and concentrations of the organic waste.



—PROCESS OF PHOTOSYNTHETIC OXYGENATION

FIG. 3.1 (OSWALD, 1963)

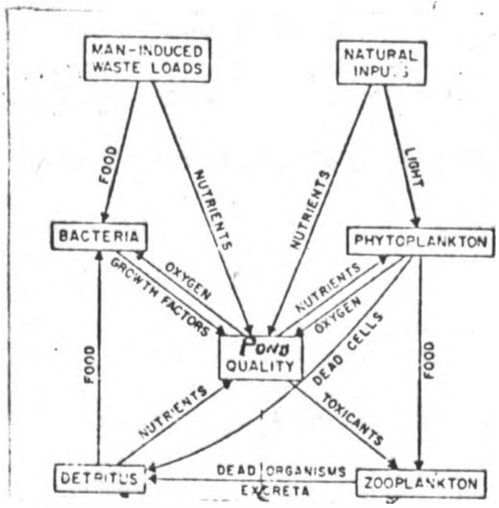


Fig. 3.2 —ECOSYSTEM DEFINITION (CHEN, 1970)

4. Temperature (air, water and sludge temperature)
5. Light intensity - its duration, penetration and algal light conversion efficiency.
6. pH
7. Mixing conditions
8. Toxicity etc.

Symbiotic conditions are attained only when the oxygen requirements of bacterial oxidation of organic matter are satisfied by photosynthetic oxygenation without need for additional aeration.

Aquatic ecosystems (see fig. 3.2) may be said to be made up of the following biological and chemical elements.

1. Abiotic substances i.e. CO_2, N_2, P, organic constituents and water
2. Decomposers i.e. bacteria
3. Producers i.e. autotrophic organisms such as algae.
4. Consumers including heterotrophic organisms such as zooplankton.

Producers, the algae, with the aid of solar energy manufacture complex organic materials from the abiotic substances(CO_2,P,NH_3) through a photosynthetic reaction, and release oxygen into the pond. These organic materials serve as food sources for herbivorous animals such as zooplankton, These biological activities generate detritus which consists of dead cells, excreta and undigested food-stuffs and refractories. Bacteria, the decomposers stabilise organic matter in the waste waters to release simple abiotic

substances for use by the algae, thus completing the cycle.

Bacterial growth, its population density, species variety and efficiency of waste stabilisation is governed by such factors as substrate assimilation patterns, prey-predator interactions, physical and environmental factors. In general substrate assimilation or degradation of organic wastes takes place in a pattern that enables the organisms to multiply at the fastest attainable specific growth rate and, at the same time, achieve the maximum possible yield of biomass (Ghosh et al, 1972). However, protozoal grazing, through selective feeding on the more substrate efficient substrate - utilising bacteria, may enhance the survival of poorly endowed bacteria, resulting in less efficient degradation of the organic matter and production of carbon dioxide (Canale et al, 1973; Curds 1974).

Algal growth, maximum standing crop achieved, species variety and oxygen production is dependent on physical and environmental factors such as, light intensity, its duration, periodicity etc as well as on a constant and adequate supply of carbon dioxide, and other growth nutrients. It has been demonstrated that the uptake of carbon dioxide during a cell cycle is variable, starting from minimum to a maximum and back to minimum during one life-cycle of an algal cell (Brockway et al, 1970). The principal supply of carbon dioxide utilised for algal growth in waste stabilisation ponds is derived from the biological stabilisation of the organic wastes. It has been postulated (Goldman et al, 1972) that algal growth in waste stabilisation ponds is limited by

the supply of carbon dioxide. Foree and Scroggin (1973) demonstrated a contrast between carbon dioxide enriched and carbon dioxide deficient cultures growing on diluted sewage treatment plant effluent. They concluded:-

> "Even in sewage treatment plant effluent, which contained significant concentrations of both organic and inorganic carbon, algal growth was limited by the availability of carbon dioxide. As much as ten-fold increase in growth was observed when excess carbon dioxide was artificially supplied to the sewage effluent growth medium."

The quantity produced will be related to the chemical oxygen demand removed, the degree of oxidation and will depend on the oxidation level of the substrate (Brockway et al, 1970). Estimation of the quantity produced by bacterial activity is complicated by factors such as chemical reactivity in the pond, secretion and exchange phenomena within the biota, photolysis of compounds and autolysis of organisms in the water body.

Oxygen production in aquatic ecosystems is dependent on algal growth. The algae fix carbon dioxide for growth and release oxygen. The rate of oxygen production will vary with environmental conditions, availability of the essential nutrients for algal growth, the bio-kinetic properties of the algae, algal species variety and predominance.

3.2 OBJECTIVE OF PRESENT STUDY

The purpose of the present study is to investigate the dynamics of algal growth, substrate consumption, oxygen

production and consumption in a laboratory batch system (as described in chapter 4) and relate the experimental results to the behaviour of aquatic systems in which algae and bacteria occur.

The objectives of the present study may be stated as follows:-

1. Study of dynamics of
   (a) algal growth
   (b) substrate consumption
   (c) oxygen production and consumption in the above mentioned system.
2. Establish basic relationships between algal growth, substrate consumption, oxygen production and consumption within the system.
3. Relate the experimental findings to the behaviour of aquatic ecosystems in which algae and bacteria occur.

4. EXPERIMENTAL METHODS AND PROCEDURES

4.1 MODEL BATCH REACTORS

Two types of batch reactors were used during the study. In experiment one and two, 250 ml glass measuring cylinders were used as biomass growth reactors. The reactors were supported and kept in the upright position with the aid of plastic base holders to which a 5 mm thick layer of cork had been glued. The biomass growth reactors were mixed by means of magnetic stirrers. The purpose of the cork was to minimise heat exchange between the magnetic stirrers and the reactor contents. The reactors were closed with rubber stoppers and sealed with vaseline, such that no exchange of gases between the biomass growth units and the surrounding room air could take place. In each experimental run one and two, five reactors were set up and were illuminated with a periodicity of twelve hours light period and twelve hours dark period.

Eight reactors were used in experiments three, four and five. Four of these were illuminated with a periodicity of 0.50 and four were unilluminated and covered with aluminium foil. The reactors were 250 ml. glass measuring cylinders as described above. However, the reactors were closed in such a manner that evolved carbon dioxide and oxygen could be collected in 250 ml. conical flasks as shown in the figure in appendix 1. In this case also, there was no gaseous exchange between the biomass growth units and the surrounding room air.

In experiments six, seven, eight and nine, a specially designed batch reactor was used. A model hexagonally shaped batch reactor was made from 6 mm thick clear perspex sheeting. The model (see Appendix II), jointed and sealed with chloroform, was made of parts and to the specifications shown in Appendix II.

The batch reactor was designed to operate as a completely mixed, closed system. The inside of the base plate was fitted with a glass plate, on which the magnetic follower rotated, thus avoiding the erosion, caused by the rotating magnetic follower, on the perspex sheeting. The central, outside part of the base plate, where it comes to rest on the magnetic stirrer, was made such that an insulating cushion of air was interposed between the base plate and the magnetic stirrer. This helped to minimise heat transfer between the magnetic stirrer and the reactor. The reactor was provided with parts fitted with probes for measurement of dissolved oxygen (liquid phase and air space above pond liquid) and hydrogen ion concentration pH. In addition the reactor had a pressure port connected to a manometer for detecting changes in the pressure of the air above the pond liquid, as well as a sampling port for withdrawing pond liquid samples for analysis. All the fittings for the probes, sampling tube and pressure connection, were made such that no exchange of air between the interior of the reactor and the surrounding room air could take place.

The total volume of the closed batch reactor was 40.65

litres and in each experiment the reactor was filled with a mixture of synthetic sewage and an algal-bacterial innoculum to the 25 litre mark. The air-space above the pond liquid thus occupied a volume of 15.65 litres.

The batch reactors were housed in the weighing room situated off the main research laboratory. Natural day light was excluded from the room and artificial illumination provided by four 1.2m, 40w flourescent lamps, one[1] of which was suspended above the surface of the reactors. In all experiments the lights were switched on and off by an automatic timer[2]. Except in experiments eight and nine, the lights were switched on at 08.00 hours and off at 20.00 hours. In experiment eight the lights were switched on at 20.00 hours and off at 08.00 hours. In experiment nine, they were switched on at 06.00 hours and off at 22.00 hours. All experiments, except experiment nine, had a periodicity of 0.50 i.e. twelve hours light period and twelve hours dark period. Experiment nine had a periodicity of 0.67 i.e. sixteen hours light period and eight hours dark period. The average light intensity at the surface of the batch reactors was a 1000 lumens/sq.m. A maximum - minimum thermometer was used to measure the mean-air temperature in the room.

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1 Tropical, Daylight, Quickstart, 6500°K
2 Sangamo, 24MD

4.2 SYNTHETIC SEWAGE

In experiments one and two synthetic sewage consisting of glucose and tap water was used as a growth medium. i.e. glucose - 80 mg/l; NH_4 CI - 63 mg/l; K_2HPO_4 - 40.3 mg/l .

In the rest of the other experiments synthetic sewage of the following composition was prepared.

Table 4.1

Components	Concentration, mg/l
Yeast Extract	158
Glucose	80
Starch	80
NH_4Cl	63
$CaCl_2.2H_2O$	10
$FeSO_4.7H_2O$	10
$MnSO_4.H_2O$	10
$MgSO_4.7H_2O$	300
K_2HPO_4	40.3
$CaCO_3$	20
Tap water	to 1 litre.

This type of synthetic sewage was chosen because it is reported to approximate the characteristics of domestic sewage (Humenik, 1971). For each experiment fresh synthetic sewage was prepared.

4.3 CULTURE DEVELOPMENT

The biomass growth medium was made up of synthetic

sewage and an algal-bacterial inoculum obtained from Embakasi Oxidation ponds. In the early experiments i.e. experiments one to six, direct inoculation of Embakasi pond algal bacterial seed was practised. This was later discontinued because in September, 1975, adverse weather conditions as evidenced by prolonged cloud cover, resulted in poor seed from the Embakasi ponds. Both the primary and secondary Embakasi ponds at this time, emitted relatively large amounts of gas (probably methane, Hydrogen sulphide, carbon dioxide etc) and the colour of the pond liquid was a murky dull green. This was probably due to the existence of anaerobic conditions in the greater part of the ponds, increased bottom sludge fermentation and resuspension of settled sludge. In an attempt to prepare an algal-bacterial inoculum of the same type and characteristics for all subsequent experiments, the Embakasi pond liquid samples were aerated in the laboratory and fed with synthetic sewage several days before a new experimental run was begun. In this way, an algal bacterial seed acclimated to the synthetic sewage and consisting predominantly of aerobic bacteria was developed.

4.3.1 START-UP PROCEDURES

Embakasi algal-bacterial seed was mixed with synthetic sewage made up of glucose and tap water in the ratio of one to four in experiments one and two. The growth medium was put into five biomass growth reactors and illuminated on a

periodicity of 0.50. Initial measurements and analysis for temperature, pH, chlorophyll, chemical oxygen demand, alkalinity etc were made and repeated at daily intervals for four days. The same ratio of algal-bacterial seed to synthetic sewage was used in experiments three, four and five. Eight batch reactors were set up in which four were illuminated and four unilluminated. The four unilluminated. reactors were covered with aluminium foil to completely exclude light. Parameters as stated above were measured and analysed for at the beginning of each experimental run and at daily intervals. Daily measurements, analyses and microscopic examinations involved one illuminated and one unilluminated reactor.

In experiment six, five litres of Embakasi ponds algal-bacterial seed were mixed with twenty litres of unaerated synthetic sewage. The biomass growth unit was then closed and sealed. Observation, sampling, analysis and measurement for parameters such as colour intensity of the algae, dissolved oxygen, chemical oxygen demand, pH, chlorophyll algal species and temperature were carried out and continued at definite intervals, though not necessarily at the same frequency for all parameters. This direct method of seed preparation and inoculation proved unsatisfactory as the reactor contents turned immediately anaerobic and were unable to re-establish aerobic conditions for the duration of the experiment. This was probably due to:-

1. an inadequate bacterial seed, as well as a seed

unacclimated to the synthetic sewage, resulting in non-production of carbon dioxide and thus die-off of algae.

2. Prolonged oxygen deficit conditions resulting in change of microflora to predominantly anaerobic bacteria and production of acids.

In experiments seven, eight and nine, the seed for inoculation was prepared in the laboratory by aeration of the Embakasi oxidation pond liquor and fill-and draw feeding with synthetic sewage to obtain, as previously stated, a seed acclimated to the synthetic sewage. In addition the synthetic sewage itself was aerated separately before mixing with the inoculum in the biomass growth unit, and initiating observation, sampling, analysis and measurement of parameters of interest. Synthetic sewage aeration time was the same in all experiments and limited to half a day.

In experiments eight and nine, the biomass growth medium (mixture of the algal-bacterial inoculum, prepared as stated above, and the synthetic sewage) was artificially aerated in the illuminated reactor and dissolved oxygen measurements taken at regular intervals until a high DO concentration had been established. Aeration was then stopped, the growth reactor closed and sealed and observation, sampling analysis and measurement of parameters of interest instituted.

Day-light variations of parameters were monitored in all experiments except experiment eight in which night-time variations of parameters of interest were monitored.

4.3.2 GROWTH REACTOR MIXING CONDITIONS

Mixing conditions in the specially constructed batch growth unit were investigated, using tap-water and potassium permanganate crystals, at different settings of the magnetic stirrer dial. Observation of the time it took to attain uniform colouration of the growth reactor contents was used to assess mixing conditions. Results showed that complete mixing was established in less than two minutes at magnetic stirrer dial setting at 4. However, tests conducted with the algal-bacterial inoculum and synthetic sewage mixture indicated that some settlement was observed only at the periphery of the base plate and was not continuous. Settings of the control dial at positions higher than four, and therefore at higher speeds, resulted in unstable rotation of the magnetic follower and subsequently the magnetic follower was thrown out of action. Thus, throughout the series of experiments, the magnetic stirrer speed was maintained at dial setting four. It was further observed that at this setting, the settling particles consisted almost entirely of undissolved calcium chloride crystals; the algae remained in the suspended state. Mixing was greater at the centre of the growth reactor and diminished towards the periphery of the biomass growth reactor. This permitted the development of attached growths, especially just below the surface of the biomass pond liquid and near the end of each experimental run.

Mixing conditions were further investigated by colorime-

tric measurement of changes in colour intensity of the reactor contents. $KMnO_4$ was introduced with a pipette, at a point just above the magnetic follower. Samples of the liquid were withdrawn at regular intervals from several points in the reactor. The colour intensity of the pond liquid was determined in a colourimeter. A graph of colour intensity versus time was drawn for each point.
The results of these investigations are given in appendix IV.

4.4 ANALYTICAL PROCEDURES AND MEASUREMENTS

Operation of the biomass growth reactors was monitored by several analytical procedures and measurements.

pH

A direct reading pH meter[3] was used to measure hydrogen ion concentration changes in the biomass growth reactors. In experiments one to five pH measurements were carried out on a daily basis. In experiments six to nine the pH electrodes were permanently suspended in the growth unit and readings made at two hourly intervals.

Temperature

Temperature readings were recorded on a daily basis in experiments one to five and at two hourly intervals in experiments six, to nine. In the last four experiments the temperatures were recorded for the gaseous and the liquid phases by means of two temperature probes attached to two dissolved oxygen meters. The mean room air temperature

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3 Vibret pH Meter Model 3920

was recorded daily using a maximum-minimum thermometer.

Dissolved Oxygen

Dissolved oxygen measurements were made using a dissolved oxygen meter[4] probe. Where it was permanently installed into the reactor, measurements were made at two hourly intervals. In experiments one to five readings were taken on a daily basis. For experiments six to nine dissolved oxygen concentrations were monitored in the growth medium and oxygen concentrations were determined in the air-space above the growth medium at two hourly intervals. The dissolved oxygen meter readings in percent saturation were converted to milligrammes oxygen per litre, taking into account the pond liquid temperature and using a nomograph supplied with the dissolved oxygen instrument by the manufacturers.

In experiments three, four and five the evolved oxygen concentration was measured by means of an Orsat Apparatus[5] i.e. a gas analysis apparatus using alkaline pyragallol for oxygen absorption. Determinations of oxygen concentration were made on a daily basis. However, precise readings of oxygen concentrations could not be obtained as values recorded lay beyond the graduate scale of the measuring burette of the apparatus. Rough estimates were made.

Carbon Dioxide

The amount of free carbon dioxide present in the growth

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4. Electronic Instruments, Richmond, Surrey, U.K.
5. Baird and Tatlock Ltd. - Three Bulb Type

medium at any equilibrium was calculated with the following equation derived by Harvey, 1957, and Park 1969 from basic equilibrium theory.

$$X = \frac{aH^2}{K_1(H + 2K_2)} \qquad (4.1)$$

where X = millimoles H_2CO_3 (including free CO_2

a = milli-equivalents carbonate alkalinity $[(HCO_3^-)] + 2[(CO_3^=)]$

H^+ = Hydrogen ion activity as measured by a pH meter

K_1 = first dissociation constant of carbonic acid

K_2 = second dissociation constant of carbonic acid.

Free carbon dioxide was calculated from measurements of pH, alkalinity and the use of the dissociation constants K_1 and K_2 (Temperature adjusted). The above method was used in determining the free carbon dioxide in experiments three, four, five and six. In experiments seven and eight, the nomographic method for determination of carbon dioxide (Standard methods, 1971) was used.

Chemical Oxygen Demand

Chemical oxygen demand analyses were made of samples withdrawn from the growth reactor at the beginning of each experiment and at intervals of one day in experiments one to eight and at intervals of four hours in experiment nine. The analysis was done as described in standard methods (1971).

For each determination, three samples of unfiltered growth medium were analysed and the average of the values obtained taken as the true value for the unfiltered growth medium. Three other samples were filtered through two whatman filter papers before determination of the COD. The average of the three values was taken as the values of the chemical oxygen demand of the filtered medium.

Chlorophyll

Algal biomass was monitored by analysis for chlorophyll concentrations in the biomass growth units. Samples for chlorophyll analysis were withdrawn once daily in experiments one to five; four times daily in experiments six, seven, and eight and five times daily in experiment nine. Analysis of the samples were performed in triplicate and the average of the three chlorophyll concentration values obtained, was taken as the true value.

The procedure used for analysis of samples was an adaptation of the method described in standard methods (1971), i.e. Trichromatic method for chlorophyll of attached communities) Samples were filtered through 0.45μm membrane filters[6] using a pyrex millipore vacuum filter holder assembly. After filtration, the membrane filters, together with the algal residue were frozen for about an hour. This promoted the easy break down of the algal cell walls and facilitated subsequent chlorophyll extraction. Extraction was done over a period of 24 hours with 90% acetone and kept in a fridge at a temperature of about 4°C. The samples were then

centrifuged and optical density readings at wave lengths of 665, 645 and 630 mm were determined using a spectrophotometer.[7] Concentrations of chlorophyll were calculated using the trichromatic formulae (Standard Methods, 1971),

5. EXPERIMENTAL RESULTS

5.1 GENERAL OBSERVATIONS

Nine experiments were set up during the studies of the dynamics of algal growth, oxygen production-consumption and substrate utilisation in laboratory scale closed batch systems. Two types of growth mediums were used. In experiment one and two glucose and tap-water enriched with micro-nutrients (see 4.2, Chapter 4), and in experiments three to nine a synthetic sewage the composition of which is given in table 4.1 Chapter 4 were used.

Growth studies in experiments one to five, were carried out in 250 ml measuring cylinder batch reactors illuminated with a periodicity of 0.50 (i.e. twelve hours light and twelve hours dark periods). Experiments three to five had, in addition to the illuminated reactors, unilluminated batch reactors covered with aluminium foil as described in section 4.1 Chapter 4.

Experiments six to nine were long-term growth studies conducted in a specially designed growth reactor (see section 4.1, Chapter 4). Growth parameters were investigated over a longer period (over ten days) and some samples and readings taken at shorter intervals than in experiments one to five.

All the illuminated experiments maintained aerobic conditions except experiment six. In this particular experiment, the initial rapid depletion of dissolved oxygen was followed by the onset and progression of anaerobic conditions. This was evidenced by the formation of a

persistent scum-layer and smell of septic-tank sewage. For the duration of the experiment dissolved oxygen concentrations remained at zero and the culture was unable to restore anaerobic conditions. As an unacclimated algal-bacterial inoculum was mixed with an unaerated synthetic sewage, the following factors probably adversely affected the ability of the biomass culture to establish symbiotic growth conditions.

1. Delay in the production of carbon dioxide by the unacclimated bacteria and by a bacterial fauna derived from a largely anaerobic pond.
2. Toxicity.
3. Algal shock or algal growth paralysis resulting from sudden introduction of the culture into a new environment.

Experiments one to eight were operated as batch experiments in which a feed was introduced at the beginning of the experiment and no additional feed added during the duration of the experimental run. Experiment nine, was a fill and draw experiment. The biomass, after the initial inoculation with three litres algal bacterial mixture to twenty-three litres synthetic sewage, was allowed to grow until a satisfactory dissolved oxygen concentration was reached. Then three litres of the growth reactor contents were siphoned off and three litres freshly prepared synthetic sewage added and the growth parameters measured and analysed.

5.1.1 VISUAL OBSERVATION

Visual observation of the colour and colour intensity of the biomass culture growth units made it possible to

speculate about the growth patterns of the biomass culture. In general, inoculation and start-up of the biomass growth unit was followed by a number of distinct phases as determined by visual observation of the algal green colour developed in the reactor. Immediately after inoculation and complete mixing of the inoculum with the synthetic sewage, the green colour of the biomass growth unit rapidly disappeared or in some cases greatly diminished in intensity. The reactor took on the same colour as the colour of the synthetic sewage alone or a very faintly green colour. The disappearance of the green colour was most pronounced in experiments six to nine. This colour fading phase had different durations in different experiments. It lasted a very short period in experiments one and two (about half a day) and about one and a half days in experiments three, four and five. In experiments seven, eight and nine it lasted about two and a half days. During this phase, in general, there was a rapid reduction in chemical oxygen demand and the dissolved oxygen concentration dropped to zero.

This phase was followed by the reappearance of the green colour which, at first gradually and then rapidly intensified, imparting a lush green colouration to the reactor contents. In some experiments, especially seven to nine, after an additional day or two, a change in the type of green colour took place. The lush green colour became progressively dark green. This colour change period probably encompassed part of the stationery and part of the declining growth phases and was probably indicative of a change in algal species or a shift in the type of algal species (ankistrodesmus to Euglena). In experiments seven and eight, the colour change phase was later

followed by the appearance of attached growths on the sides of the growth reactor. These attached growths were concentrated and limited to areas near the surface of the growth reactor contents. With the passage of time the reactor took on a yellow-green colouration and became more transparent to light. Suspended matter i.e. algae tended to clump together into clearly identifiable flocks which floated on the surface of the growth reactor medium.

Observation of the colour changes taking place in the reactor was helpful in following the overall growth phases of the algae, especially when these observations are taken together with the results of microscopic examinations of the batch reactor contents.

5.1.2 MICROSCOPIC EXAMINATION

Microscopic examinations were made of the Embakasi oxidation pond algal-bacterial seed and on the inoculated batch reactor contents. They were made at the beginning of each experimental run and at daily intervals during the course of each experiment. The results of microscopic examinations are presented in Tables 5.1 and 5.2. Algal species identified in the Embakasi oxidation pond seed were Euglena, chlorella and/or westella, ankistrodesmus as well as other micro-organisms such as paramoecium and rotifers. The above named algal species were present in almost all Embakasi oxidation pond samples examined although each species abundance differed on different occassions.

Microscopic examination of the reactor contents showed

that in the phase following the inoculation of the algal-bacterial seed, there was, in all experiments, a change in the algal species variety as well as in the overall numbers of algae present. For example in experiment four algal species identified and inoculated into the synthetic sewage were Euglena spp, chlorella sp and/or westella, ankistrodesmus (Table 5.1). At this point in time, Euglena sp was predominant in the seed. After a day, there were only three algal species i.e. ankistrodemus, chlorella and/or westella. Euglena had completely disappeared and ankistrodesmus was the abundant algal species although the actual numbers present were small. In addition no rofiters and paramoecium, which had been observed at the start of the experiment were found present. At this stage also, chlorella and/or westella were observed to be in a decided minority. Examination of Table 5.1 and 5.2 shows that in all experiments, ankistrodesmus always enjoyed an initial and early growth advantage over the other micro-organisms identified. In most cases the motile algae, present in the inoculum, always disappeared during the lag and early log growth phases. They either died out or encysted. The non-motile algae, experienced an initial reduction in numbers then a period of lag growth and then growth. Under the experimental conditions which were maintained, growth in biomass reactors proceeded, in all experiments (except experiment six) from a predominance of non-motile algae (ankistrodesmus growing at a faster rate than chlorella and/or westella), through a mixture of non-motile and motile types (Euglena and in some

TABLE 5.1 MICROSCOPIC EXAMINATIONS

EXPERIMENTS ONE TO FIVE

EXP NO / TIME HRS	ONE		TWO		THREE		FOUR		FIVE	
	PRS	PRD	PRS	PRD	PRS	PRD	PRS	PRD	PRS	PRD
0	Eu Chl Wes Ank Rot	Ank	Eu Chl Wes Ank Rot	Chl Wes	Eu Chl Wes Ank Rot	Chl Wes	Eu Chl Wes Ank Par Rot	Eu	Eu Chl Wes Ank Par Rot	Eu
45.50	Chl Wes Ank	Ank	Chl Wes Ank	Ank	Chl Wes Ank	Ank	Chl Wes Ank	Ank	Chl Wes Ank	Ank
69.50	Ank Chl Wes Rot	Ank	Ank Chl Wes Rot	Ank	Ank Chl Wes Rot	Ank	Ank Chl Wes Rot	Ank	Ank Chl Wes Rot	Ank
93.50	Ank Rot	Ank	Ank Rot	Ank	Ank Chl Wes Rot	Ank	Ank Chl Wes Rot	Ank	Ank Chl Wes Rot	Ank
117.50	Ank Rot	Ank	Ank Rot	Ank	Ank Eu Chl Rot Par	Ank	Ank Eu Chl Rot Par	Ank	Ank Eu Chl Rot Par	Ank

N.B. Eu Euglena; Chl Chlorella
Wes Westella; Ank Ankistrodesmus
Rot Rotifer; Par Paramoecium
Prs Present; Prd Predominant species.

TABLE 5.2 MICROSCOPIC EXAMINATION

EXPERIMENTS SIX TO EIGHT

TIME DAYS	SIX		SEVEN		EIGHT	
	PRESENT	PRED.	PRESENT	PRED.	PRESENT	PRED.
0	Eu Chl Wes Ank Par Rot	Eu	Eu Chl Wes Ank Rot	Ank	Eu Chl Wes Ank Rot Par	Eu
1 - 4	Ank Chl Wes	Ank	Ank Chl Wes	Ank	Ank Chl Wes	Ank
5 - 8	Algal Debris	-	Ank Chl Wes Eu Rot	Ank	Ank Chl Wes Eu Rot	Ank
9 -10	Algal Debris		Ank Chl Wes Eu Rot Lep Par	Eu	Ank Chl Wes Eu Rot Lep Par	Eu
10+			Par Rot Eu Algal Debris	Eu	Par Rot Eu Algal Debris	Eu

experiments lepocinclis was present but in smaller numbers) to a predominance of Euglena.

Another phenomenon observed was that paramoecium and rotifers present in the inoculum always disappeared during the period following inoculation. At some stage during the explosive growth of ankistrodesmus, rotifers appeared followed by the appearance of paramoecium. The average size of the mature rotifers increased with time being biggest towards the end of the experiment when there was a greater accumulation of dead algae. The rotifers observed, had sets of cilia which they used for achieving two observed purposes (see plate No 3 and 4 (Appendix V)

1. The cilia were used to catch algae brought within reach of the rotifer by currents created by what appeared to be rotational and vibratory movements of the cilia.

2. The cilia were also used as a sieving or selecting mechanism for excluding unwanted algae and particulate substances.

Pictures of some of the observed micro-organisms are presented in plates No. 1 to 6 (Appendix V)

5.2 CULTURE DEVELOPMENT

5.2.1 ALGAL GROWTH

Algal growth as measured by spectrophotometrically determined chlorophyll concentrations is graphically depicted in figures 5.1 to 5.14, and in tabulated form shown in Tables 5.3 to 5.22 for all experiments. Figures 5.1

to 5.3 and figures 5.5, 5.7, 5.9, 5.10 depict the day to day variation of chlorophyll concentrations (mg/l) in the batch systems. Figures 5.12 and 5.14 show the day-time hourly variations and figure 5.13 the night-time hourly variations of chlorophyll concentration. Figures 5.4, 5.6 and 5.8 are graphical presentations of chlorophyll concentration daily variation in unilluminated batch systems (i.e. the algal controls in experiments three to five).

From the curves it can be seen that algal growth as determined by chlorophyll is characterised by lag, logarithmic, stationery and declining growth phases. All the phases were present in experiments seven and eight (figs. 5.9 and 5.10) and at least lag and logarithmic, and in some cases, stationery growth phases were present in the other experiments. In all the illuminated experiments the growth phase sequence was lag, logarithmic, stationery and declining growth. It is interesting to note that in experiment seven there were two logarithmic and two stationery growth phases in addition to the lag and declining growth phases (fig. 5.9).

A summary of the results obtained in experiments one to five is shown in Table 5.23. From the graphs (Figs. 5.1 to 5.8) and tables 5.3 to 5.7 and 5.23, it can be seen that algal growth is governed by a complex relationship of a number of factors. The substrate, its complexity and organic content i.e. its composition and concentration, physical and biological factors affect the rates of algal growth and the maximum standing crop produced. A simple

TABLE 5.3 EXPERIMENT ONE - 5th MAY TO 10th MAY 1975

PARAM SAMPLE	TIME HRS	TEMP. °C	PH	DO % SAT	DO mg/l	CODF mg/l	CODU mg/l	CHLOR. mg/l
EOP	0	20.00	7.40	17.00	1.80	-	-	0.288
G.M.	0	18.00	7.30	35.00	3.60	120	140	0.0246
1	21.50	24.20	6.80	5.00	0.80	71.00	86.00	0.1650
2	45.50	24.50	6.60	36.00	3.05	32.00	53.00	0.3450
3	69.50	24.40	6.50	50.00	4.45	26.00	41.00	0.4050
4	93.50	24.60	6.40	56.00	5.08	36.00	42.00	0.3850
5	117.50	24.50	6.40	49.00	4.45	41.00	51.00	0.3100

ILLUMINATION PERIODICITY - 0.50

TABLE 5.4 EXPERIMENT TWO - 12TH MAY TO 17TH MAY 1975

PARAM. SAMPLE	TIME HRS.	TEMP. °C	PH	DO % SAT	DO mg/l	CODF mg/l	CODU mg/l	CHLOR. mg/l
EOP	0	21.00	7.20	20	2.20	-	-	0.3020
G.M.	0	18.50	7.00	34	3.40	118	146	0.0226
1	22.00	23.80	6.80	8.00	1.10	76.00	93.00	0.1850
2	45.50	24.00	6.60	22.00	2.25	26.00	65.50	0.2100
3	69.50	24.30	6.50	42.00	3.85	17.00	46.50	0.3600
4	93.50	24.40	6.45	55.00	4.90	31.00	42.00	0.4050
5	117.50	24.40	6.30	54.00	4.85	27.00	42.00	0.4250

ILLUMINATION PERIODICITY - 0.50

TABLE 5.5 EXPERIMENT THREE - 22nd MAY to 27TH MAY 1975

PARAM. SAMPLE	TIME HRS.	TEMP °C	PH	DISSOL-VED OXYGEN % SAT.	DO mg/l	ALK. $CaCO_3$ mg/l	EQ.CO_2 mg/l	CODF mg/l	CODU mg/l	CHLOR. mg/l
INNOCUL.	0	20.50	7.75	4.80	0.82	-	-			0.2980
GROWTH MEDIUM	0	17.80	7.40	45.60	4.70	-	-	242.00	340.20	0.0384
1	45.50	25	7.00	34	3.10	84.60	17.29	151	250	0.0911
2	69.50	25	6.50	83	7.10	99.00	61.60	140	199.28	0.1423
3	93.50	26	6.60	105	9.35	96.00	49.33	78.70	148.00	0.2689
4	117.50	25	6.60	124	11.20	90.40	46.25	65.10	167.00	0.8170
5	45.50	26	6.90	5	0.75	108.00	27.81	165.5	276.00	0.0511
6	69.50	24.40	6.40	3	0.65	122	99.37	117	265.00	0.0293
7	93.50	27	6.40	0.5	0.25	120	97.74	91.45	205.00	0.0373
8	117.50	26	6.30	0.05	0.00	112	115.22	74	126.08	0.0138

SAMPLES 1 to 4 ILLUMINATED REACTORS; PERIODICITY 0.05

SAMPLES 5 to 8 UNLLUMINATED REACTORS.

TABLE 5.6 EXPERIMENT FOUR - 5TH JUNE TO 10TH JUNE 1975

PARAM. SAMPLE	TIME HRS.	TEMP °C	PH	DISSOLVED OXYGEN % SAT	D.O. mg/l	ALK. as $CaCO_3$ mg/l	EQ. CO_2 mg/l	CODF mg/l	CHLOR. mg/l	CODU mg/l
INNOCUL.	-	20	7.60	5.00	0.80	-	-	-	0.344	-
GROWTH MEDIUM	0	15.50	7.30	51.00	5.40	-	-	266.80	0.0469	350.80
1	45.50	25.00	6.30	5.00	0.75	79.50	81.42	122.00	0.2399	256.80
2	69.50	24.60	6.30	56.50	5.00	93.90	95.66	70.40	0.2835	200.40
3	93.50	24.20	6.30	108.50	9.60	89.00	91.15	93.30	0.4884	170.45
4	117.50	24.40	6.50	102.00	9.30	87.00	56.28	99.50	0.5391	127.34
5	45.50	24.60	6.25	1.80	0.50	96.00	110.44	185.60	0.0968	320.08
6	69.50	24.60	6.19	1.95	0.58	105.00	138.73	188.00	0.1082	300.01
7	93.50	24.60	6.10	1.00	0.45	103.50	168.22	111.50	0.1080	288.80
8	117.50	25.00	6.10	0.05	0.00	101.50	133.66	73.35	0.0795	193.26

SAMPLES 1 to 4 ILLUMINATED REACTORS - PERIODICITY - 0.5

SAMPLES 5 to 8 UNILLUMINATED REACTORS

TABLE 5.7

EXPERIMENT FIVE - 16TH JUNE TO 21ST JUNE, 1975

PARAM. / SAMPLE	TIME HRS.	TEMP. °C	PH	DISSOLVED OXYGEN % SAT.	D.O. mg/l	ALK. as $CaCO_3$ mg/l	EQ. CO_2 mg/l	CODF mg/l	CHLOR. mg/l	CODT mg/l
INNOCUL.	-	21	7.50	5.50		-	-		0.302	-
GROWTH MEDIUM	0	16.90	7.30	53.00		-	-	282.45	0.0610	348.60
1	45.50	25.50	6.35	10.00	1.10	103.070	94.20	120.00	0.3600	220.00
2	69.50	26.00	6.30	45.00	4.68	87.770	90.01	92.00	0.3500	183.50
3	93.50	26.50	6.40	104.00	9.09	118.450	96.48	73.00	0.6525	106.20
4	117.50	27.00	6.50	112.00	9.70	59.775	48.37	78.50	0.5725	90.80
5	45.50	26.50	6.20	5.00	0.80	83.910	108.34	178.40	0.1620	230.48
6	69.50	26.50	6.10	0.00	0.00	78.100	126.94	152.00	0.1480	200.78
7	93.50	27.00	6.15	0.00	0.00	105.965	153.50	106.30	0.1324	180.20
8	117.50	27.00	6.10	0.00	0.00	106.265	172.70	82.60	0.1289	132.40

SAMPLES 1 to 4 ILLUMINATED REACTORS

SAMPLES 5 to 8 UNILLUMINATED REACTORS

substrate such as glucose is easily broken down by bacteria, whereas a multi-component substrate such as the synthetic sewage used in experiments three to nine induces phasic assimilation patterns by the bacteria. When factors such as temperature, pH, mixing conditions, intensity of light, algal species - types, concentrations, states of growth -, micro-nutrient availability etc, are equal, algal growth will greatly be influenced by the amenability of the substrate to bacterial oxidation. When the above-mentioned factors vary, the total effect may be reinforcement or retardation of growth. In such cases, results become difficult to interpret because of the complexity of the relationships involved. In general, it can be seen that there was rapid growth in experiments one and two. The bacteria quickly assimilated glucose for growth and released carbon dioxide into the reactor medium. The fairly rapid initial drop in pH (Tables 5.3 and 5.4) may be attributed to the release of carbon dioxide into the medium. In these two experiments, there was a short lag phase (12 hours), and maximum growth was attained in a short period of time. In both experiments the maximum algal chlorophyll concentration was about the same. (Tables 5.3 and 5.4 and figures 5.1 and 5.2). In experiments three to five maximum algal chlorophyll concentrations were higher than in experiments one and two. Since micro-nutrient concentrations, physical conditions (i.e. light intensities and mixing conditions) were the same for all five experiments, the higher algal concentrations attained were the result of the greater organic content of the substrate (Fuhs, 1974). The multi-

component nature of the substrate used in these experiments, requires a phasic and complex deployment of bacterial enzymes for oxidation of the substrate. This affects carbon dioxide availability to the algae. Initial growth in experiment three was slower than in experiment four and five. Equilibrium carbon dioxide concentration in the growth medium was 17.29 mg/l in experiment three after two days (shown as day one in table 5.5) compared to 81.42 and 94.20 mg/l in experiments four and five after the same period. Thus there was more carbon dioxide available for growth in experiment four and five than in experiment three at this point. This may account for the longer lag and slow initial growth phases of experiment three. Though the COD reductions at this point are comparable, it would appear that in experiment three, degradation had not reached the stage of significant release of carbon dioxide to the growth medium. This however, cannot be taken as fully accounting for the growth patterns observed since the physiological states of the micro-organisms constituting the seed, the ratios of active to inactive organisms, and their individual and community responses to the same environment may differ and are not easy to elucidate.

A comparison of experiment three, four and five shows that though the initial chlorophyll concentration was less in experiment three than in experiments four and five, a greater maximum chlorophyll concentration was attained in experiment three than in experiments four and

EXPERIMENT ONE

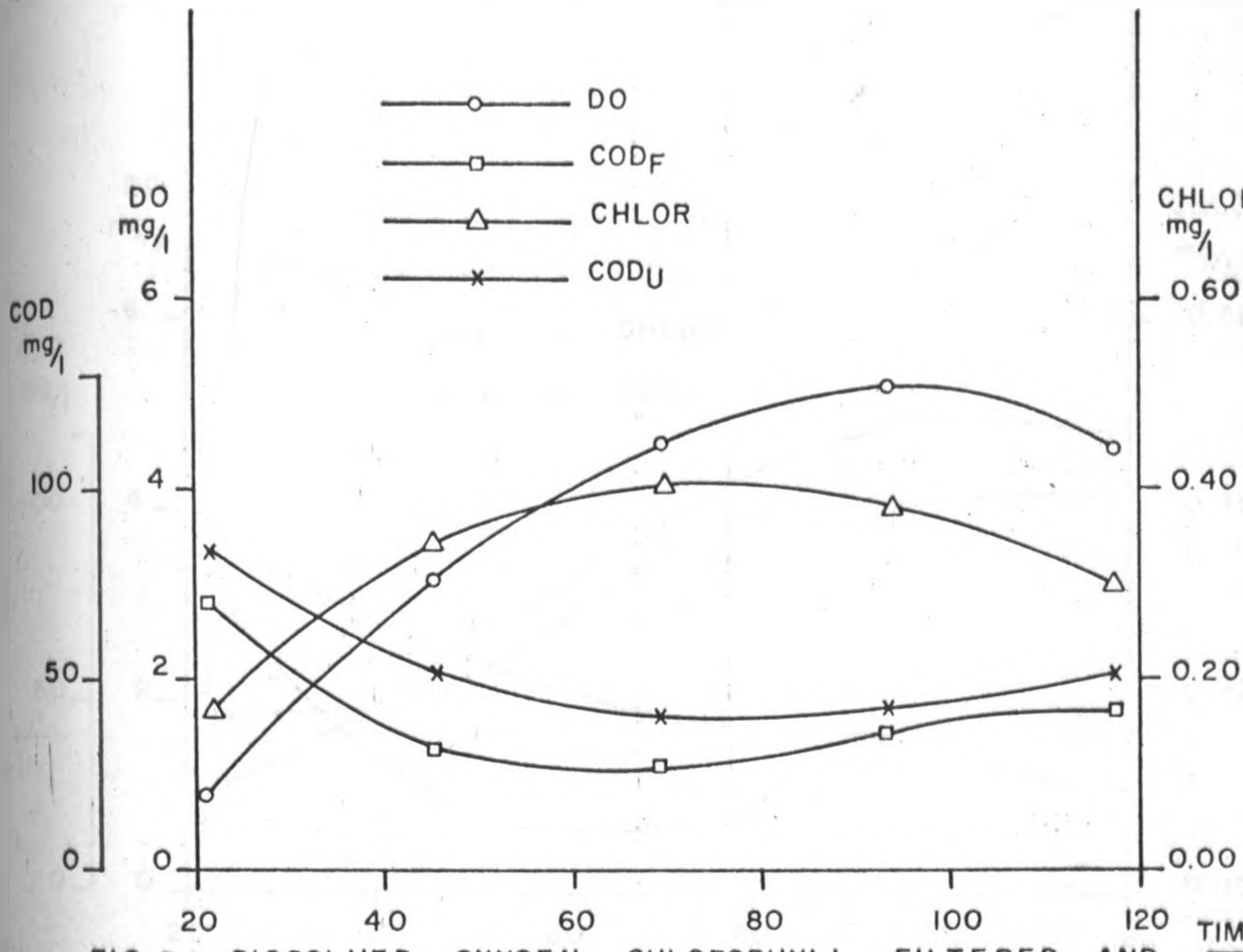


FIG. 5.1: DISSOLVED OXYGEN, CHLOROPHYLL, FILTERED AND CHEMICAL OXYGEN DEMAND VARIATION

EXPERIMENT TWO

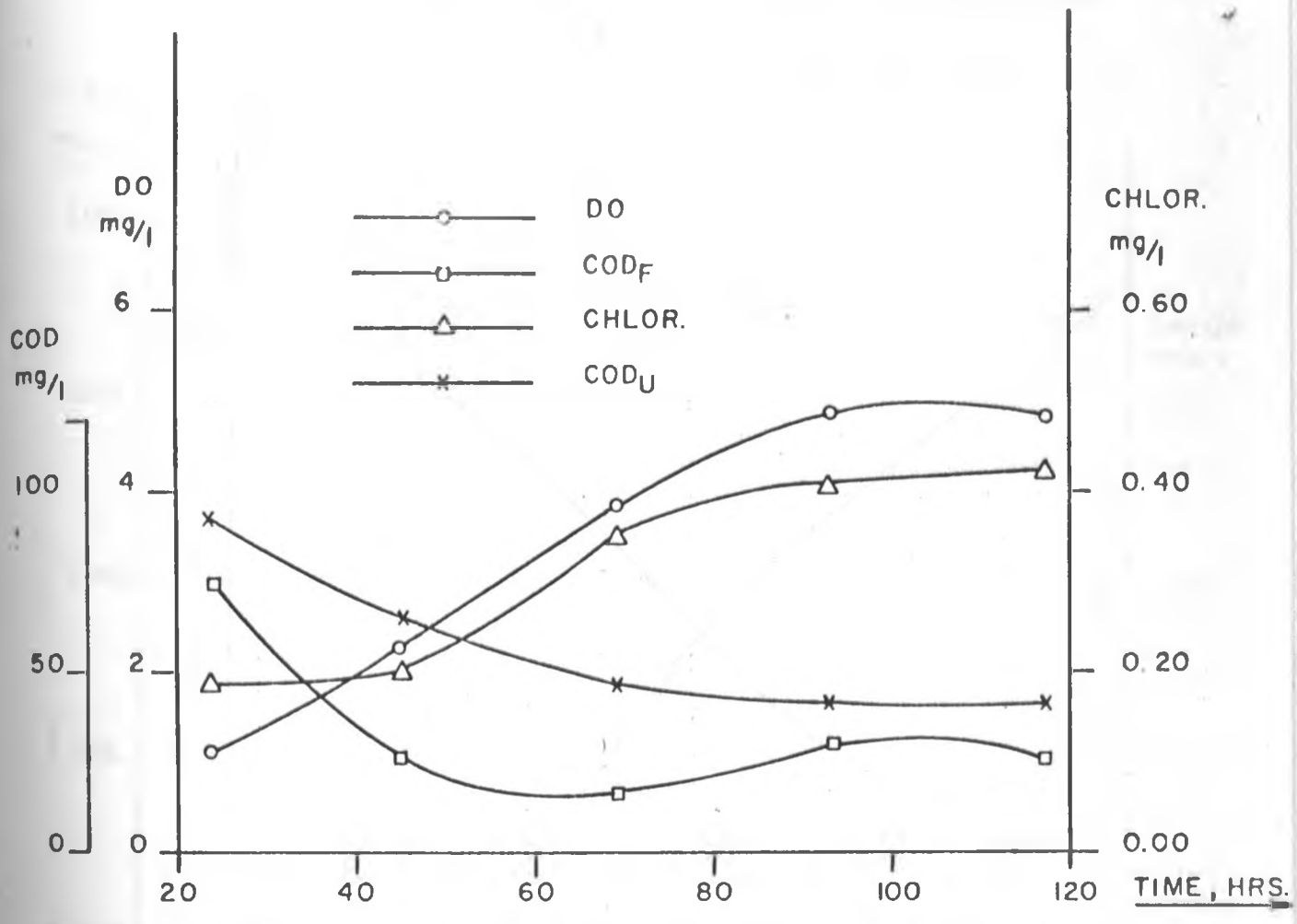


FIG. 5.2: DISSOLVED OXYGEN, CHLOROPHYLL FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

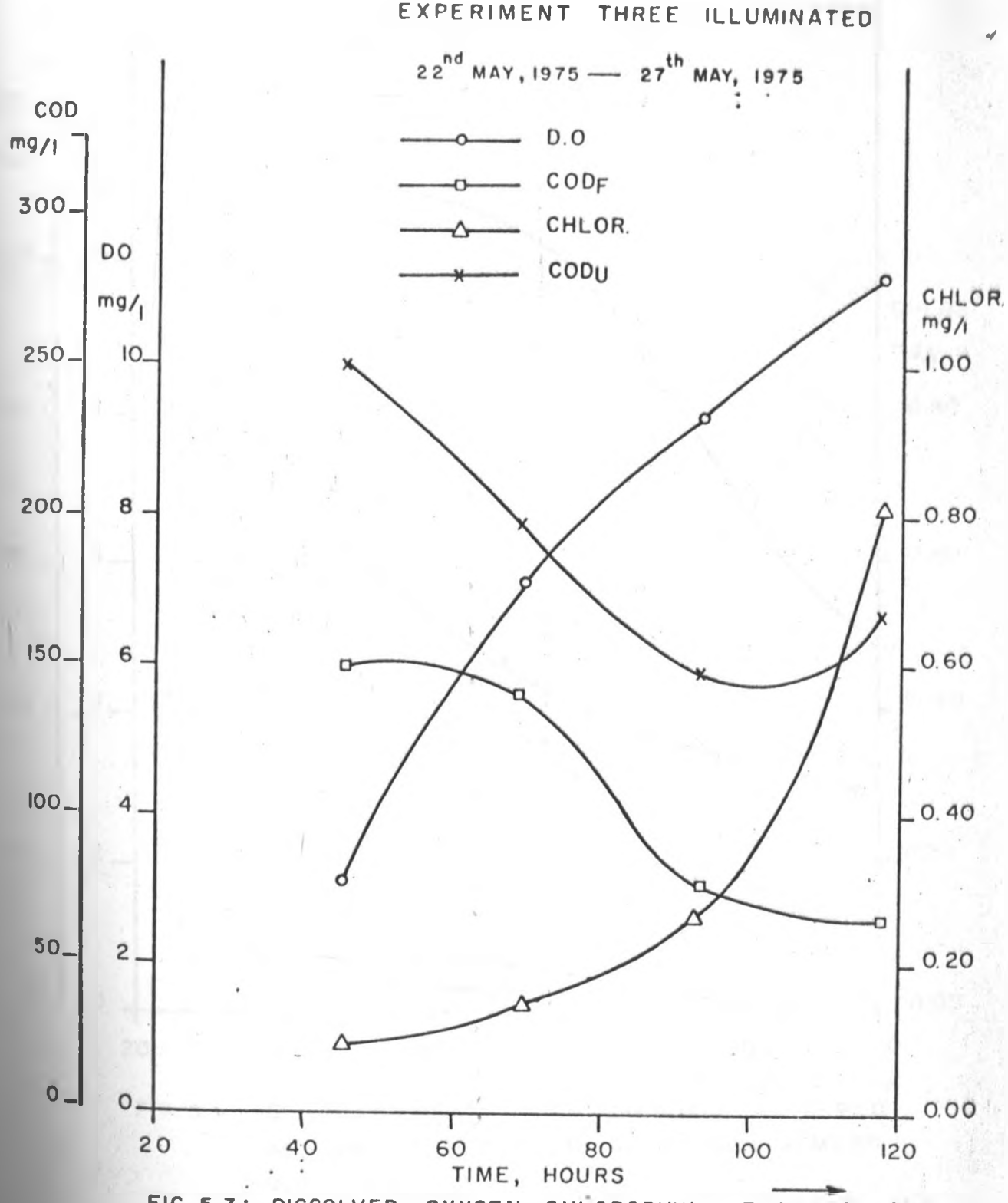


FIG. 5.3: DISSOLVED OXYGEN, CHLOROPHYLL FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

EXPERIMENT THREE UNILLUMINATED

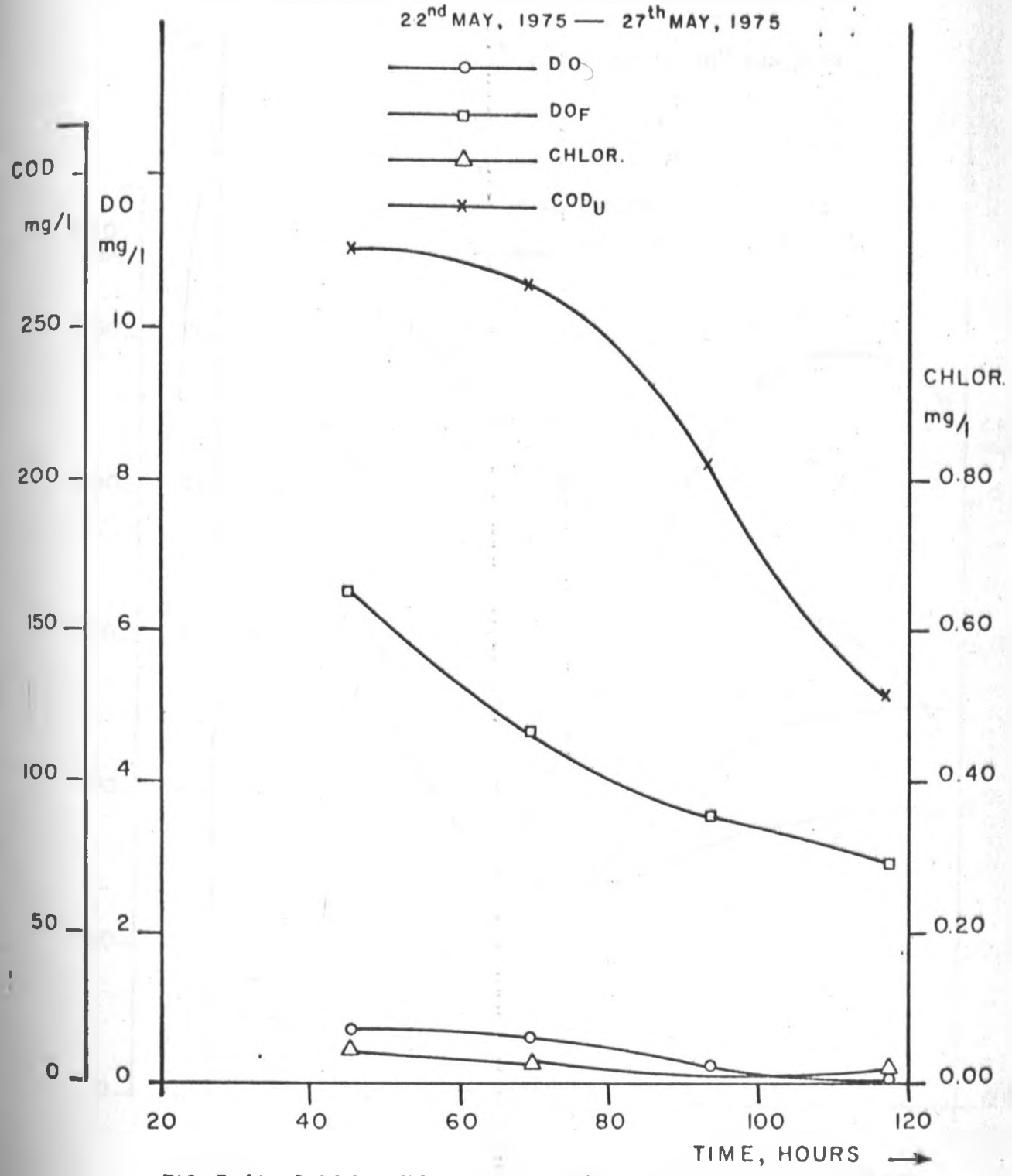


FIG. 5.4: DISSOLVED OXYGEN, CHLOROPHYLL, FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

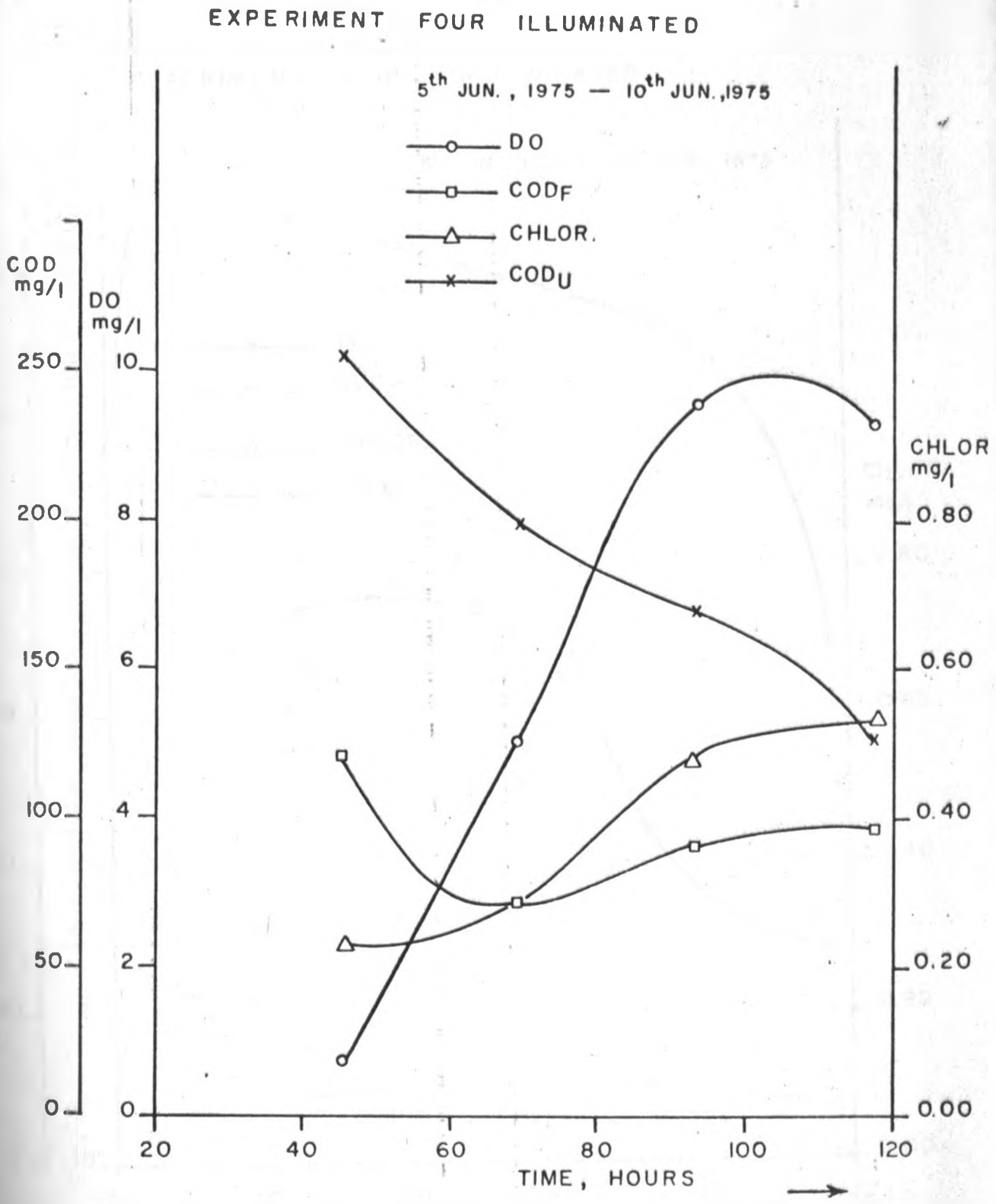


FIG. 5.5: DISSOLVED OXYGEN, CHLOROPHYLL, FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

EXPERIMENT FOUR UNILLUMINATED

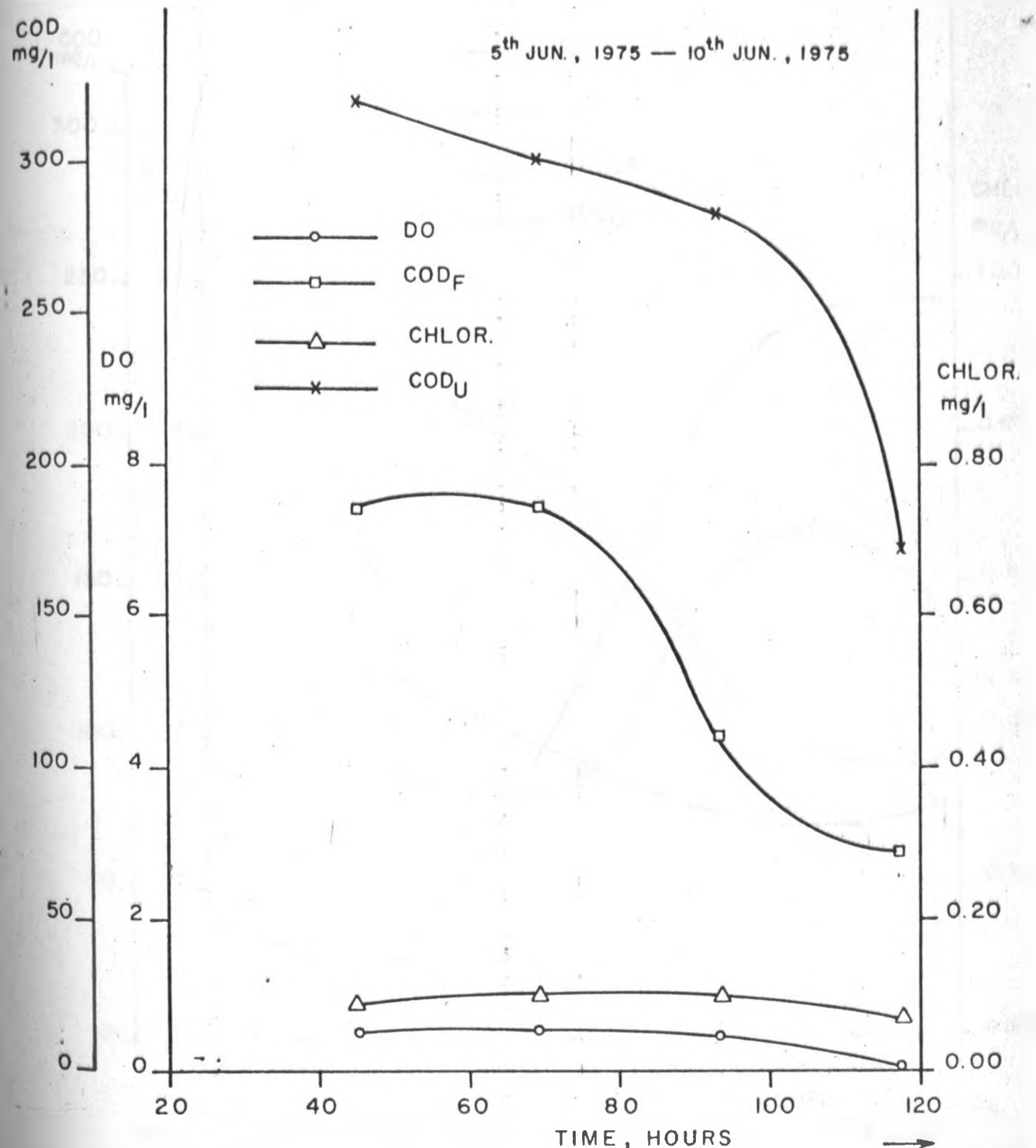


FIG. 5.6 : DISSOLVED OXYGEN, CHLOROPHYLL, FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

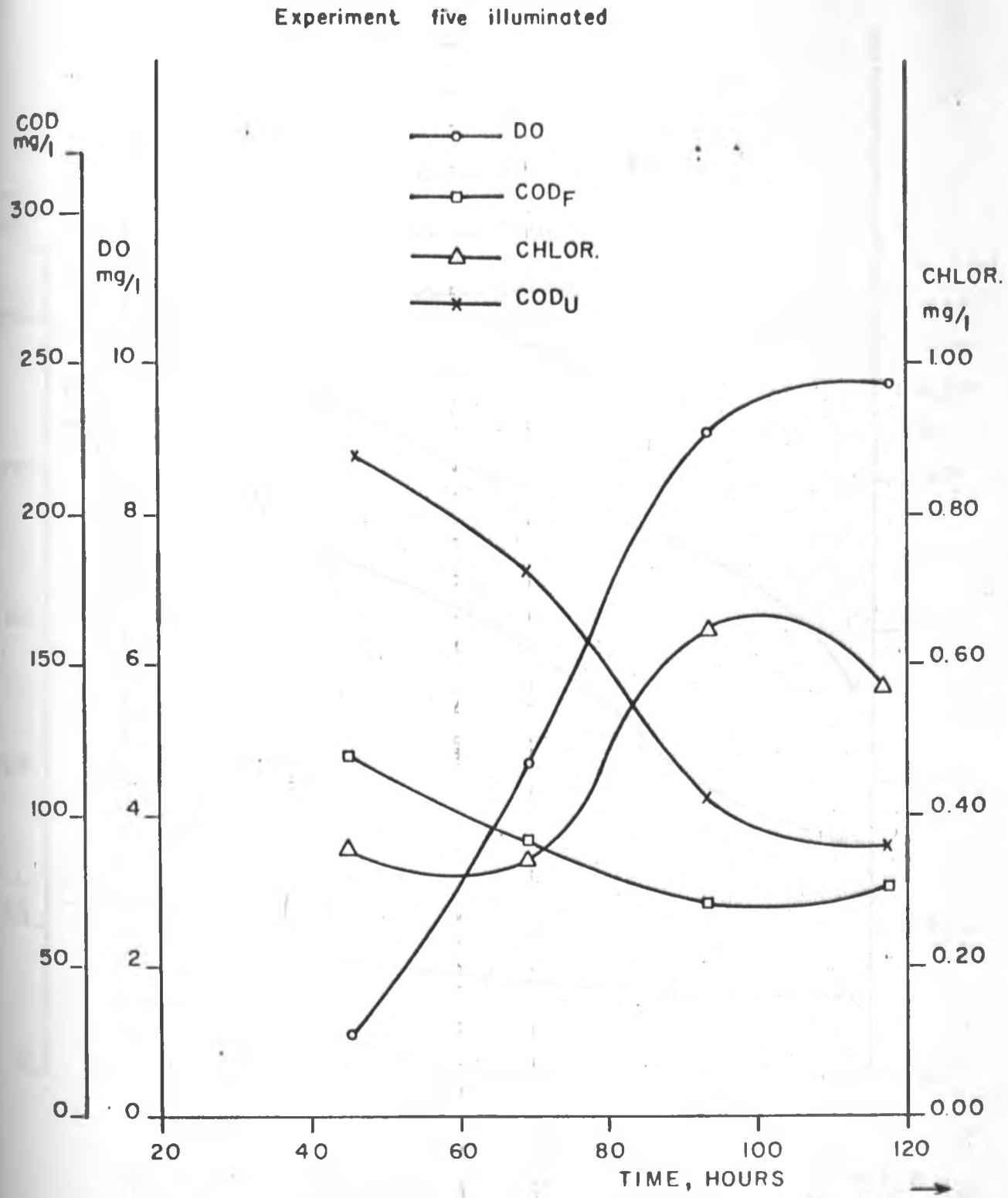


FIG. 5.7 : DISSOLVED OXYGEN CHLOROPHYLL FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

Experiment Five Unilluminated

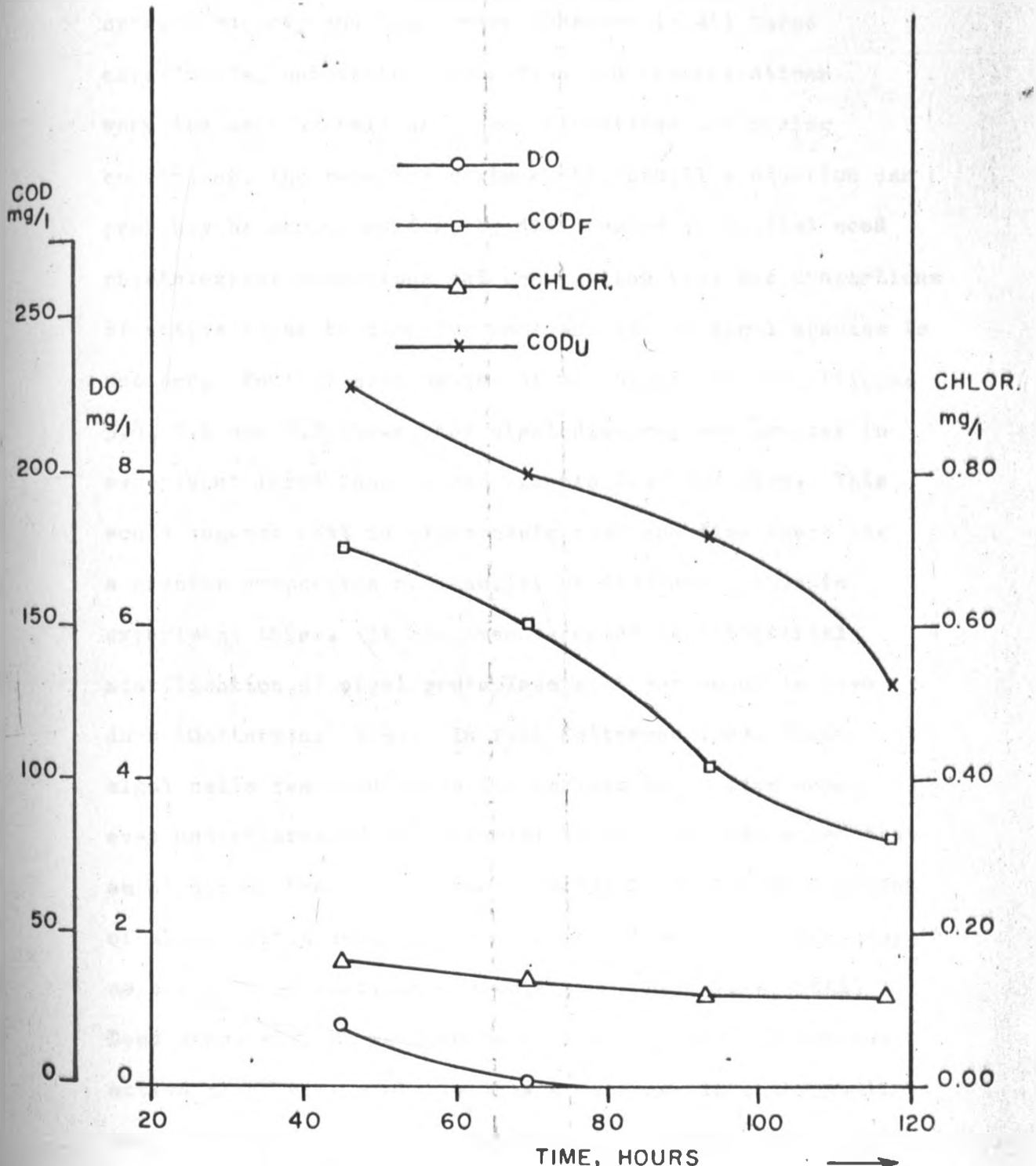


FIG 5.8 : DISSOLVED OXYGEN, CHLOROPHYLL, FILTERED AND UNFILTERED CHEMICAL OXYGEN DEMAND VARIATION

five. (Tables 5.5, 5.6 and 5.7). Since the same algal species variety and types were observed in all three experiments, substrate composition and concentrations were the same as well as light intensities and mixing conditions, the reported maximum chlorophyll production can probably be accounted for, by differences in initial seed physiological conditions and composition i.e. the proportions of active algae to inactive ones and of one algal species to another. Further examination of the algal controls, figures 5.4, 5.6 and 5.8, shows that algal die-away was greater in experiment three than in experiments four and five. This would suggest that in experiments four and five there was a greater proportion of inactive or dead algae than in experiment three. It has been reported that bacterial stabilisation of algal protoplasm will not occur in five days (Golterman, 1964). In fact Golterman 1964, found that algal cells remained alive for periods up to five days, even under"intense" ultra-violet light. He also noted that autolysis of these algae must precede bacterial utilisation of algal protoplasm. Under dark conditions algal die-away as a result of continuous respiration.(Golterman, 1964) Dead algae will be removed by bacterial oxidation whereas active algae will probably show a decrease in chlorophyll concentration as a result of continuous respiration and as a result of encysting. The illuminated experiments show that experiment three had only two phases i.e. lag and log growth whereas experiment four and five went through more phases in the same period of time. Since the experiments had a duration of only 120 hours, it is likely that food and

nutrients had not been exhausted in the growth medium and that the algae were still in the process of multiplication. This is supported by the fact that at the end of the experiments there were still high equilibrium carbon dioxide concentrations in the growth reactor medium.

The high rate of growth and the high yield attained in experiment three is probably the result of the state of growth of the algae and carbon dioxide availability to the growing algae. Temperature does not appear to be a significant factor. However, a comparison of the growth curves for experiments four and five show that the higher temperature in experiment five had some influence on the growth rate.

Results of algal growth are shown in Tables 5.8 to 5.13 for experiment six. No algal growth was recorded in this experiment. As previously stated (see section 4.3 chapter 4) prolonged cloud cover at the Embakasi oxidation ponds had promoted anaerobic conditions at the ponds. This algal bacterial seed was unable to grow and establish aerobic conditions in the laboratory growth reactors. Although analysis of the growth reactor contents for chlorophyll yielded results, they probably reflect chlorophyll contained in dead algae, i.e. algal debris.

The results of experiments seven, eight and nine are shown in figures 5.9 to 5.14; Tables 5.14 to 5.22 and in appendix III (Tables 1 to 20). The algal growth curves shown are also characterised by lag, logarithmic, stationery and declining growth phases (figures 5.9 to 5.11). In experiment seven and

TABLE 5.8 EXPERIMENT SIX - 2/9/76 to 8/9/75

PARAM. / DATE	TIME HRS.	pH	T°C	DO % Sat.	Eq.CO_2 mg/l	COD_F	COD_F	CHLOR. mg/l
2/9/75	16.00	7.00	19.40	28.70	10.734	272	312	0.0834
	18.00	6.91	20.00	21.00				0.0648
	20.00	6.81	20.50	9.00				0.0545
	22.00	6.70	20.80	0.50				0.0321
	24.00	6.70	20.90	0.50				0.0339

TABLE 5.9 EXPERIMENT SIX - 2/9/75 to 8/9/75

PARAM / DATE	TIME HRS.	PH	T°C	DO % SAT	EQ.CO_2 mg/l	CCDF	CODU	CHLOR. mg/l
3/9/75	02.00	6.70	20.90	0.30				0.0349
	04.00	6.70	20.90	0.30	-			0.0266
	06.00	6.65	20.90	0.30	-			0.0437
	08.00	6.50	21.40	0.00	33.64	114	270	0.0220
	10.00	6.70	21.10	0.00	-			0.0112
	12.00	6.70	21.50	0.00	20.40			0.0175
	14.00	6.80	19.90	0.00	-			0.0105
	16.00	6.75	22.30	0.01	18.19			0.0248
	18.00	6.80	22.50	0.02	-			
	20.00	6.80	22.60	0.01	16.209			0.0226

PARAM / DATE	TIME HRS	PH	T°C	DO % SAT	EQ.CO_2 mg/l	CODF	CODU	CHLOR. mg/l
4/9/75	02.00	6.80	22.80	0.00	-			0.0196
	04.00	6.80	22.80	0.00	-			0.0231
	06.00	6.80	23.00	0.00	-			0.0222
	08.00	6.80	22.50	0.00		175	240	0.0287
	10.00	6.85	23.50	0.00				0.0437
	12.00	6.80	23.50	0.00				0.0483
	14.00	6.80	23.00	0.00				0.0437
	16.00	6.80	22.60	0.00				0.0442
	18.00	6.70	23.50	0.00				0.0581
	20.00	6.71	23.60	0.00				0.0590
	22.00	6.70	23.60	0.00				0.0283
	24.00	6.71	24.00	0.00				0.0364

TABLE 5.11 EXPERIMENT SIX - 2/9/75 to 8/9/75

PARAM. / DATE	TIME HRS.	PH	T_{oC}	DO % SAT	EQ.CO_2 mg/l	CODF	CODU	CHLOR. mg/l
5/9/75	02.00	6.70	23.00	0.00				0.0531
	04.00	6.70	22.80	0.00				0.0615
	06.00	6.70	23.50	0.00				0.0730
	08.00	6.70	23.60	0.00		135	280	0.0060
	10.00	6.70	23.60	0.00				0.0390
	12.00	6.70	24.00	0.00				0.0860
	14.00	6.65	23.20	0.00				0.193
	16.00	6.70	23.50	0.00				0.0395
	18.00	6.65	23.50	0.00				0.0465
	20.00	6.68	23.50	0.00				0.0542
	22.00	6.60	23.80	0.00				0.0591
	24.00	6.60	23.50	0.00				0.0536

TABLE 5.12 EXPERIMENT SIX 2/9/75 to 8/9/75

PARAM. / DATE	TIME HRS	PH	T°C	DO % SAT	EQ.CO_2 mg/l	CODF	CODU	CHLOR. mg/l
6/9/75	02.00	6.65	24.50	0.00				0.0458
	04.00	6.50	24.00	0.00				0.0420
	06.00	6.60	23.90	0.00				0.0463
	08.00	6.60	23.50	0.00		115	275	0.0553
	10.00	6.60	23.60	0.00				-
	12.00	6.60	23.50	0.00				0.0351
	14.00	6.60	23.90	0.05				0.0519
	16.00	6.65	24.00	0.05				0.0459
	18.00	6.65	24.25	0.05				-

TABLE 5.13 EXPERIMENT SIX - 2/9/75 to 8/9/75

PARAM / DATE	TIME HRS	PH	T°C	DO % SAT	EQ.CO_2 mg/l	CODF	CODU	CHLOR. mg/l
7/9/75	08.00.	6.55	23.90	0.05		110	220	0.0434
	10.00	6.55	24.00	0.05				0.0482
	12.00	6.55	24.10	0.00				0.0594
	14.00	6.55	24.20	0.00				0.0326
	16.00	6.50	24.40	0.00				0.0090
	18.00	6.50	24.20	0.00				0.0434
8/9/75	08.00	6.20	24.30	0.00		110	240	0.0219
	10.00	6.20	24.30	0.00				0.01934
	12.00	6.30	24.30	0.00				0.01436

eight initial algal chlorophyll concentrations were 0.2107 and 0.2491 mg/l respectively. After inoculation, the reactors were illuminated continuously until the dissolved oxygen concentrations had built up to 8.40 mg/l and 6.45 mg/l in experiments seven and eight respectively. Thereafter a dark light sequence of illumination was begun with a periodicity of twelve hours dark and twelve hours light period. At that point, the algal chlorophyll concentrations were 1.0060 and 1.1108 mg/l in experiments seven and eight respectively. Maximum algal chlorophyll concentrations attained were 1.7805 mg/l in experiment seven and 1.3780 mg/l in experiment eight. Day-time algal chlorophyll concentration variation and night-time chlorophyll concentration variation are shown in figures 5.12 and 5.13 for experiments seven and eight respectively. The night time algal chlorophyll concentration variation graphs show that at night, there is no algal growth (Fig. 5.13). The form of the daily algal chlorophyll concentration variation graphs is determined by factors such as the growth phase. There is almost a linear relation during the logarithmic growth phase. Figure 5.11 depict the results of the chlorophyll variation in the fill and draw experiment nine. The biomass culture was grown as described for experiments seven and eight. When a dissolved oxygen concentration of 3.6 mg/l was attained a dark-light sequence of illumination was started. A periodicity of sixteen hours light and eight hours dark period was used. Then on the day following the start of the light-dark sequence of illumination, three litres of growth medium were withdrawn from the reactor and replaced with three litres of

TABLE 5.14 EXPERIMENT SEVEN

DATE	CHLOR. mg/l	D.O. mg/l	CODU mg/l	Δ CODU mg/l	%Δ CODU	CODF mg/l	Δ CODF mg/l	%Δ CODF
24/9/75	0.2107	7.10	320.00	-	-	183.34	-	-
25/9/75	-	0.40	152.80	167.20	52.25	120.00	63.34	34.55
26/9/75	-	-	119.69	200.31	62.60	110.00	73.34	40.00
27/9/75	0.2833	-	105.07	214.93	67.17	96.67	86.67	47.27
28/9/75	0.5559	-	96.67	223.33	69.79	83.34	100.00	54.54
29/9/75	0.7833	0.80	92.65	227.35	71.05	74.67	108.67	59.27
30/9/75	1.0060	5.69	94.88	225.12	70.35	68.00	115.34	62.91

DATE	TIME HRS.	CHLOR. G mg/l	O_2PROD. mg/l/t	O_2UTIL. mg/l/t	CODU mg/l	Δ CODU mg/l/t	%Δ CODU	CODF mg/l	Δ CODF mg/l/t	%ΔCODF
1/10/75	08.00	1.0765			96.66		69.79	58.67		68
	12.00	1.0819	3.50	1.60		223.34			124.67	
	16.00	1.0912	7.45	3.20						
	20.00	1.0980	11.45	4.80						
	-	-								
2/10/75	08.00	1.0796			104.09		67.47	67.08		57.96
	12.00	1.0897	3.7820	1.7320		215.91			116.26	
	16.00	1.1189	7.6640	3.464						
	20.00	1.1729	11.7960	5.196						

DATE	TIME HOURS	CHLOR. G mg/l	O_2PRCD. mg/l/t	O_2UTIL. mg/l/t	CODU mg/l	ΔCODU mg/l/t	%ΔCODU	CODF mg/l/t	ΔCODF mg/l/t	%ΔCODF
3/10/75	08.00	1.1600			98.67		69.17	65.34		64.30
	12.00	1.2450	4.0680	1.8668		221.33			118.00	
	18.00	1.4068	8.3360	3.7337						
	20.00	1.5692	12.0040	5.6004						
4/10/75	08.00	1.5608			94.67		70.42	60.00		67.27
	12,00	1.6891	2.9000	1.7000		225.33			123.34	
	16.00	1.7608	5.6000	3.4000						
	20.00	1.7789	9.4500	5.1000						

DATE	TIME HRS	CHLOR. a. mg/l	O_2PROD mg/l/t	O_2UTIL. mg/l/t	CODU mg/l	Δ CODU mg/l/t	%ΔCODU	CODF mg/l	ΔCODF mg/l/t	%ΔCODF
5/10/75	08.00	1.7805			91.18		71.51	53.36		70.90
	12.00	1.7209	3.2500	1.700		228.82			129.98	
	16.00	1.5998	7.0500	3.4000						
	18.00	1.4532	9.7500	5.1000						
6/10/75	08.00	1.4409			81.22		74.62	43.34		76.36
	12.00	1.2873	3.9140	1.8668		238.78			140.00	
	16.00	1.2053	7.1282	3.7337						
	18.00		9.7920	5.6004						

TABLE 5.18 EXPERIMENT EIGHT

DATE	CHLOR. mg/l	D.O. mg/l	CODU mg/l	Δ CODU mg/l	%Δ CODU	CODF mg/l	Δ CODF mg/l	%Δ CODF
23/10/75	0.2491	4.53	226.68	-	-	149.34	-	-
24/10/75	-	0.50	117.33	109.35	48.24	101.34	48.00	32.14
25/10/75	-	-	96.16	130.52	57.58	86.67	62.67	41.96
26/10/75	0.4278	-	85.74	140.94	62.17	74.00	75.34	50.45
27/10/75	0.5680	1.95	80.00	146.68	64.71	69.34	80.00	53.57
28/10/75	0.6891	5.00	86.19	140.49	61.98	66.67	82.67	55.36
-	-	-	-	-	-	-	-	-

TABLE 5.19 EXPERIMENT EIGHT

DATE	CHLOR. mg/l	O_2PROD. mg/l/day	CODU mg/l	ΔCODU mg/l/day	%ΔCODU	CODF mg/l	ΔCODF mg/l/day	%ΔCODF
29/10/75	1.1108	8.0580	84.27	142.41	62.82	62.66	86.68	58.04
30/10/75	1.2116	7.2492	86.27	140.41	61.94	60.00	89.34	59.82
31/10/75	1.2744	7.5504	86.18	140.50	61.98	53.34	96.00	64.28
1/11/75	1.3686	7.6104	95.60	131.00	57.83	52.67	96.67	64.73
2/11/75	1.3709	9.4560	100.00	126.68	55.88	47.34	102.00	68.30
3/11/75	1.3429	9.1308	116.70	109.98	48.52	42.00	107.34	71.88
4/11/75	1.3159	8.9400	120.80	105.88	46.71	36.67	112.67	75.45
5/11/75	1.2123	8.1720	113.34	113.34	50.00	32.67	116.67	78.12

TABLE 5.20 EXPERIMENT NINE

DATE	TIME HRS.	CHLORA mg/l	DO mg/l	O_2PROD. mg/l/t	O_2UTIL. mg/l/t	CODU mg/l	ΔCODU mg/l/t	% ΔCOD	CODF mg/l	ΔCODF mg/l/t	% ΔCOD
19/1/76											
	10.00	1.1722	2.65			116.40	6.40	5.5	75.60	1.20	1.59
	14.00	1.2092	1.00			110.00	9.40	8.08	74.40		9.52
	18.00	1.2320	1.80			107.00	12.40	10.70	68.40	7.20	23.54
	22.00	1.2930	3.20			104.00	14.77		57.80	17.80	
20/1/76	06.00	1.2901	0.60	2.90	1.30	101.63	17.31	12.69	65.55		13.29
	10.00	1.3158	2.20	5.70	2.60	99.09	28.40	14.87	65.17		13.80
	14.00	1.3321	3.70	9.10	3.90	88.00	39.40	24.40	70.56		6.67
	18.00	1.3720	5.80	12.02	5.20	80.98		31.27	69.02		8.70
	22.00	1.4341	7.42			77.00			63.00		

TABLE 5.21 EXPERIMENT NINE

DATE	TIME HRS	CHLOR.a mg/l	O_2PROD. mg/l/t	O_2UTIL. mg/l/t	CODU mg/l	Δ CODU mg/l/t	%ΔCODU	CODF mg/l	ΔCODF mg/l/t	% Δ CODF
21/1/76	06.00	1.3750	3.71	2.41	89.84	11.65	22.82	60.92	6.94	19.42
	10.00	1.3976	7.98	4.82	78.19	12.72	32.83	53.98	10.79	28.60
	14.00	1.4235	11.79	7.50	77.12	15.72	33.75	50.13	12.72	32.37
	18.00	1.4602	14.95	9.64	74.00		36.43	48.20		36.24
	22.00	1.5059			67.00			44.50		47.10
22/1/76	06.00	1.4903			72.00		38.14	40.00		25.20
	10.00	1.4503			92.83		6.06	69.59		28.93
	14.00	1.4598			87.20		14.22	52.05		
	18.00	1.4680			79.63			49.56		
	22.00	1.4802			71.50			47.30		

TABLE 5.22 EXPERIMENT NINE

DATE	TIME HRS.	CHLOR. mg/l	O_2PROD. mg/l/t	O_2UTILIS. mg/l/t	CODU mg/l	Δ CODU mg/l/t	%Δ COD_u	CODF mg/l	ΔCODF mg/l/t	%ΔCODF
23/1/76	06.00	1.4782	2.0260	1.2760	84.56	1.3600	8.91	52.56	4.3300	24.47
	10.00	1.4820	4.0220	2.5520	83.20	-1.9400	10.37	48.23	7.4700	30.69
	14.00	1.4809	5.9280	3.8280	86.50	-5.0400	6.82	45.09	13.3000	35.21
	18.00	1.4750	7.8040	5.1040	89.60		3.48	39.26		43.58
	22.00	1.4120			89.60			40.00		

TABLE 5.23 EXPERIMENTS ONE TO FIVE

EXP. NO / PARAM	ONE	TWO	THREE ILL.	FOUR ILL.	FIVE ILL.	THREE UNILL.	FOUR UNILL.	FIVE UNILL.
SUBSTRATE	GLUCOSE	GLUCOSE	SYN S.	SYN. S.	SYN. S.	SYN. S.	SYN. S.	SYN. S.
AVE. T°C	24.44	24.18	25.25	24.55	26.25	25.85	24.80	26.75
INITIAL PH	7.30	7.00	7.40	7.30	7.30	7.40	7.30	7.30
AVE. PH	6.54	6.53	6.68	6.35	6.39	6.50	6.16	6.14
INITIAL CHLOR.mg/l	0.0246	0.0226	0.0384	0.0469	0.0610	0.0384	0.0469	0.0610
MAX/MIN CHLOR.mg/l	0.4050	0.4250	0.8170	0.5391	0.6525	0.0138	0.0795	0.1289
INITIAL EqCO_2 mg/l								
FINAL Eq.CO_2mg/l			46.25	56.28	48.37	115.22	183.66	172.70
CO_2 EVOLVED								

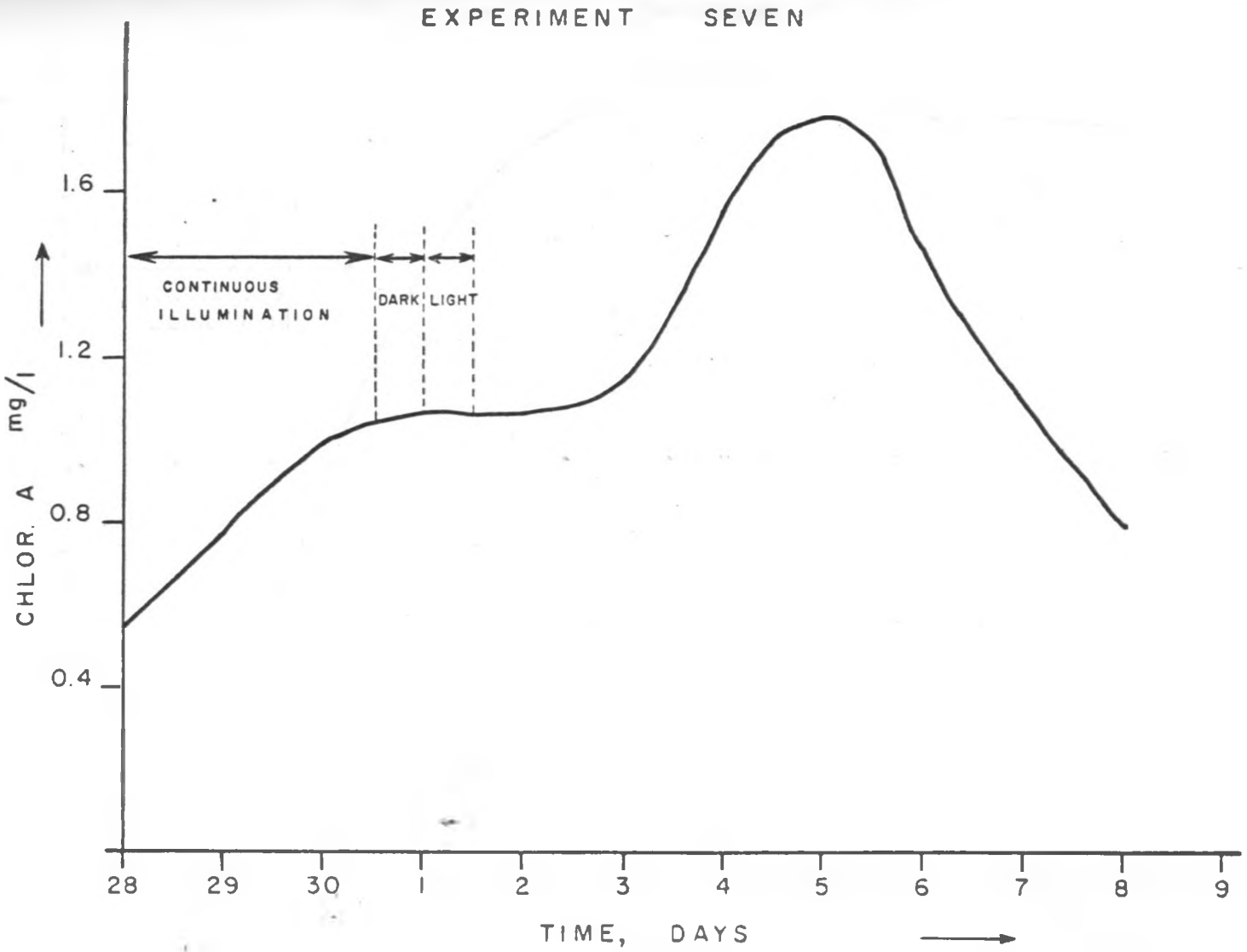


FIG. 5.9: DAILY CHLOROPHYLL VARIATION

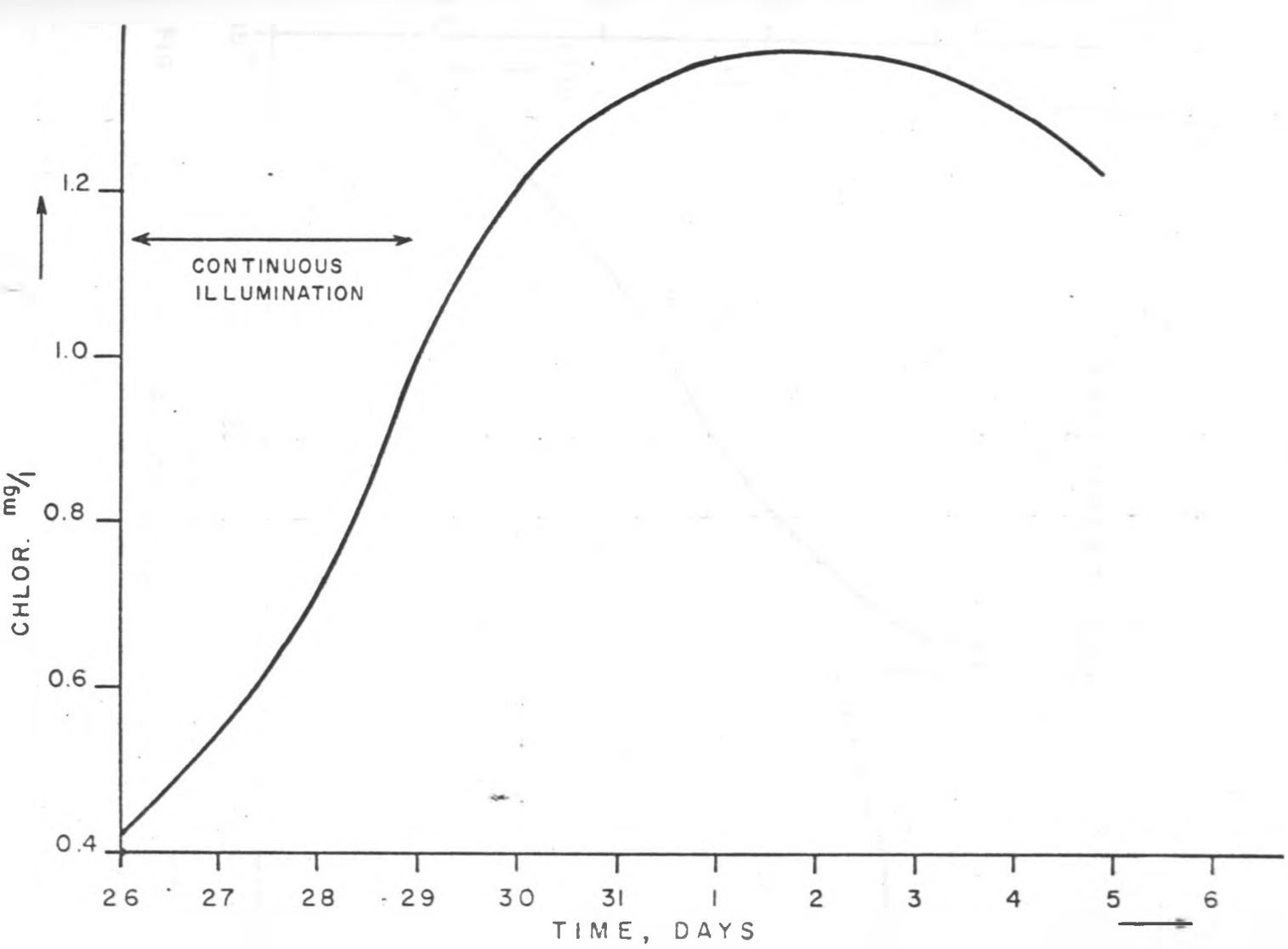


FIG. 5.10: DAILY CHLOROPHYLL VARIATION

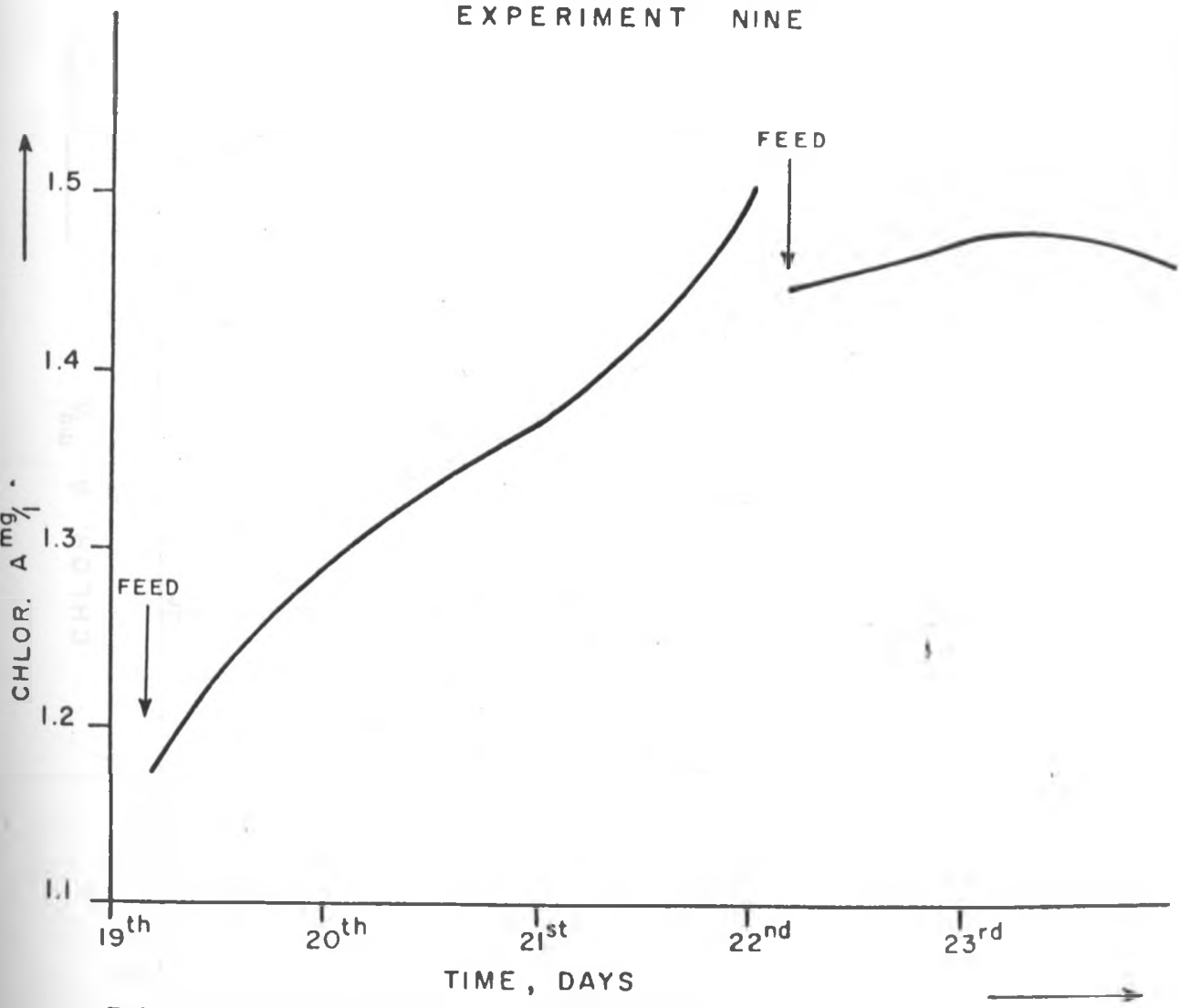


FIG. 5.11: CHLOROPHYLL A VARIATION

EXPERIMENT SEVEN

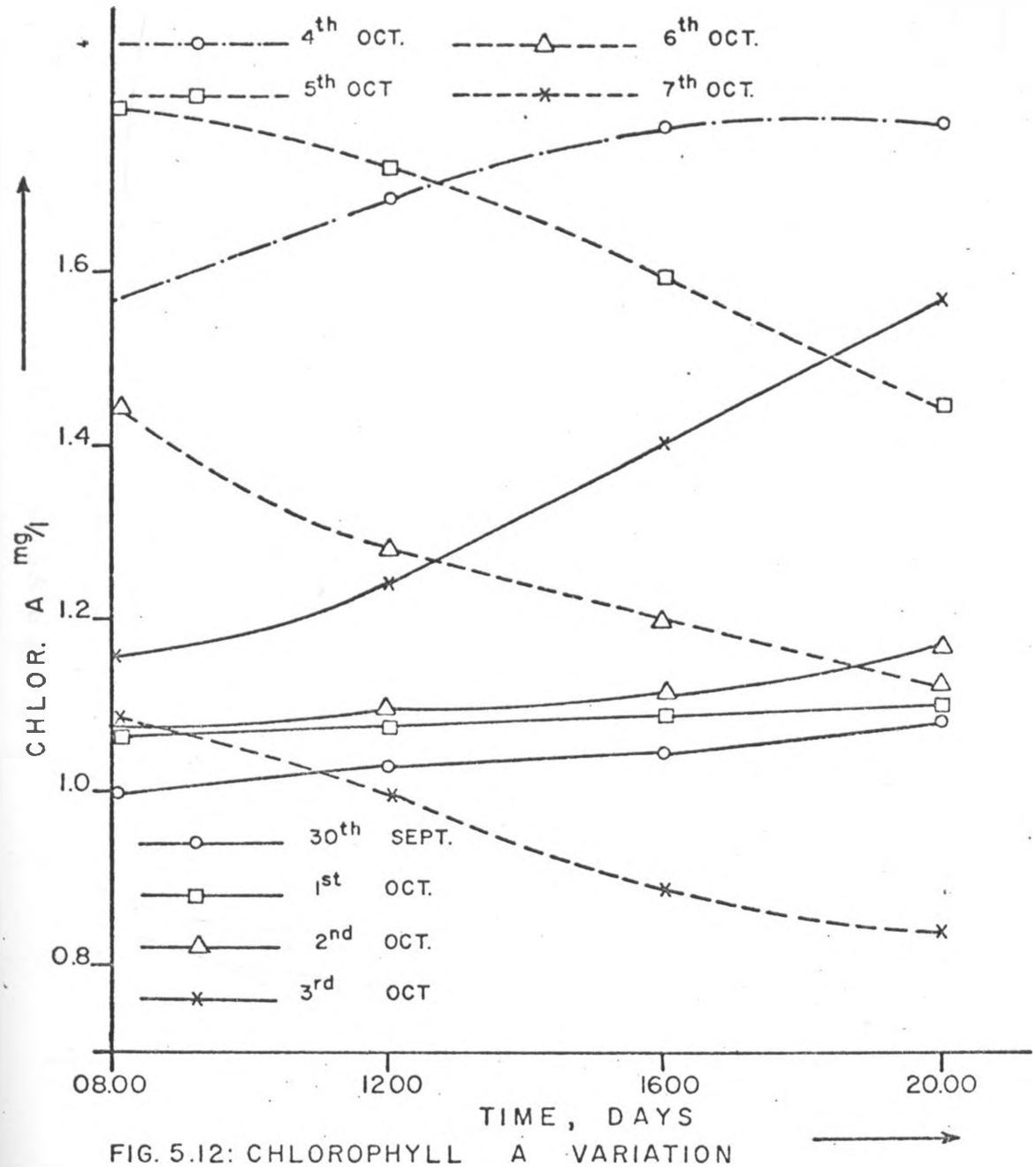


FIG. 5.12: CHLOROPHYLL A VARIATION

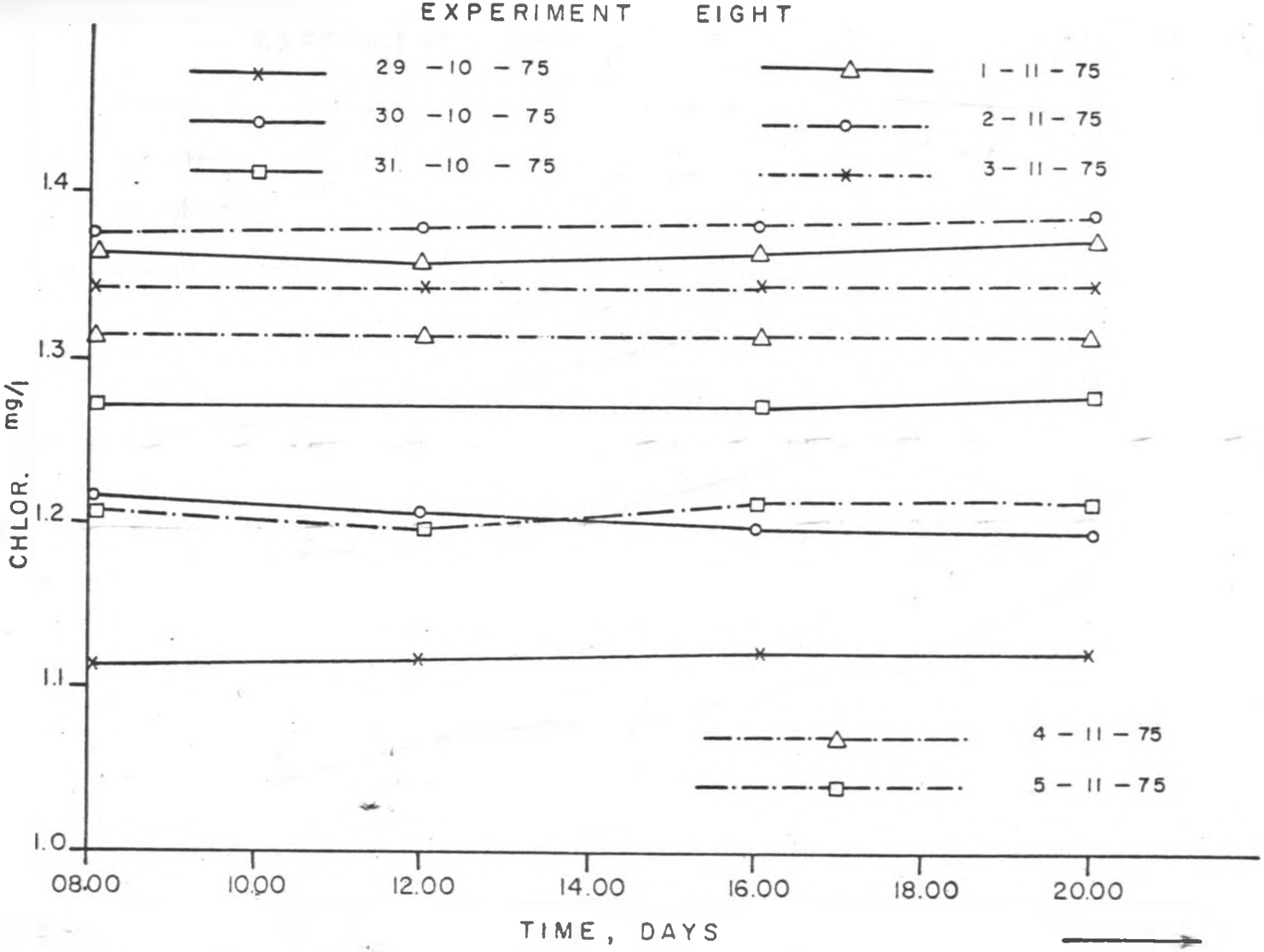


FIG. 5.13: DARK PERIOD - CHLOROPHYLL VARIATION

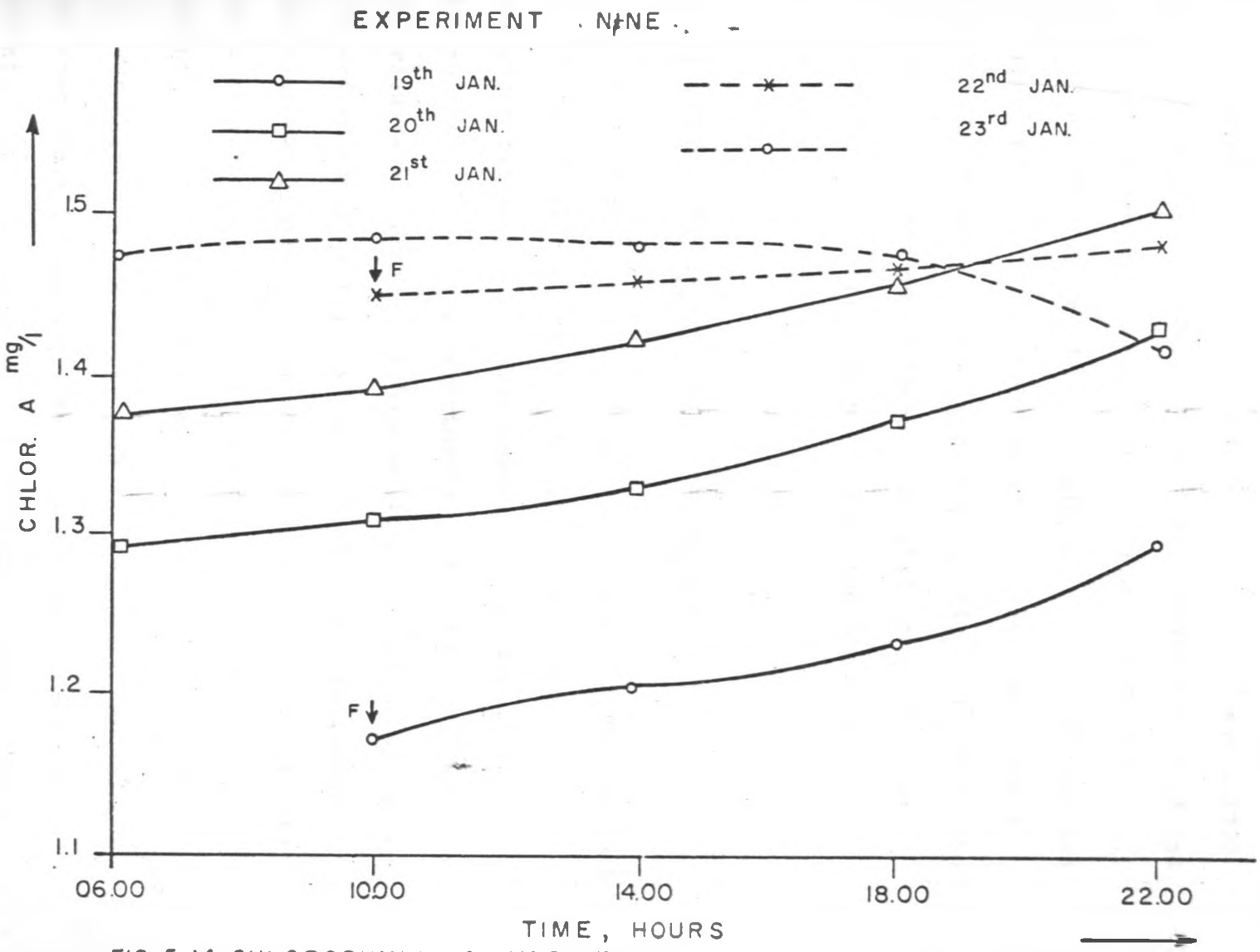


FIG. 5.14: CHLOROPHYLL A VARIATION

growth medium were withdrawn from the reactor and replaced with three litres of freshly prepared synthetic sewage. At this point the algal chlorophyll concentration was 1.1722 mg/l. After the first feed, logarithmic growth continued and a maximum chlorophyll concentration of 1.4341 mg/l was attained before a second feed was added. The second feed had the effect of "shifting" the growth phase from logarithmic growth to stationery growth (fig. 5.11). Fig. 5.14 shows the four hourly variations of algal chlorophyll concentration. There is an almost linear change during the logarithmic growth phases.

5.2.2 OXYGEN PRODUCTION/CONSUMPTION

Experimental results of dissolved oxygen concentrations in the biomass growth reactors are presented graphically in figures 5.1 to 5.8 for experiments one to five, and figures 5.15 to 5.20 for experiments seven to nine. The same results are shown in tabulated form in tables 5.3 to 5.18 and in appendix III, tables 1 to 20, for experiments one to nine. Examination of the graphs and tables shows that the dissolved oxygen content of the biomass growth reactor changed significantly with time.

Inoculation and start up of the biomass growth reactor was immediately followed by deoxygenation of the reactor contents. Dissolved oxygen concentrations in the reactors rapidly dropped to zero. The dissolved oxygen concentration remained at zero for some period of time. This time interval

varied from experiment to experiment e.g. in experiment seven it was three days and in experiment eight it was two days (Table 5.14 and 5.18). This period of zero dissolved oxygen concentration was followed by a day to day increase in the dissolved oxygen content of the biomass growth reactor. In most experiments, the "phase" of increasing concentration in dissolved oxygen was followed by a short period, during which there was no change in the dissolved oxygen concentration of the culture. In experiments which did not develop a "stationery dissolved oxygen period" the experimental runs were too short to allow for observation of the change mentioned above. In experiments seven and eight (Fig. 5.15 and 5.16) the "stationery" period was followed by a time of decreasing dissolved oxygen concentrations of the biomass growth units.

Initial deoxygenation of the biomass culture was followed, in experiments one to five, by rapid increases in the dissolved oxygen content of the biomass medium (figs 5.1 to 5.3 and 5.5, 5.7). Maximum dissolved oxygen concentration values recorded in experiments one and two were 5.80 and 4.90 mg/l respectively. At this time corresponding algal chlorophyll concentrations were 0.3850 mg/l in experiment one and 0.4050 mg/l in experiment two, 11.20 mg/l, 9.60 mg/l and 9.70 mg/l were the maximum dissolved oxygen concentrations in experiments three, four and five respectively. Corresponding algal chlorophyll concentrations were 0.8170, 0.4884 and 0.5725 mg/l. As already stated (see section 4.3.1 chapter 4), inoculation and biomass reactor start-up in

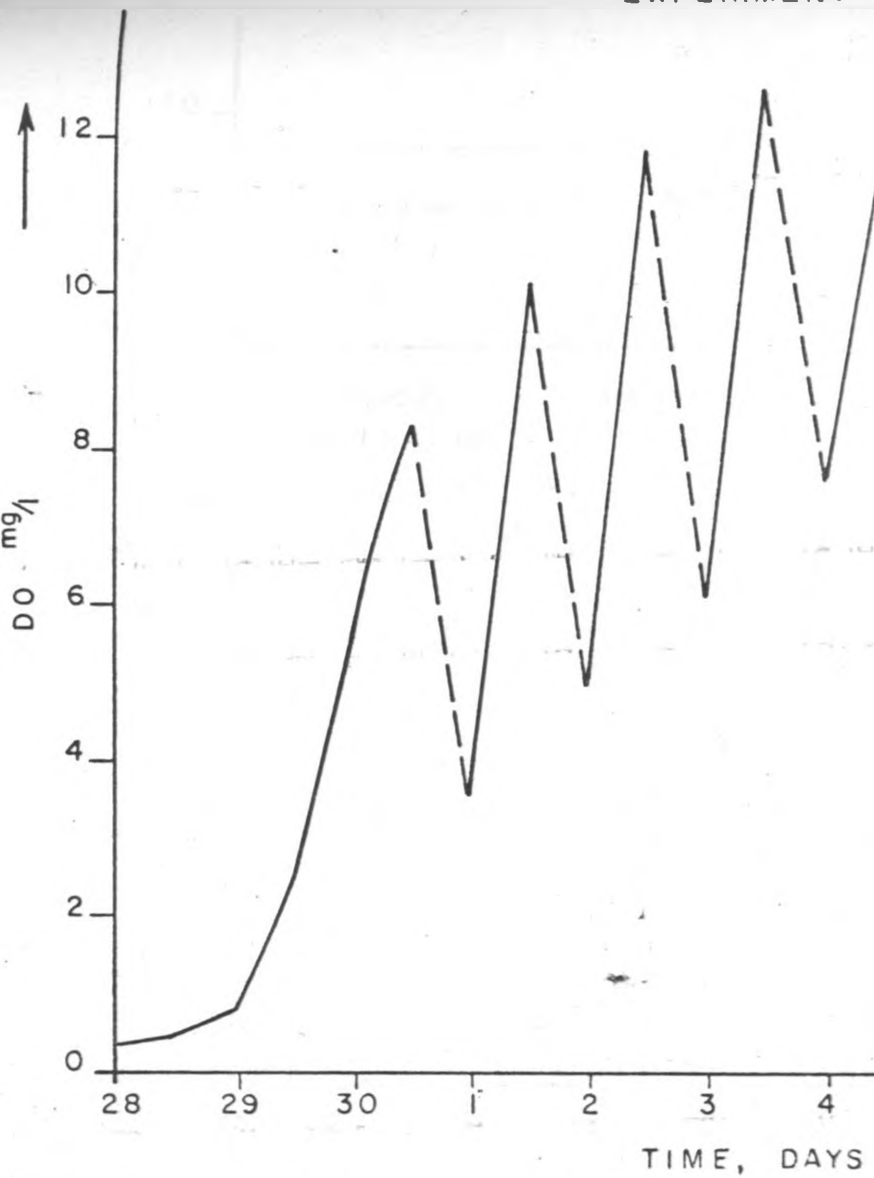
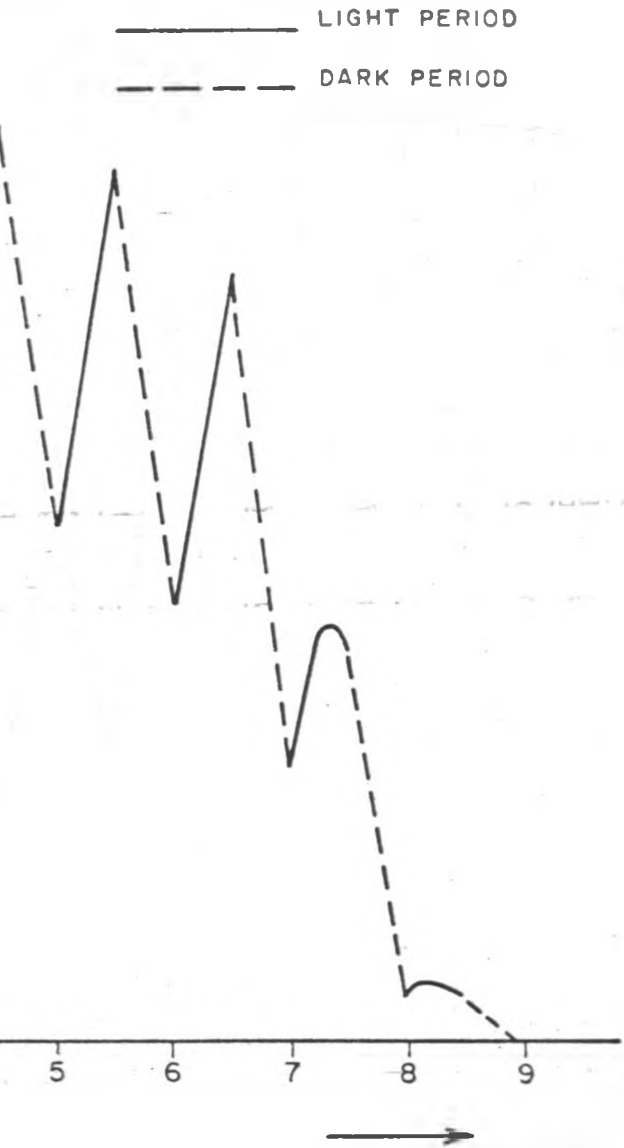


FIG. 5.15: DISSOLVED OXYGEN VARIATION

SEVEN



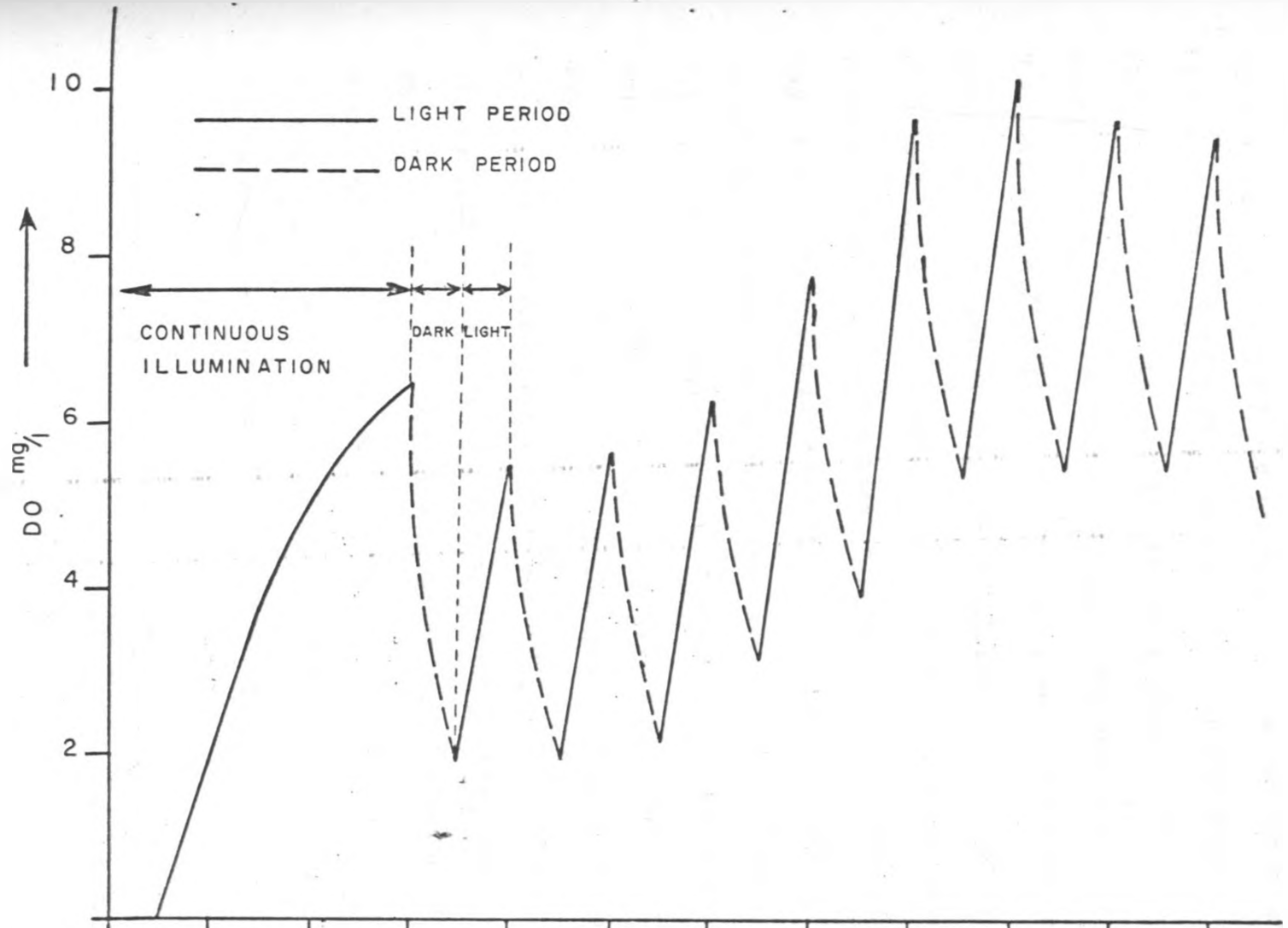


FIG. 5.16: DISSOLVED OXYGEN VARIATION

experiment six, was followed by deoxygenation of the growth medium, and then onset and progression of anaerobic conditions, (Tables 5.9 to 5.13). Experiment seven and eight followed the same pattern of initial deoxygenation of the growth medium, Initial dissolved oxygen concentrations of 7.10 and 4.53 mg/l rapidly decreased to zero in experiments seven and eight respectively. No measurable quantities of dissolved oxygen were produced for three days in experiment seven and two days in experiment eight. Measurable dissolved oxygen concentrations in both experiments lagged behind chlorophyll production by about two days (Table 5.14 and 5.18). Maximum dissolved oxygen concentrations measured during the experiments were 12.60 and 10.15 mg/l for experiments seven and eight respectively. Corresponding algal chlorophyll concentrations were 1.5192 and 1.3159 mg/l respectively. In figure 5.18 is presented the day-time hourly dissolved oxygen variation and in figure 5.19 the dark-period hourly dissolved oxygen variation or oxygen utilisation for experiment eight. The hourly dissolved oxygen variation curves can be approximately described by equations of the type:-

$$y = at + b \qquad (5.1)$$

where y = Dissolved oxygen concentration mg/l

a and b = constants

t = time, hours

e.g. for the 1st October, 1975 (Fig. 5.18)

$$y = 0.46t + 4.019 \qquad (5.2)$$

for the 21st January 1976 (Fig. 5.20)

$$y = 0.37t + 2.55 \qquad (5.3)$$

Under the experimental conditions existing in the biomass growth reactors, there was a linear variation of the dissolved oxygen concentration with time. This was observed to be especially so during the algal logarithmic growth phase. The night-time oxygen utilisation curves shown in figure 5.19 are typical. King (1970) found a similar night-time variation in the dissolved oxygen concentration of an enriched microcosm (Fig. 5.44). From the curves it can be seen that the rate of respiration is greatest at the start of the dark-period and decreases to a constant value by the end of the dark-period.

In the fill and draw experiment nine (Figs. 5.17 and 5.20), addition of synthetic sewage had the effect of decreasing the dissolved oxygen content of the biomass growth culture. On the first occasion of withdrawal and addition of feed, the immediate effect was an increase in the dissolved oxygen content of the biomass unit from 2.65 to 2.82 mg/l. This increase was due to the dissolved oxygen contained in the added synthetic sewage. Soon thereafter, the dissolved oxygen concentration decreased gradually to about 0.8 mg/l (Fig. 5.17), and then increased again. By the end of the light period the dissolved oxygen concentration had increased to 3.20 mg/l. On the second occassion, addition of feed had the same effect on the dissolved oxygen content of the growth unit as on the first time. However, on this occasion, the rate of decrease of the dissolved oxygen concentration was faster than on the previous fill and draw feeding. It was 0.80 mg/l/hr as compared to a rate of 0.50 mg/l/hr on

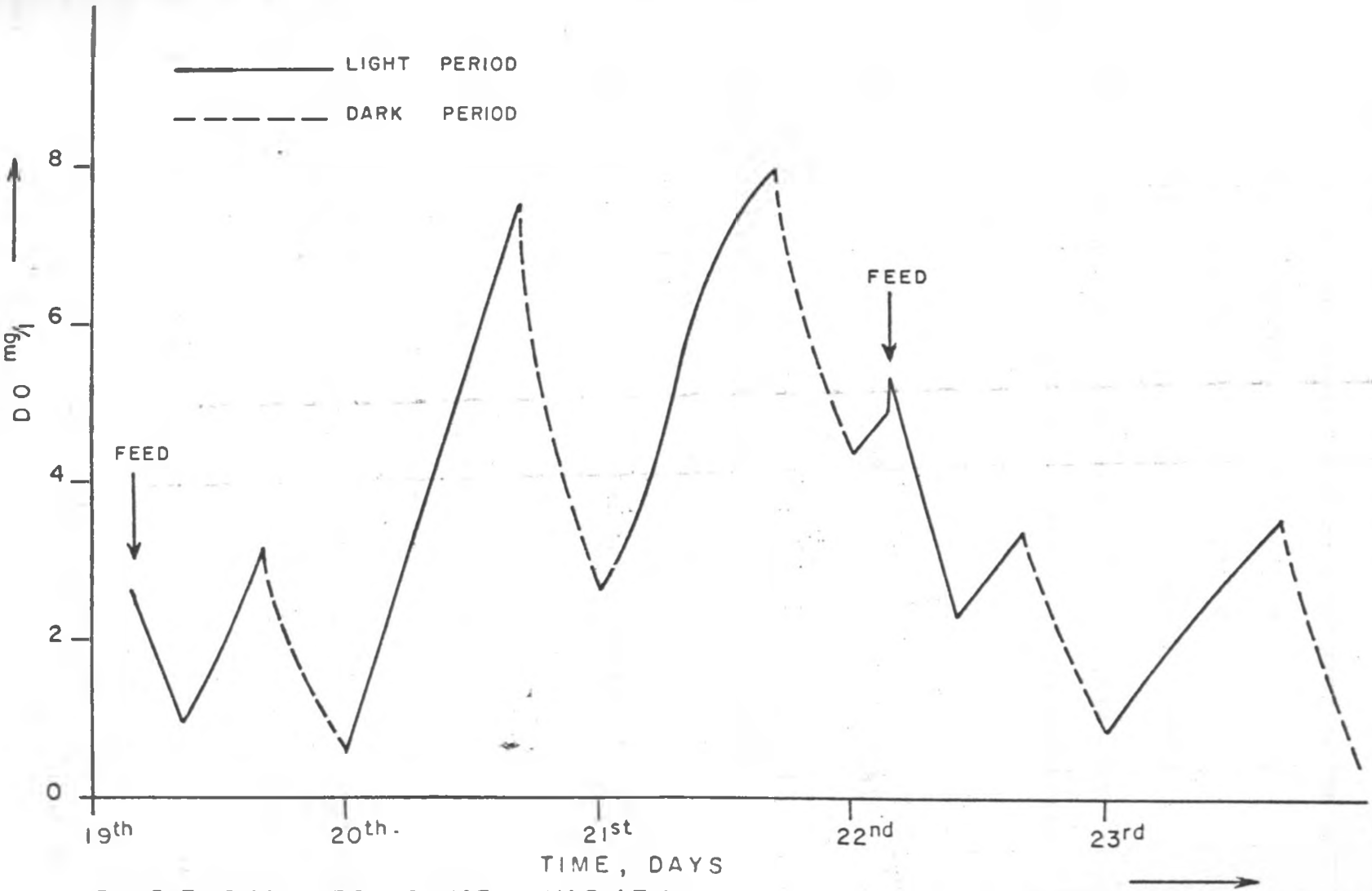


FIG. 5.17: DISSOLVED OXYGEN VARIATION

the first occasion. The subsequent increase rate in dissolved oxygen concentrations was slower (0.32 mg/l/hr) than on the first occasion (0.60 mg/l/hr). In addition the dissolved oxygen concentration at the end of the light period was lower (3.40 mg/l) than at the time of feeding (5.35 mg/l)

The rate of change in the concentration of dissolved oxygen in the reactor is described by the following differential equation.

$$\frac{d(DO)}{dt} = \frac{dP}{dt} - \frac{dR}{dt} \qquad (5.4)$$

where $\frac{d(DO)}{dt}$ = the rate of change of dissolved oxygen concentration (mg/l/t).

$\frac{dP}{dt}$ = the rate of photosynthetic oxygen production (mg/l/t)

$\frac{dR}{dt}$ = the rate of oxygen utilisation by the biota (mg/l/t).

From equation 5.4 it can be seen that when the rate of oxygen production is greater than the rate of oxygen utilisation, the rate of change of the dissolved oxygen concentration is positive, i.e. there is an increase in the dissolved oxygen content of the biomass growth reactor. A greater rate of oxygen utilisation than the rate of oxygen photosynthetic production results in a negative rate of change of dissolved oxygen concentration, i.e. a decrease in the dissolved oxygen content of the biomass growth unit. When the

EXPERIMENT SEVEN

NB: 1st OCT. $Y = 0.46t + 4.019$

2nd OCT. $Y = 0.58t + 4.7875$

3rd OCT. $Y = 0.55t + 6.1857$

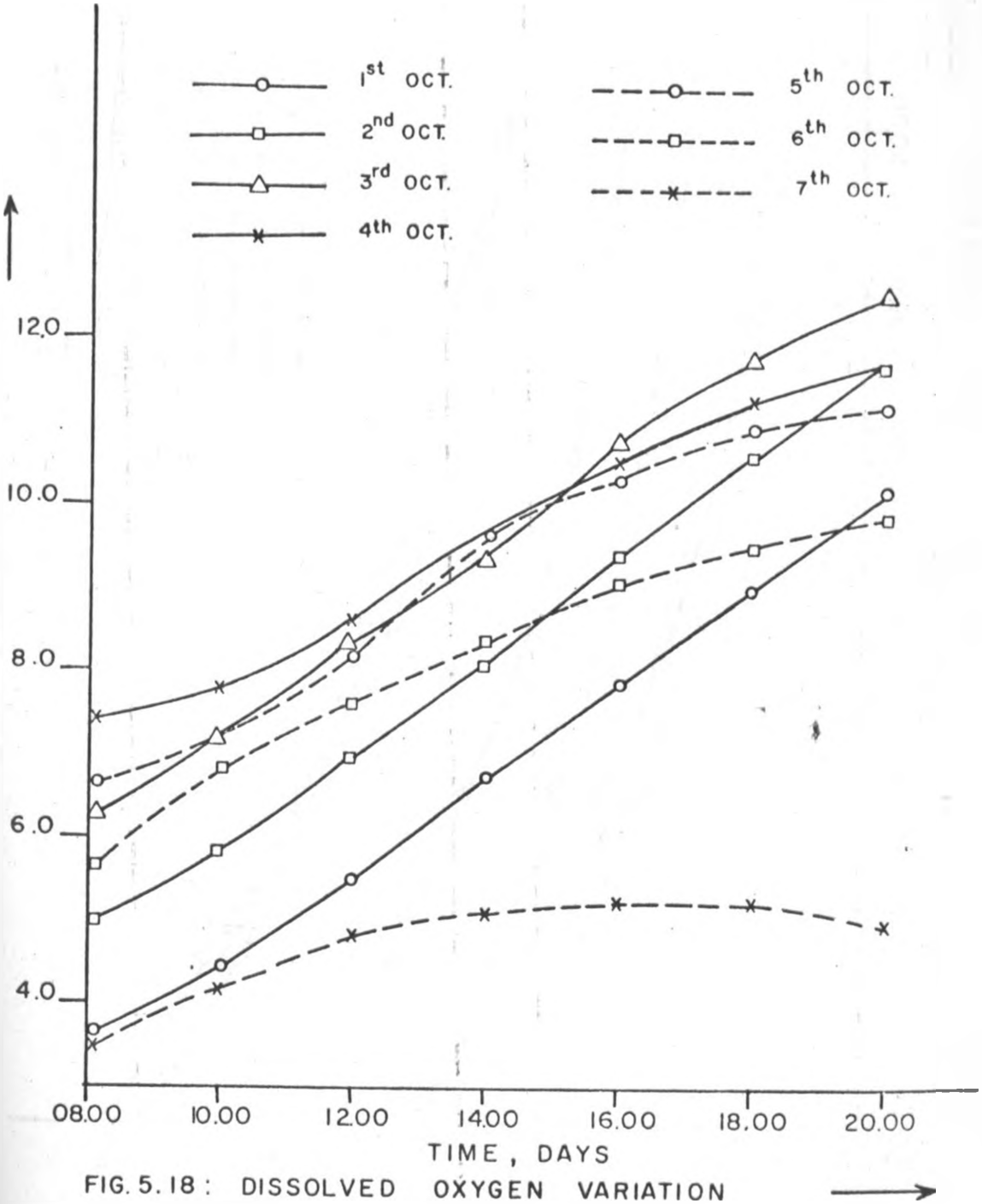


FIG. 5.18: DISSOLVED OXYGEN VARIATION

EXPERIMENT EIGHT

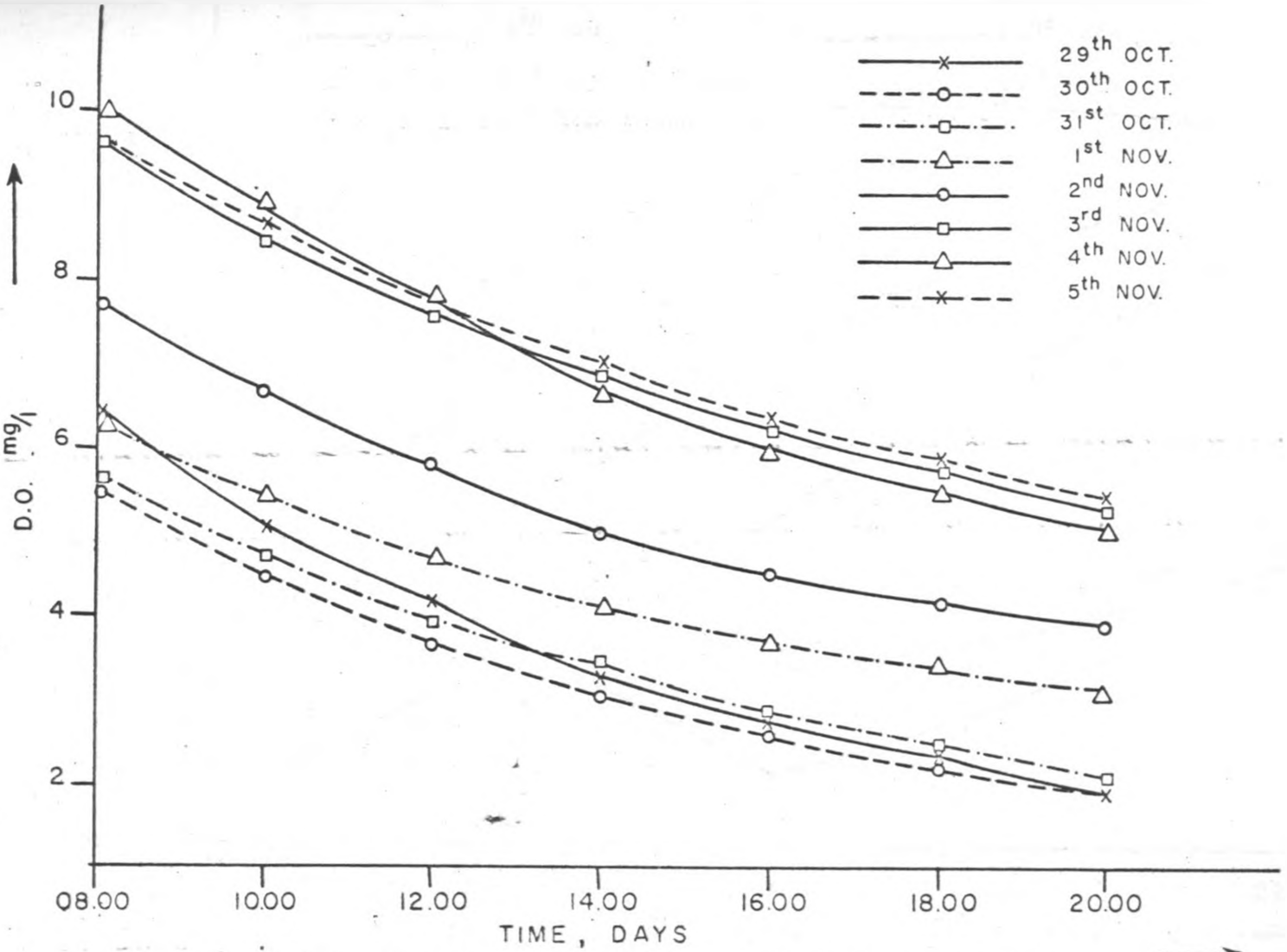


FIG. 5.19: DARK PERIOD — DISSOLVED OXYGEN VARIATION

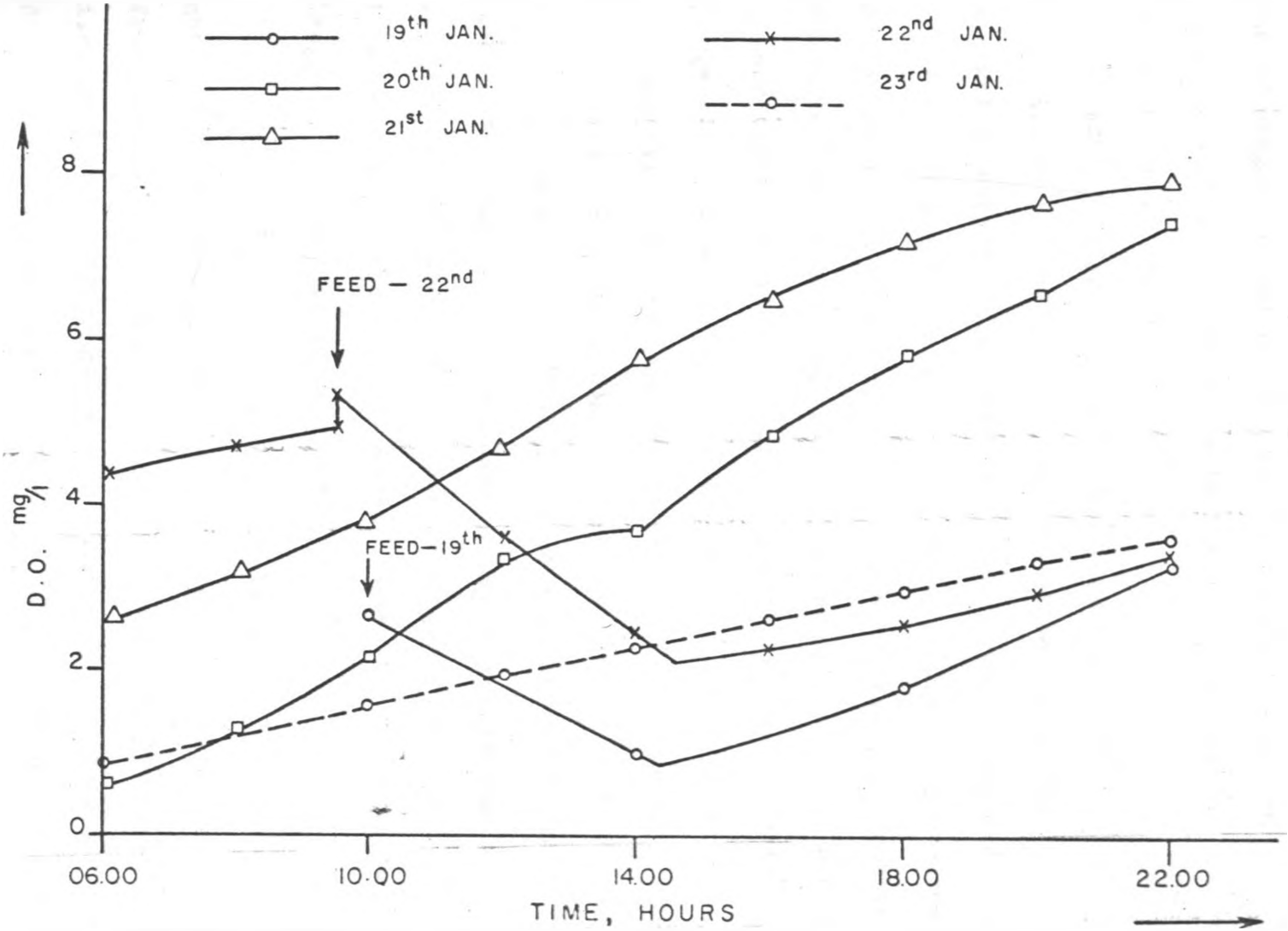


FIG. 5.20: DISSOLVED OXYGEN VARIATION

rate of oxygen production is equal to the rate of oxygen utilisation, there is no change in the dissolved oxygen concentration of the reactor.

Dissolved oxygen concentration values as shown in figure 5.1 to 5.8 and 5.15 to 5.20 cannot be correlated to algal chlorophyll concentration values because they are the result of algal photosynthetic oxygen productive processes and bacterial and algal (as well as protozoan etc) consumptive processes. In order to estimate oxygen production it is necessary to know the rates of oxygen utilisation as well as the rates of change of dissolved oxygen (see equation 5.4). Table 1 to 7 (see appendix III) and figures 5.21 to 5.29 show dissolved oxygen concentrations, oxygen production and utilisation rates during experiment seven. Table 8 to 15 (appendix III) and figures 5.30 to 5.39 depict dissolved oxygen; oxygen production and utilisation rates during experiment eight. For the fill and draw experiment nine these values are shown in tables 16 to 20 (appendix III) and in figures 5.40.

In experiment eight, the average respiration rate was obtained by finding the mean of the respiration rates calculated from the dissolved oxygen values recorded at two hourly intervals i.e. the two hourly oxygen uptake rates of the biomass for the duration of the dark period (Tables 8 to 15). This average respiration rate values was found to be the same as the average respiration rate value calculated thus:-

$$R_o = \frac{DO_I - DI_F}{T} \qquad (5.5)$$

where, R_o = average respiration rate, mg/l/t

DO_I = Dissolved oxygen concentration at the beginning of the dark-period, mg/l

DO_F = Dissolved oxygen concentration at the end of the dark period, mg/l

T = Duration of the dark period, hours.

The oxygen consumed during the dark period was determined in two ways.

1. By summation of the respiration rates and multiplying the sum by two or by summation of D.O. consumed during the two hourly periods recorded.
2. By multiplying the average respiration rate (R_o) calculated using equation (5.5), by the duration of the dark period T, or by subtracting the dissolved oxygen concentration at the end of the dark period (DO_F) from the dissolved oxygen concentration at the beginning of the dark period (DO_I).

The above methods gave nearly the same values for the average respiration rates and total oxygen utilisation.

The method adopted for calculating night respiration in experiments seven and nine, in which the oxygen uptake

rates (night-time) had not been recorded was the following.

1. Calculate the average respiration rate using equation 5.5.
2. Multiply the average respiration rate value obtained in (1) above by the duration of the dark-period in hours.

The average respiration values obtained by the methods above consist of three components.

1. The oxygen utilisation rates of the algae.
2. The oxygen utilisation rates of the bacteria
3. The oxygen utilisation rates of the protozoa and zooplankton.

For night-time respiration all the three components were operative and were correctly reflected in the average respiration rate calculated as above (equation 5.5).

Since it was difficult under the experimental conditions, to monitor the respiration of the biota during the light period, the value obtained above was assumed to reflect respiration rates obtaining in the biomass growth medium during this period. This assumption introduces some error as:-

1. During the light-period algae do not respire but instead produce oxygen
2. Photo-respiration may or may not be a factor affecting oxygen utilisation during this period
3. Bacterial respiration, under the experimental conditions, is a function of time, growth phase

(metabolic and physiological state) and the availability of biodegradable organic matter.

4. Protozoan respiration is also a function of time, bacterial population and has been shown to affect oxygen uptake rates in a heterogeneous community such as obtained during the experiment (Canale, 1974).

Since the effect of all these factors could not be assessed in the growth unit and the respiration rates obtaining during the dark period were assumed to represent respiration rates obtaining during the light period following the dark period, it was decided to use two values, representing respiration rates during the light period for calculating oxygen production and oxygen utilisation rates (see Tables 1 to 20 **appendix III)**

The first value of average respiration rate (shown in the top row in Tables 1 to 20) during the light period was assumed to be the same as the average respiration rate for the preceeding dark period and calculated using equation 5.5.

The second value of average respiration rate (shown in the bottom row in Tables 1 to 20) for the light period was calculated by determining the average of the average rates of respiration during the dark periods preceeding and following the light period using equation 5.5.

$$\text{i.e.} \quad R = \frac{R_o + R_1}{2} \qquad (5.6)$$

where R = Average rate of respiration during the light period mg/l/t.

R_o = Average rate of respiration during the dark period preceeding the particular light period calculated by equation (5.5)

R_1 = Average rate of respiration during the dark period following the particular light period calculated by equation (5.5)

Using the above values, the rates of photosynthetic oxygen production were calculated by equation 5.4.

From equation 5.4 it can be seen that a wrong estimation of the rate of respiration results in a wrong value for oxygen production rate. The two values used for respiration rates, can be regarded as permitting calculation of upper and lower limits of oxygen production rates (Tables 1 to 20).

Average respiration rates of the biota are complex functions of algal, bacterial, protozoan, zooplankton respiration rates, which in turn depend on the availability of biodegradable organic matter, their individual growth states time of day etc. The assumptions made above, make it possible to approximate the true value or at least delineate the range within which the true respiration rates lie. Thus they are useful in estimating the ranges of oxygen production and utilisation. (Tables 1 to 20).

Figures 5.21 to 5.27 show the hourly oxygen production rate variation (see also tables 1 to 7, appendix III) during experiment seven. In general all the graphs have two peak rates of oxygen production in one light period. From the beginning of the light period there is a gradual increase in

EXPERIMENT SEVEN

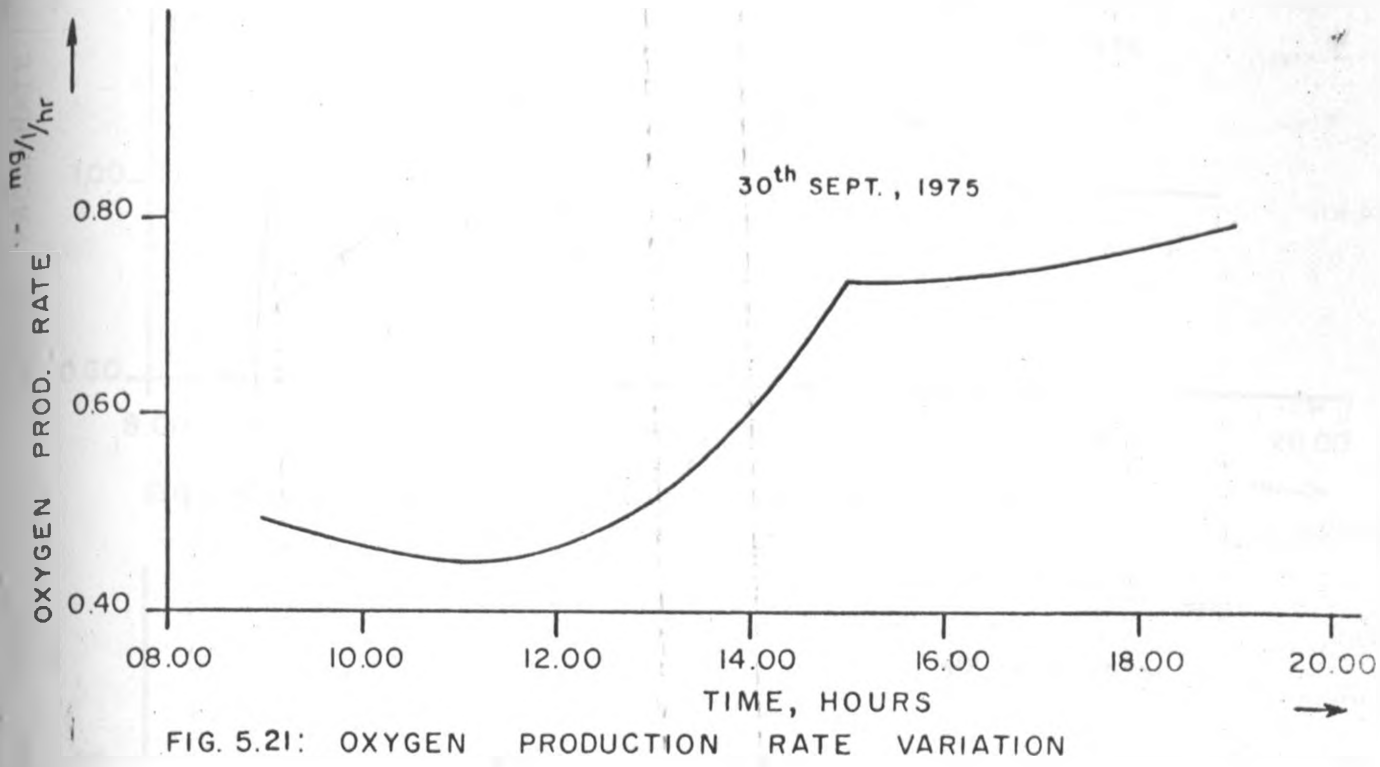


FIG. 5.21: OXYGEN PRODUCTION RATE VARIATION

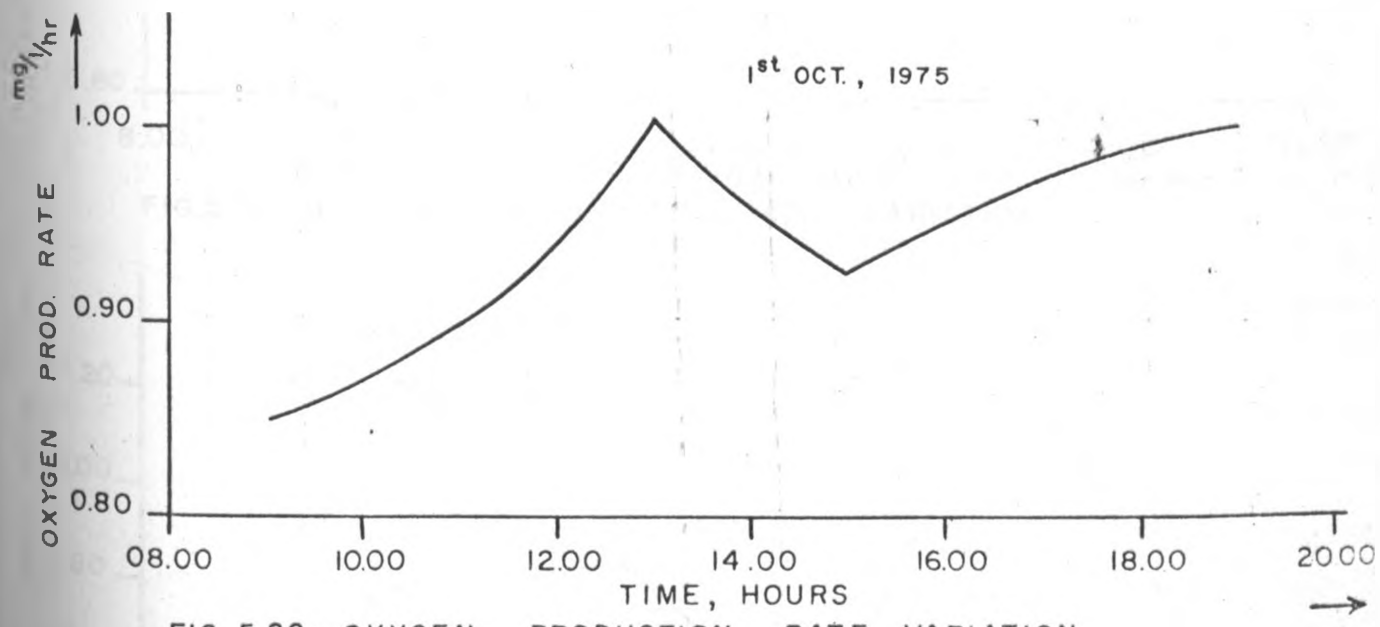


FIG. 5.22: OXYGEN PRODUCTION RATE VARIATION

EXPERIMENT SEVEN

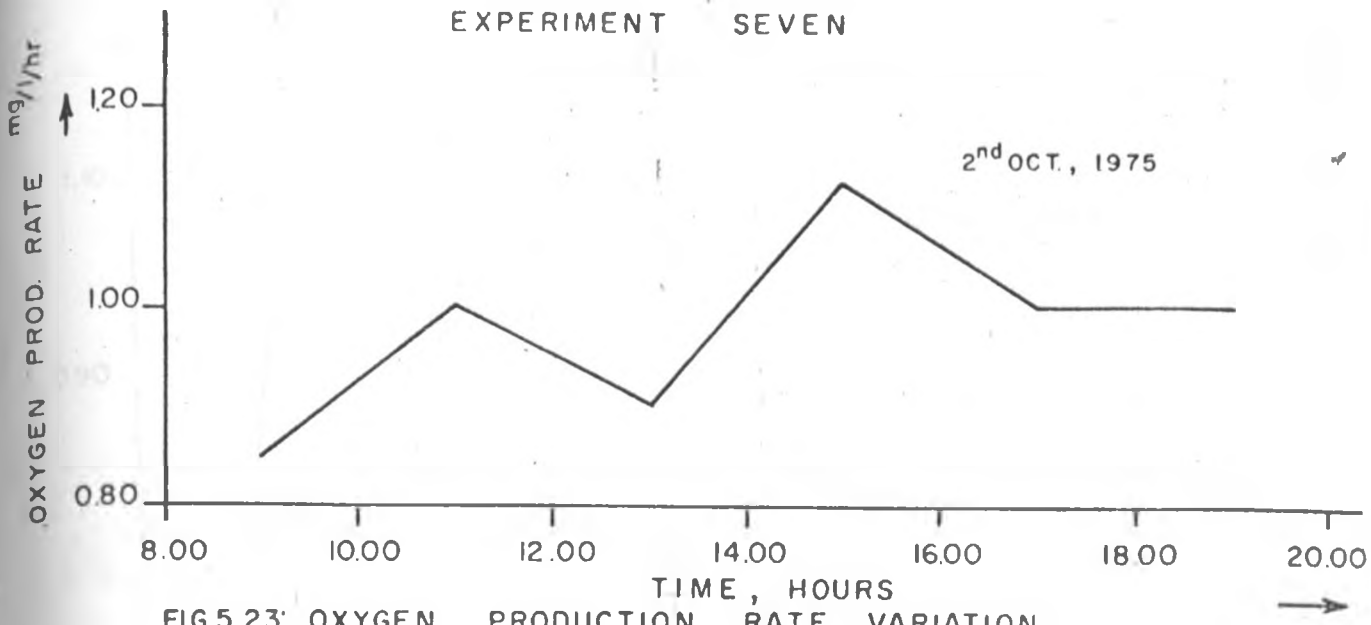


FIG.5.23: OXYGEN PRODUCTION RATE VARIATION

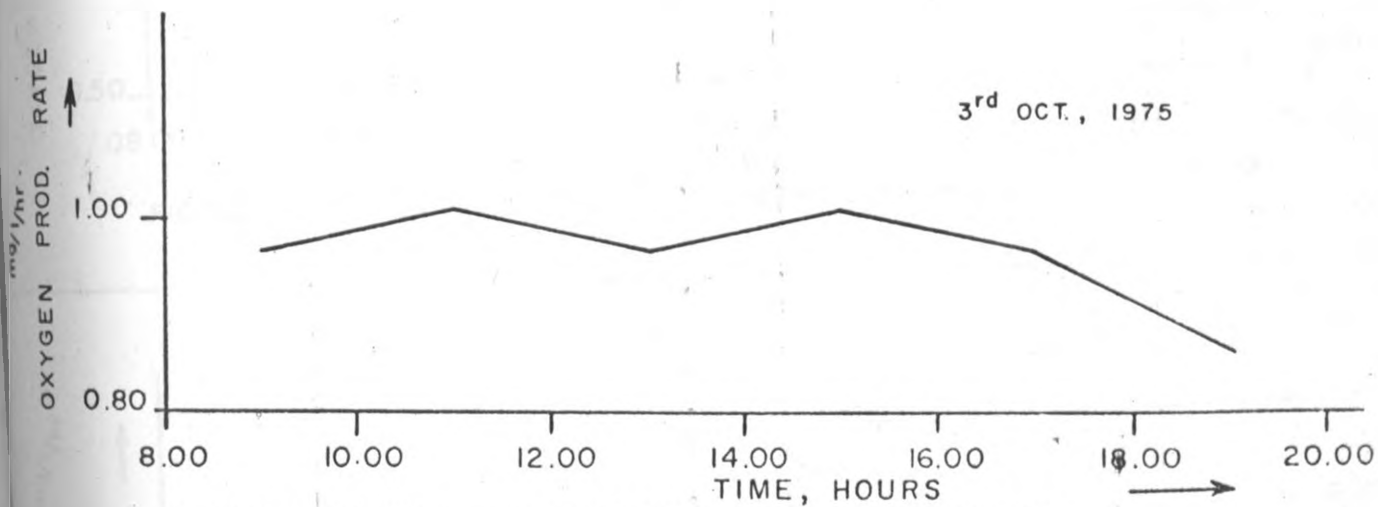


FIG. 5.24: OXYGEN PRODUCTION RATE VARIATION

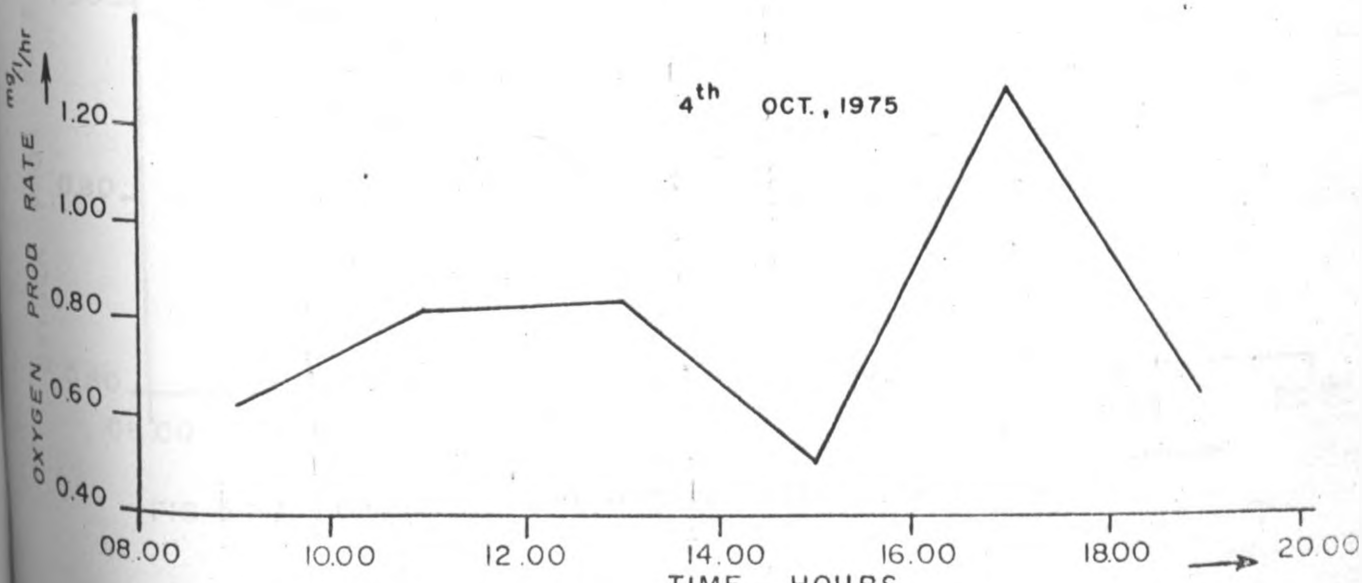


FIG. 5.25: OXYGEN PRODUCTION RATE VARIATION

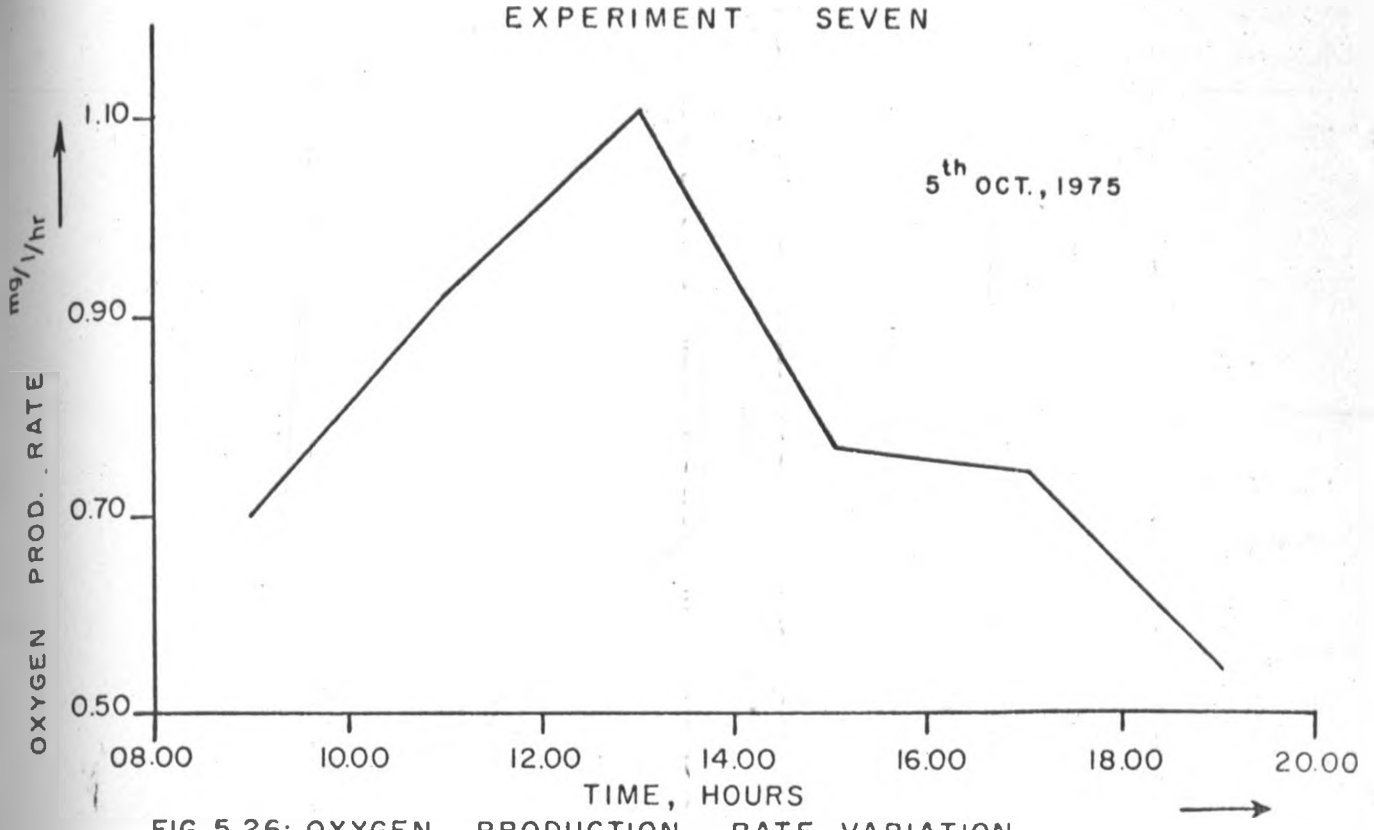


FIG. 5.26: OXYGEN PRODUCTION RATE VARIATION

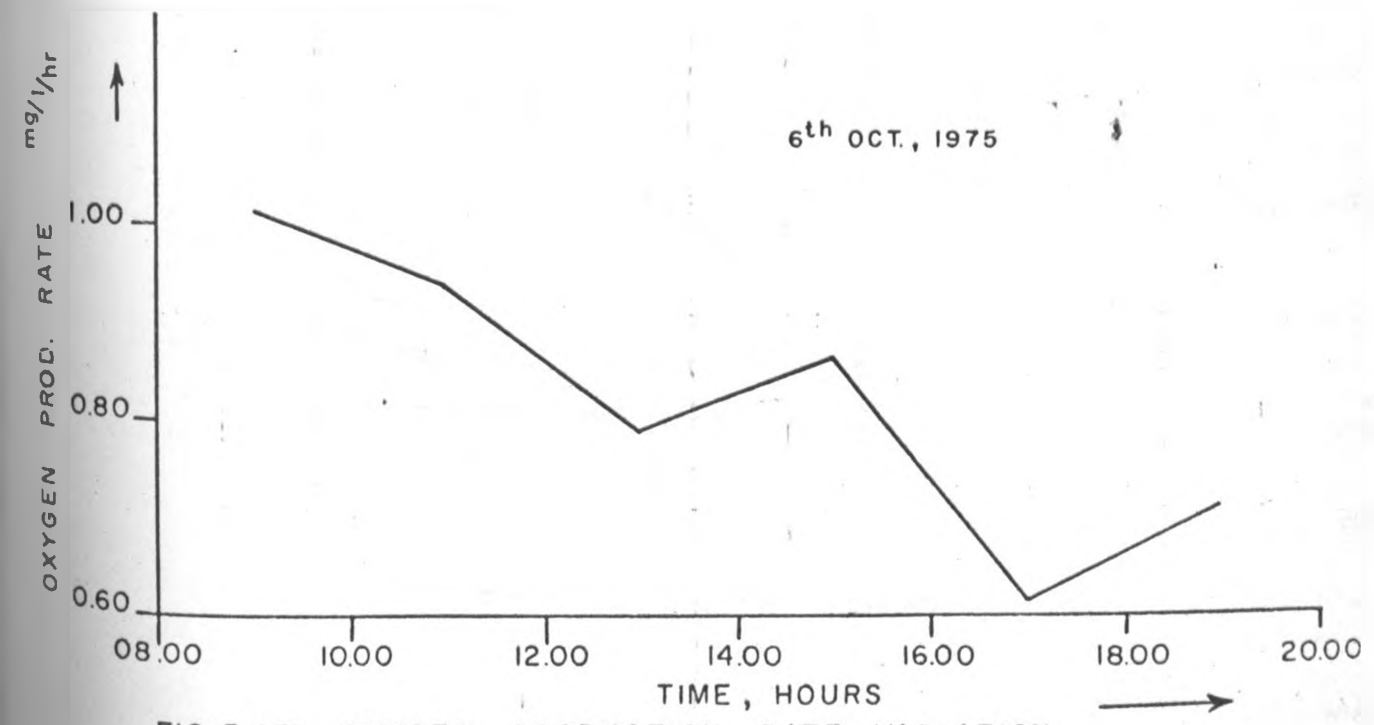


FIG. 5.27: OXYGEN PRODUCTION RATE VARIATION

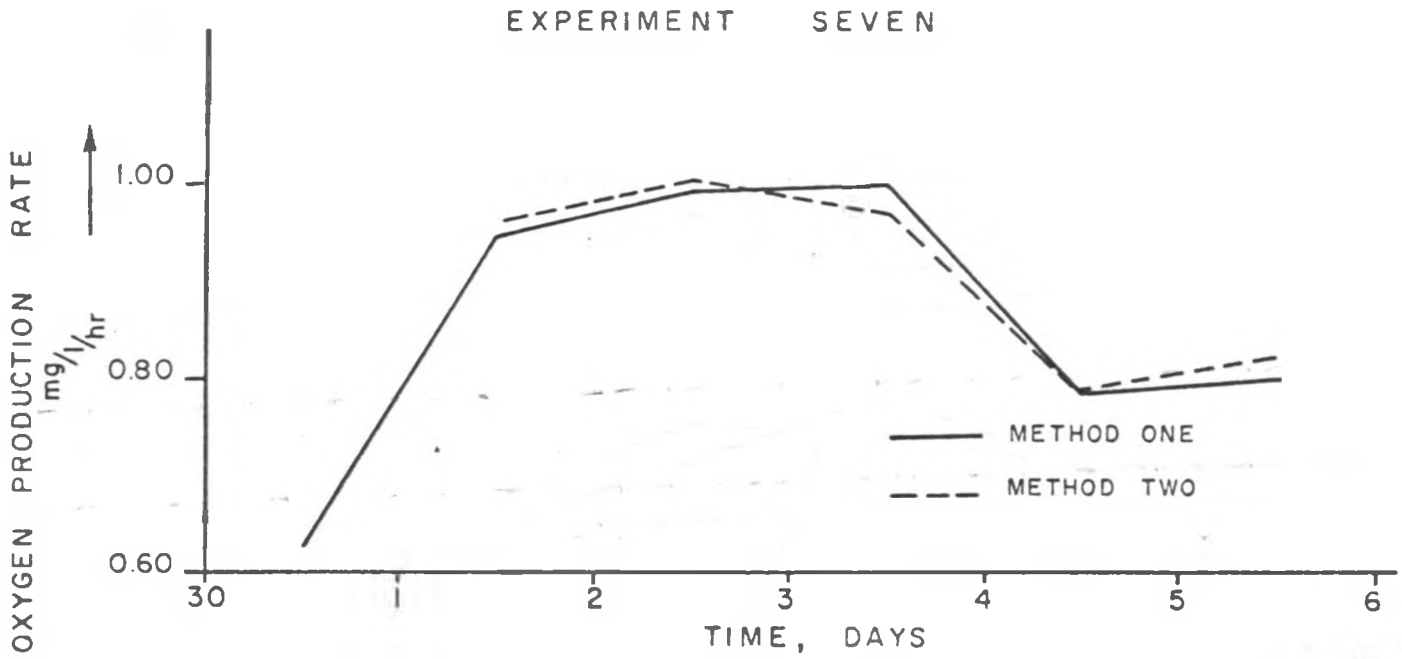


FIG.5.28: OXYGEN PRODUCTION RATE DAILY VARIATION

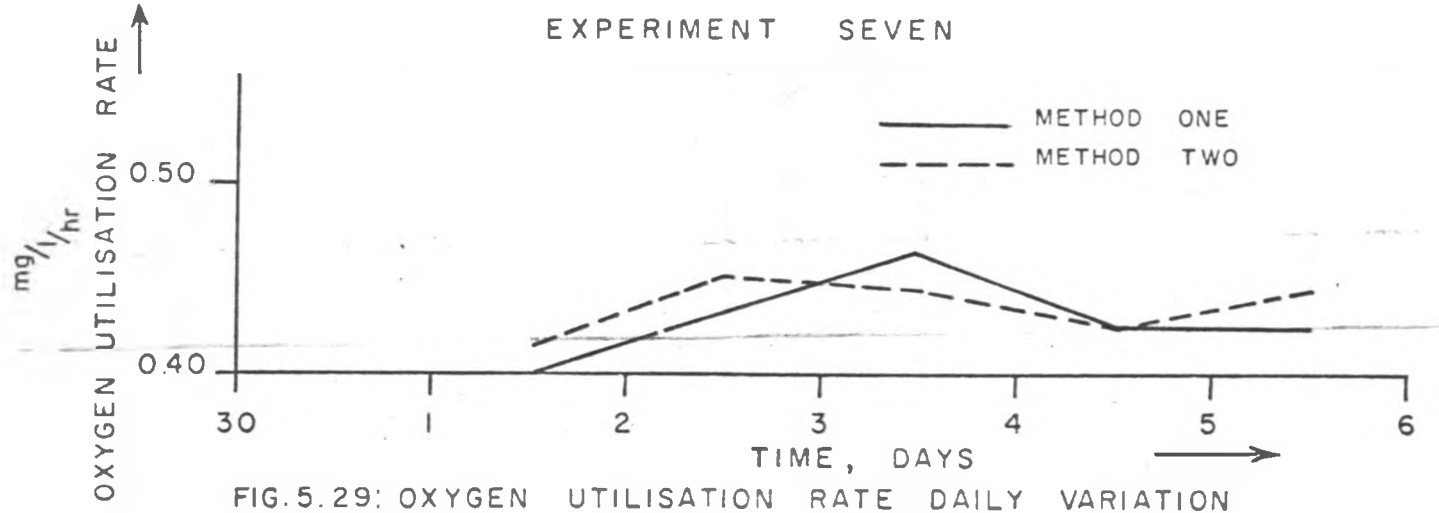


FIG. 5.29: OXYGEN UTILISATION RATE DAILY VARIATION

EXPERIMENT EIGHT

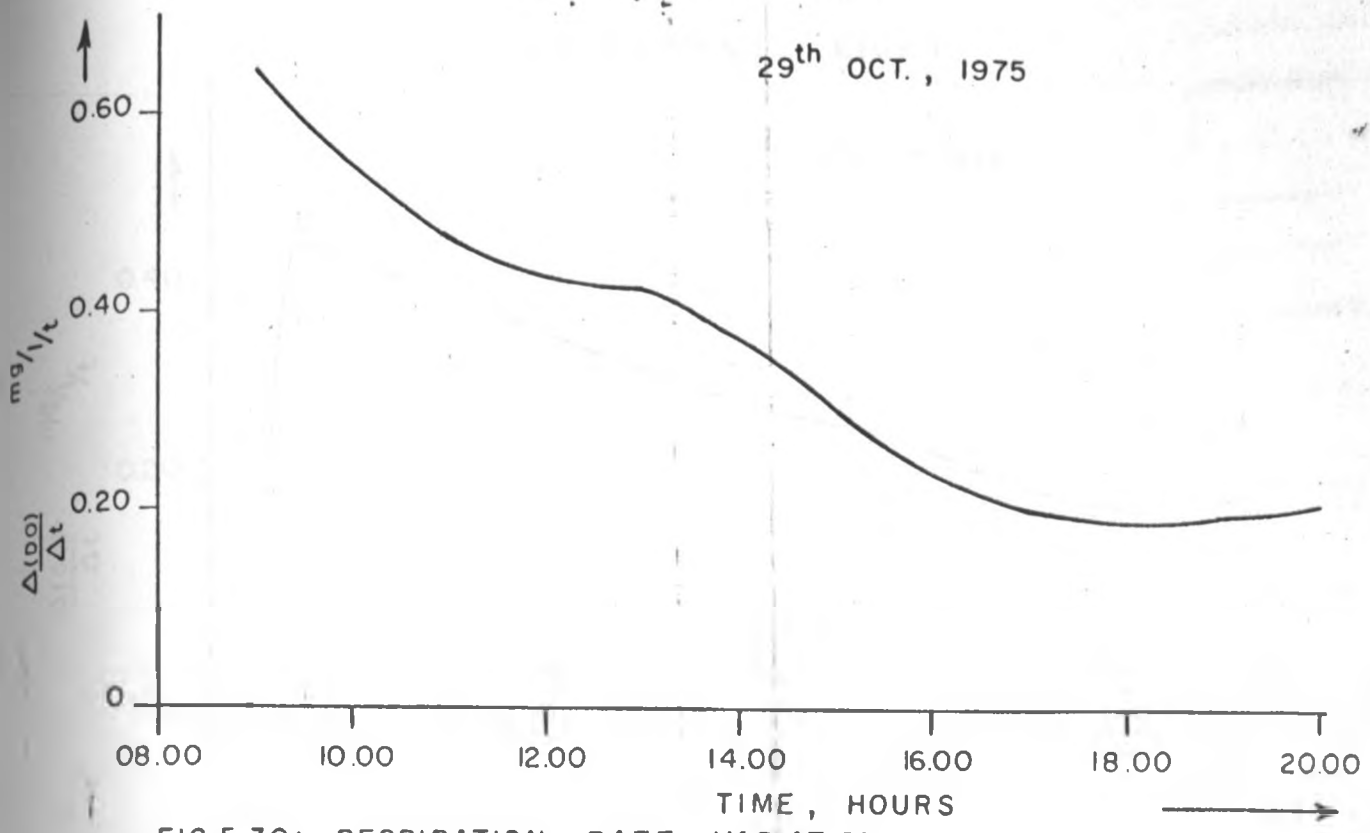


FIG. 5.30: RESPIRATION RATE VARIATION

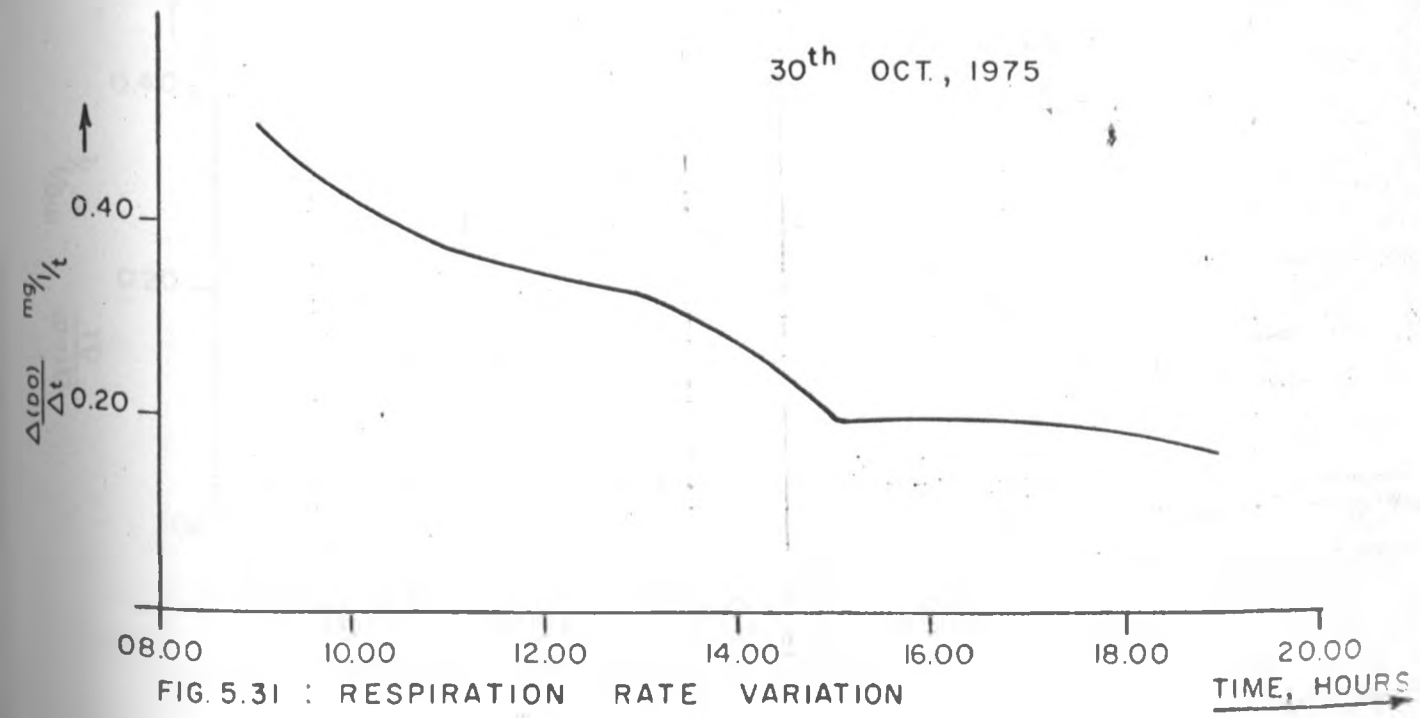


FIG. 5.31 : RESPIRATION RATE VARIATION

EXPERIMENT EIGHT

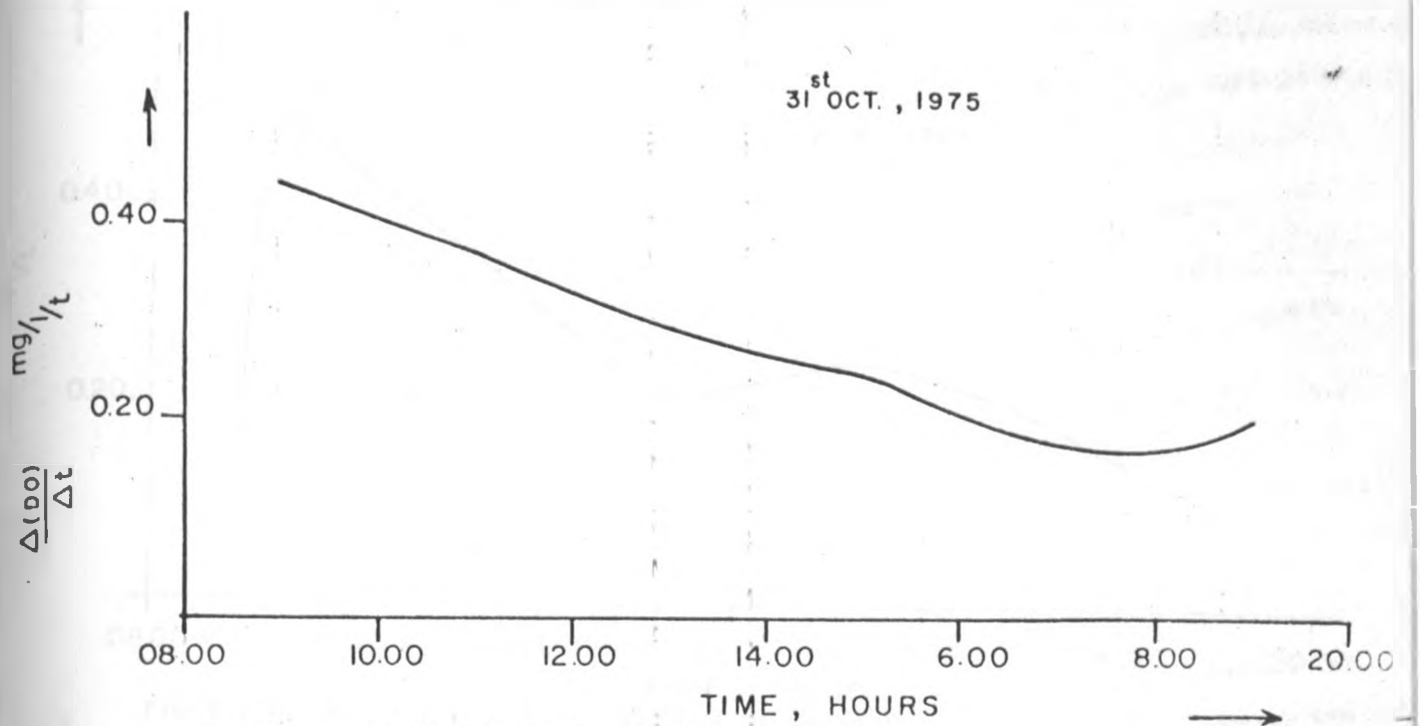


FIG. 5.32 : RESPIRATION RATE VARIATION

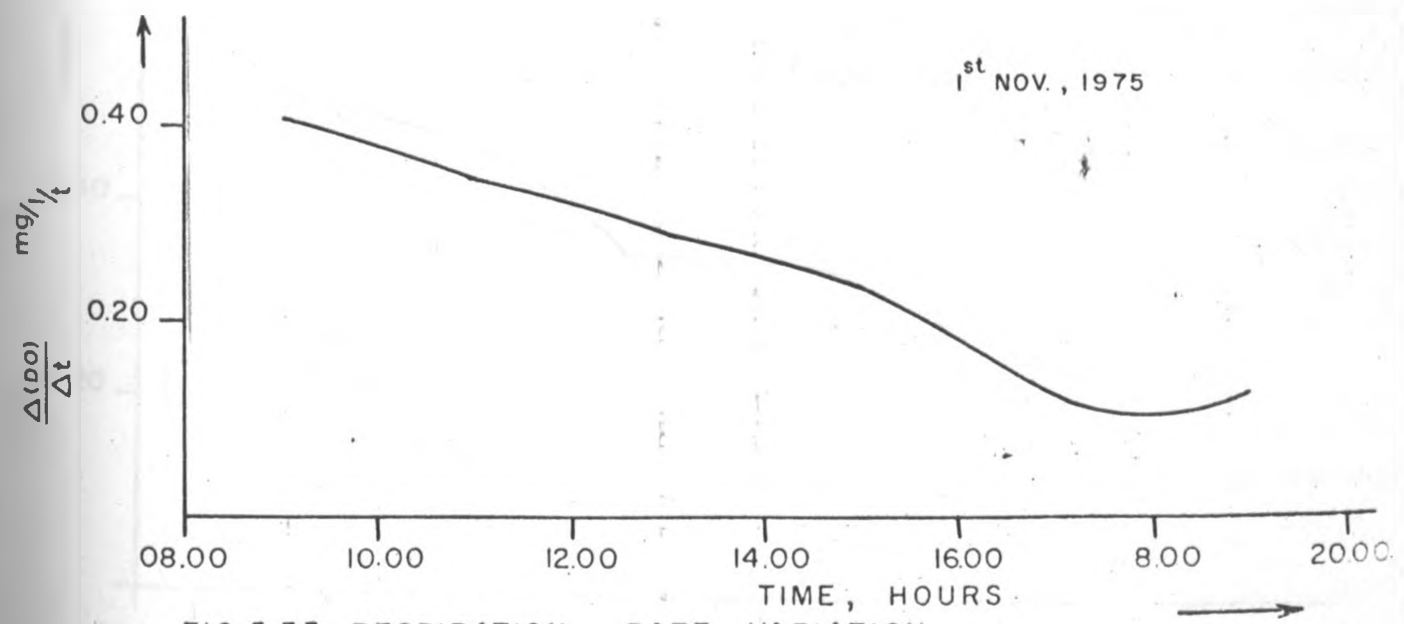


FIG. 5.33: RESPIRATION RATE VARIATION

EXPERIMENT EIGHT

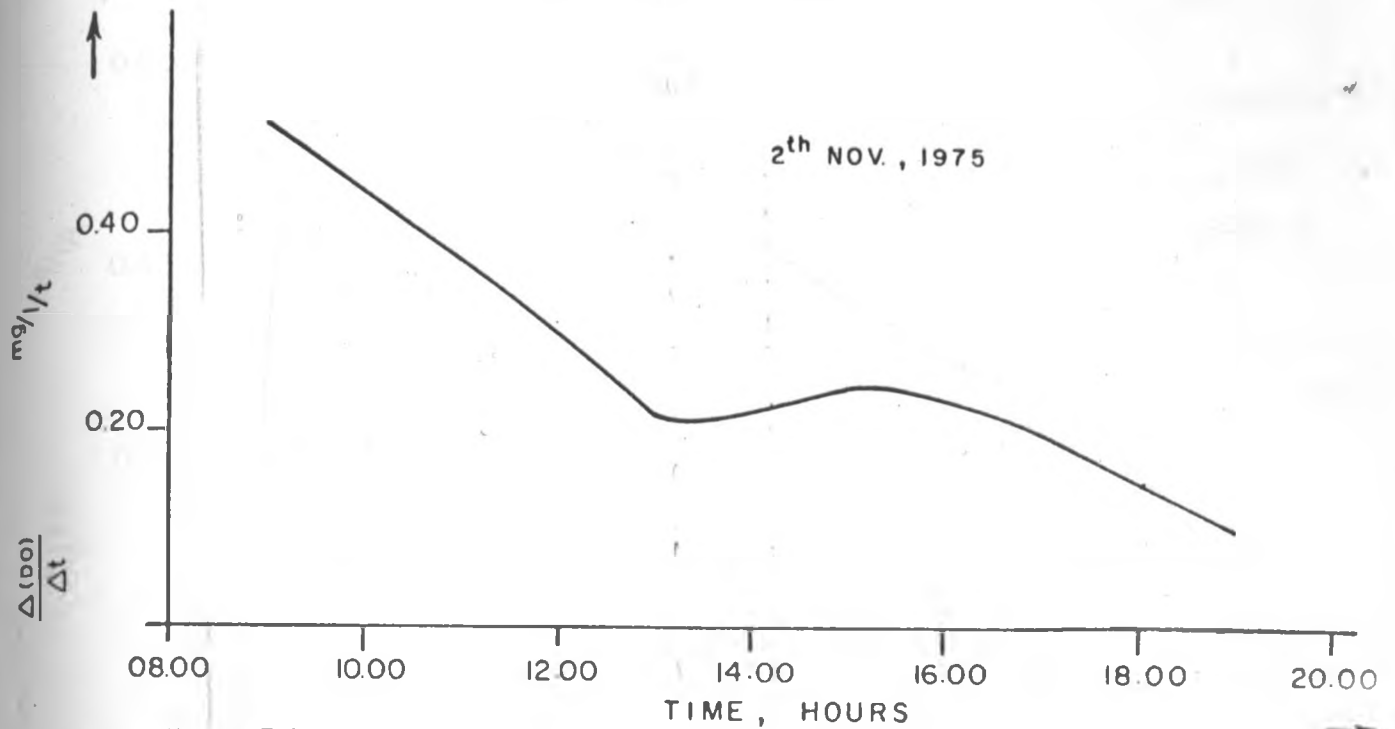


FIG. 5.34: RESPIRATION RATE VARIATION

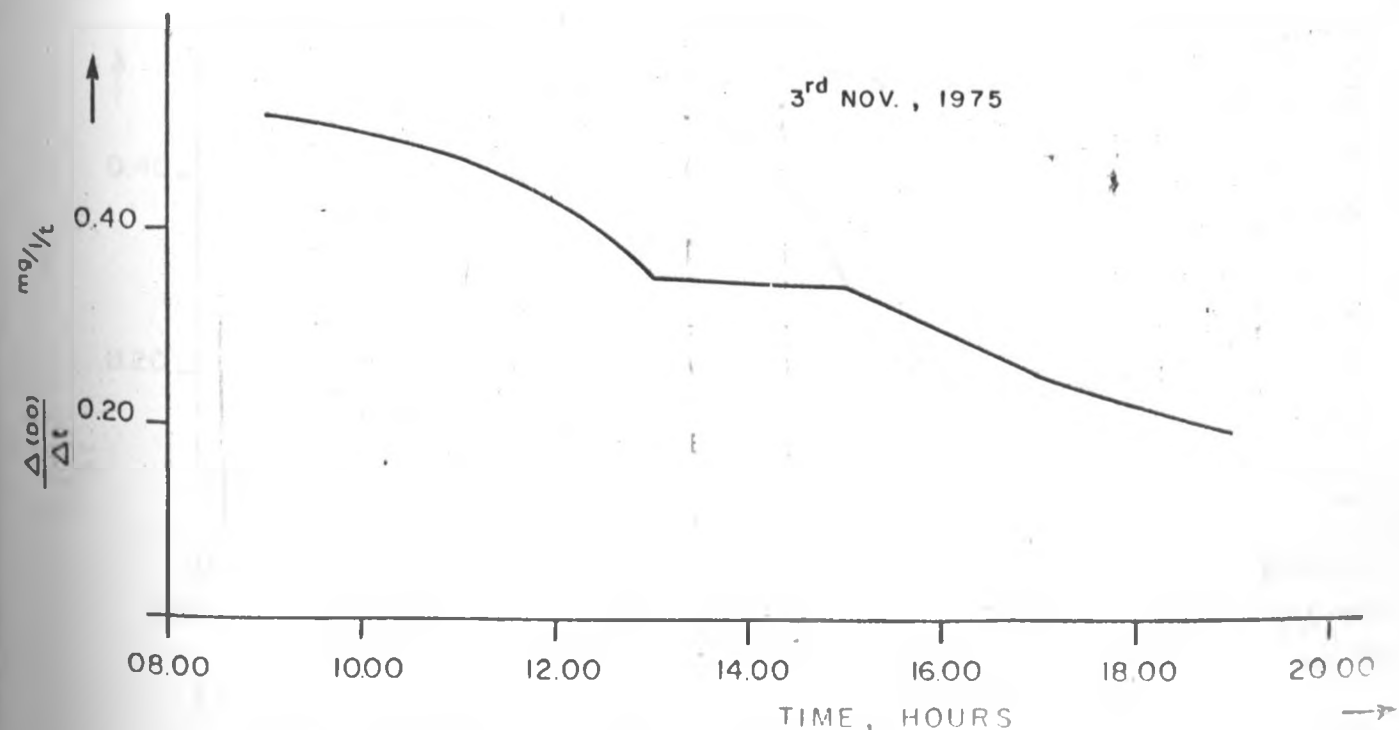


FIG. 5.35 RESPIRATION RATE VARIATION

EXPERIMENT EIGHT

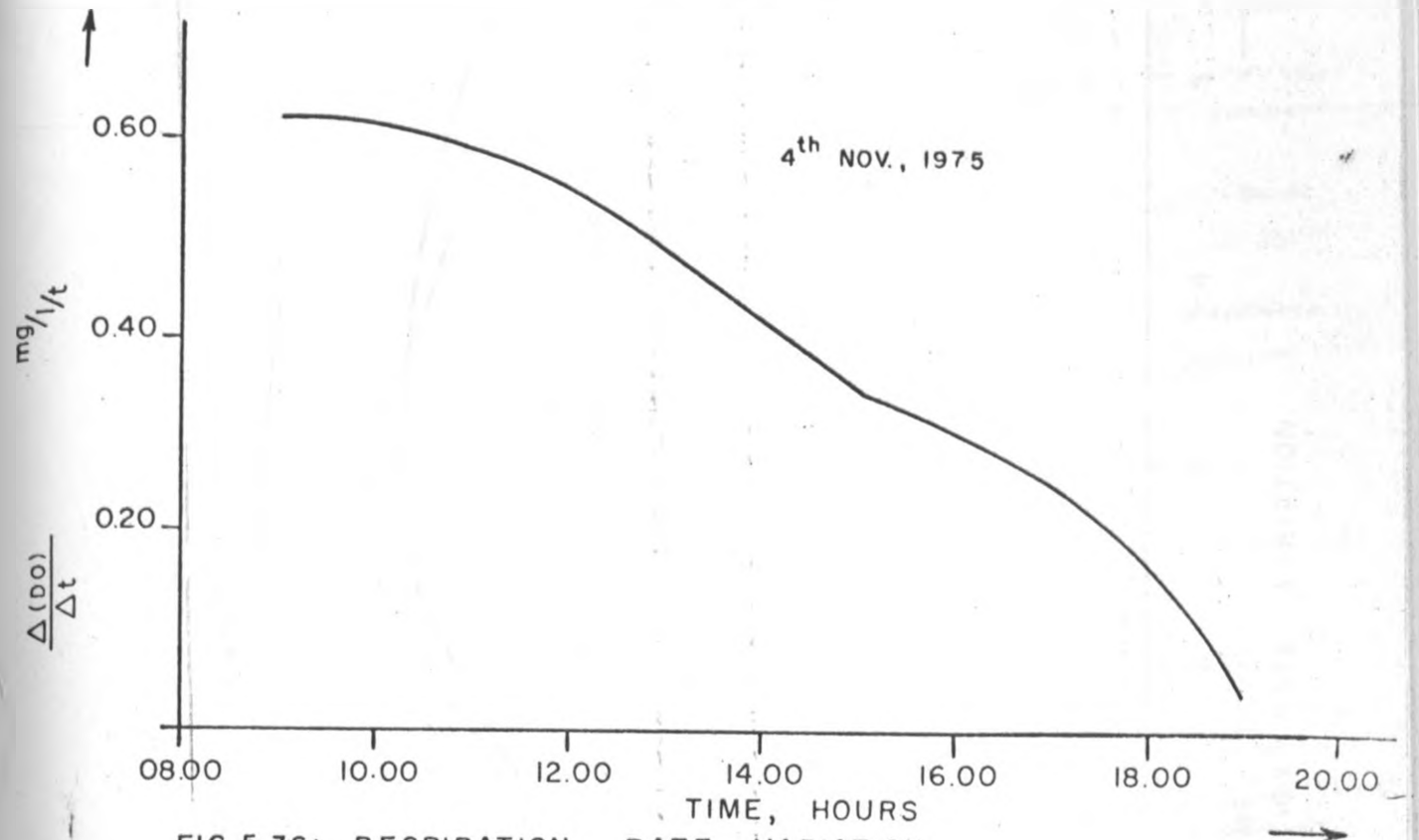


FIG. 5.36: RESPIRATION RATE VARIATION

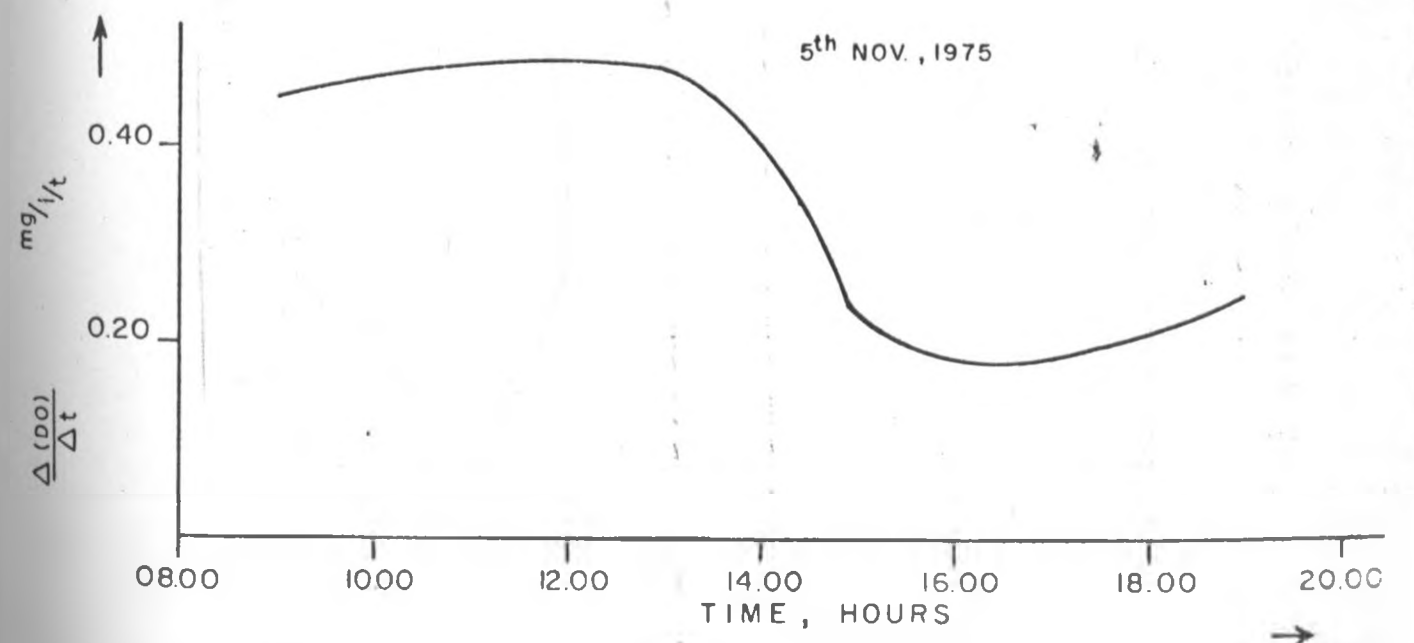


FIG. 5.37: RESPIRATION RATE VARIATION.

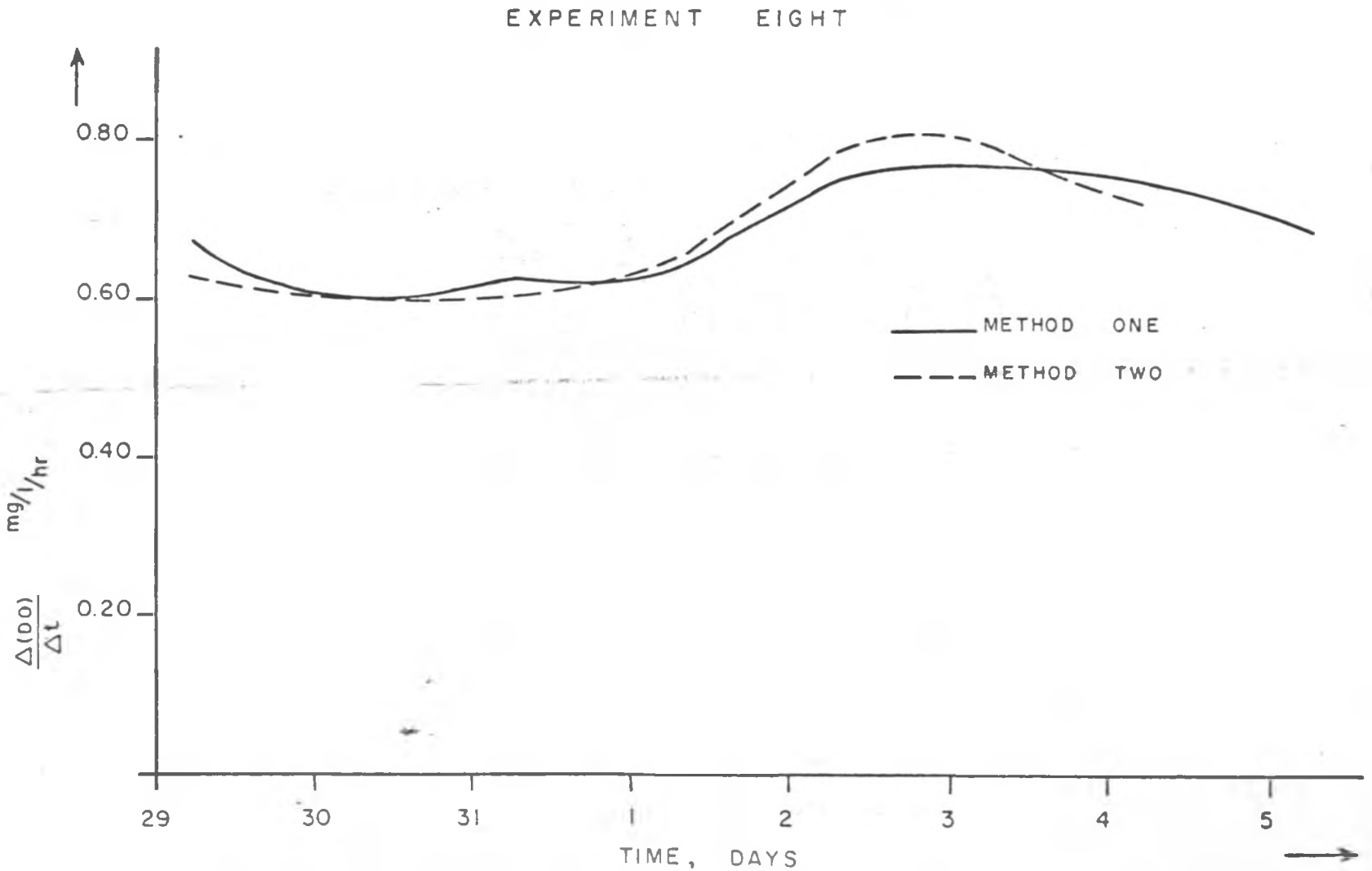


FIG. 5.38: DAILY OXYGEN PRODUCTION RATE VARIATION

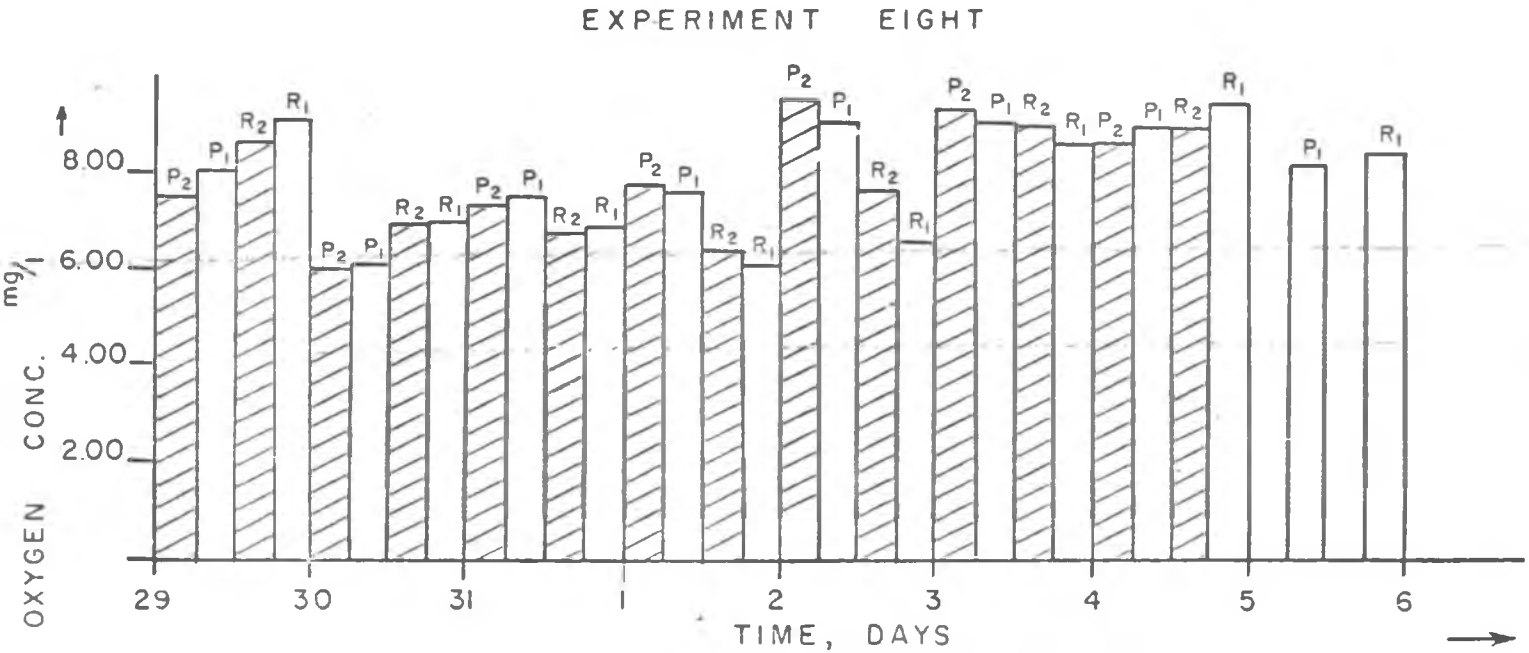


FIG. 5 39: HISTOGRAM OF DAILY OXYGEN PRODUCTION AND UTILISATION

N.B: P_1 and P_2 – Oxygen production by methods one and two

R_1 and R_2 – Oxygen utilisation by methods one and two

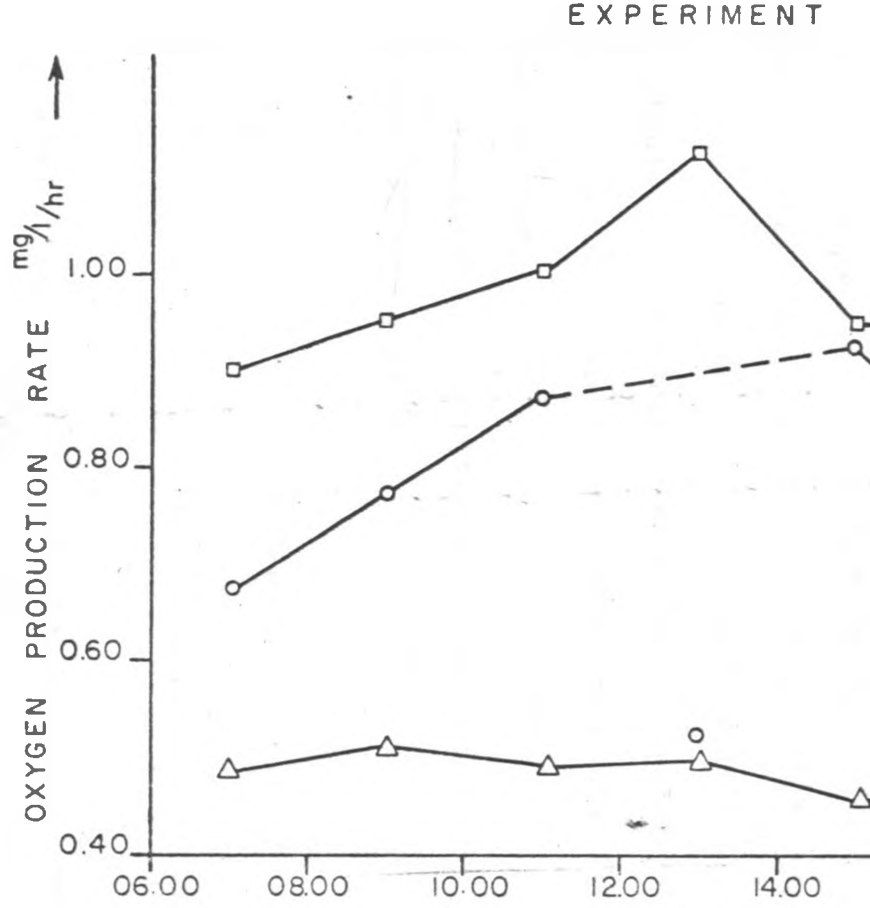
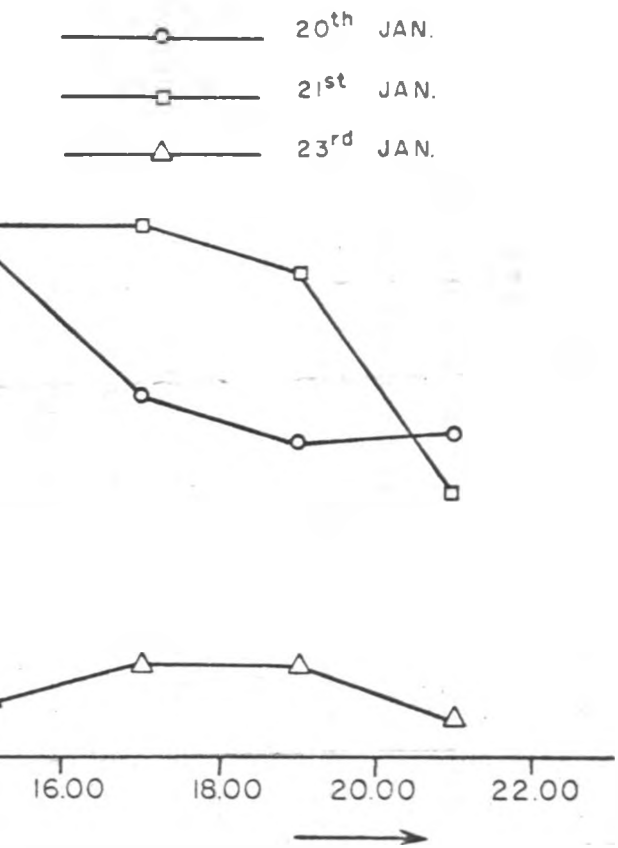


FIG.5.40: OXYGEN PRODUCTION RATE

NINE


20th JAN.
21st JAN.
23rd JAN.
16.00
18.00
20.00
22.00
HOURLY VARIATION

the rate of oxygen production until a maximum value is attained. The rate of oxygen production then decreases to a minimum value, after which it increases again to another maximum value. The maxima are not necessarily of the same value during one day-light period. Figure 5.21 may be interpreted as having three maxima. This can probably be accounted for by the fact that the data was collected during the continuous light period. The dark-light sequence of illumination was begun in the evening of the 30th September, 1975.

Algae produce oxygen by fixing carbon dioxide for growth. It has been demonstrated that the uptake of carbon dioxide during a cell cycle is variable. CO_2 uptake starts from a minimum to a maximum and back to minimum during one life cycle of an algal cell (Brockway et al, 1970). Since oxygen is produced only when carbon dioxide is fixed, oxygen production should also be variable starting from a minimum to a maximum, down to a minimum and then maximum etc. For example the following metabolic equation for chlorella pyrenoidosa illustrates the dependence of oxygen production on algal carbon dioxide fixation (Fogg, 1953).

$$2NH_4 + 11.4\ CO_2 + 68H_2O \rightarrow 2C_{5.7}H_{9.8}O_{2.3}N + 13.04\ O_2 + 2H \quad ..(5.7)$$

It is interesting to note that differences in the value of peak rates of oxygen production during one day-light period vary in magnitude. In certain cases the peaks are of the same magnitude, in other cases the first peak rate is less

less in value than the second peak and in yet others the first peak is greater than the second peak. These variations seem to depend on the state of growth of the algae, nutritional conditions and physiological characteristics of the algae.

The daily variation in average oxygen production rates are shown in figure 5.28. Fig. 5.9 shows the algal growth curve obtained during the same experiment. A comparison of the two graphs shows that algal growth resulted in an increased average daily oxygen production rate. The highest rate was attained during the first stationery growth phase. The second log growth phase resulted in an increased chlorophyll concentration of from about 1.04 mg/l to about 1.76 mg/l and yet the average daily oxygen prodution rate increased slightly for some period and then dropped very sharply. This suggests that the oxygen producing capacity of the algae was adversely affected resulting in a greater proportion of inactive cells. Shading of the algae at higher concentrations may also account for the decrease in oxygen production rate.

Figure 5.39 is a histogram of the daily average oxygen production and utilisation for experiment eight. A comparison of the above with the algal growth curves in figure 5.10 shows that oxygen production increased as the chlorophyll content of the biomass increased. The highest average daily production rate was recorded on the 2nd November, 1975. Thereafter a decrease in the algal chlorophyll content resulted in a decrease in oxygen production rate.

The oxygen consumption rate is determined by the

requirements of the biota for cell synthesis and growth as well as energy for maintenance. In general oxygen consumption rates were highest when there was the greatest reduction in chemical oxygen demand of the waste. Because at this time zero dissolved oxygen concentrations were always recorded, it was not possible to calculate the consumption rates. Figure 5.29 presents the average daily oxygen utilisation rates of the biomass growth units during experiment eight. Figures 5.30 to 5.37 depict the night-time variations of the average respiration rates of the biota. From the graphs it can be seen that the highest oxygen utilisation rate is attained at the beginning of the dark period. The average respiration rate then decreases gradually until towards the end of the dark period a more or less constant rate of respiration is maintained. The rate of oxygen utilisation is closely linked to the metabolic activities of the entire community. Though the general trend of the average rate of oxygen utilisation is from maximum rate at the beginning of the dark period to constant and minimum towards the end of the period it is subject to modification by the following factors.

1. If algal respiration is the predominant component of overall oxygen utilisation, then the oxygen utilisation curve during the dark period is more likely to be of the form shown in figure 5.30 i,e. from maximum to a minimum.
2. If bacterial oxidation of organic matter, protozoal

and zooplankton respiration are significant factors the oxygen utilisation curve will depend on whether these contributory factors are increasing, constant or decreasing, continuous or intermittent (phasic) and their magnitudes.

5.2.3 SUBSTRATE CONSUMPTION

Substrate consumption was evaluated by analysis for chemical oxygen demand reduction of the reactor growth medium. Chemical oxygen demand results are presented in tables 5.3 to 5.22 and graphically depicted in figures 5.1 to 5.8 and 5.41 to 5.44. The figures and tables mentioned above, show chemical oxygen demand values for filtered and unfiltered samples. As previously stated (section 4.4 Chapter 4) filtration was done through two whatman filter papers Number 42. Daily changes in chemical oxygen demand were determined in experiments one to eight and hourly changes in experiment nine.

The greatest reduction in chemical oxygen demand was observed to occur within the first two days after start-up of the biomass growth reactor. For example reductions of 62.60% and 40.00% for unfiltered and filtered samples, occured in experiment seven (Tables 5.14 and 5.18). In experiment eight percentage reductions of 57.58 and 31.44 for unfiltered and filtered samples were achieved. The average rates of chemical oxygen demand reduction for the first 45.50 hours in experiments one to five; 36 hours, 48 hours and 46 hours in experiments six, seven and eight respectively are shown in the table below.

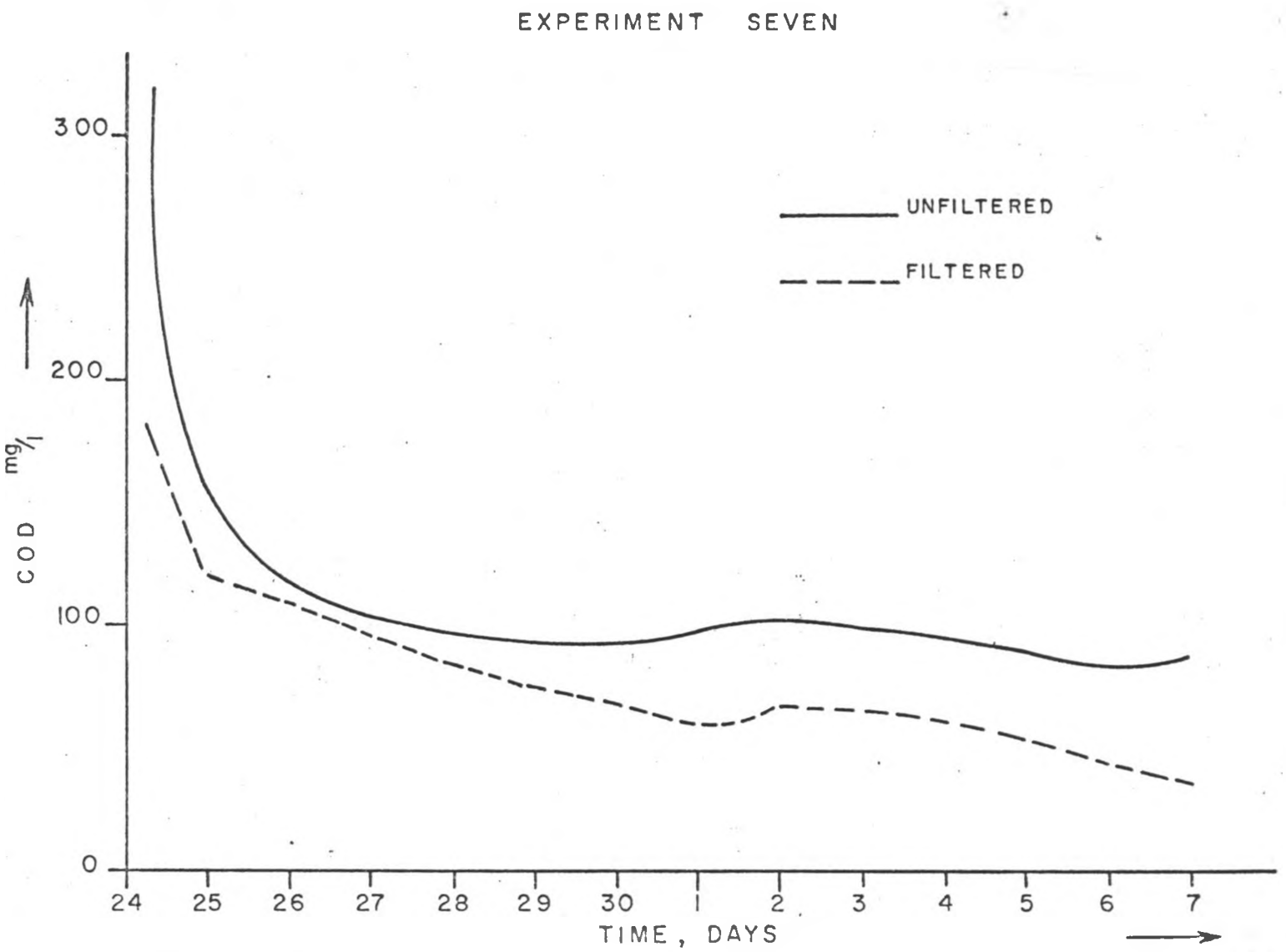


FIG. 5.41 : CHEMICAL OXYGEN DEMAND VARIATION

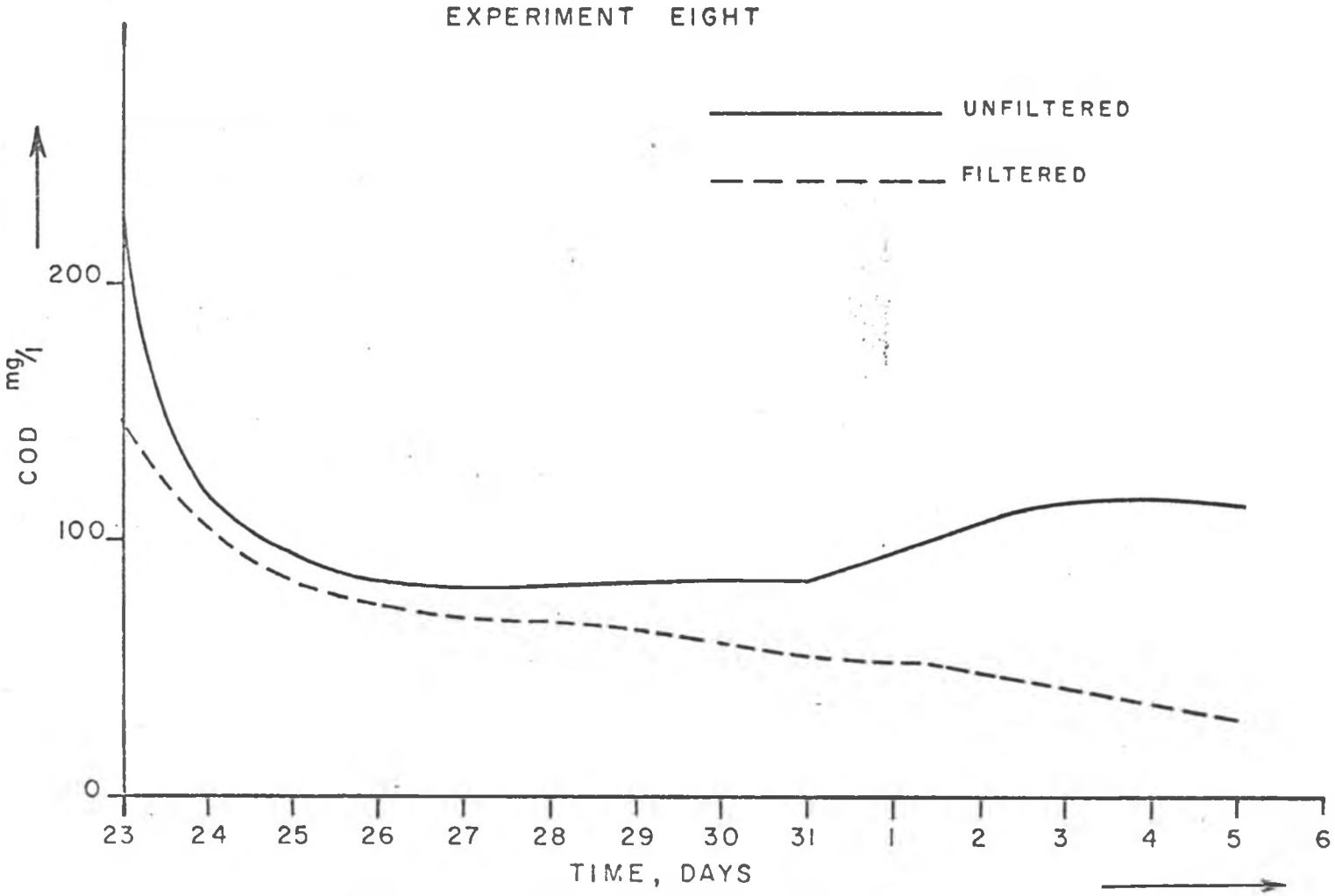


FIG. 5.42: CHEMICAL OXYGEN DEMAND VARIATION

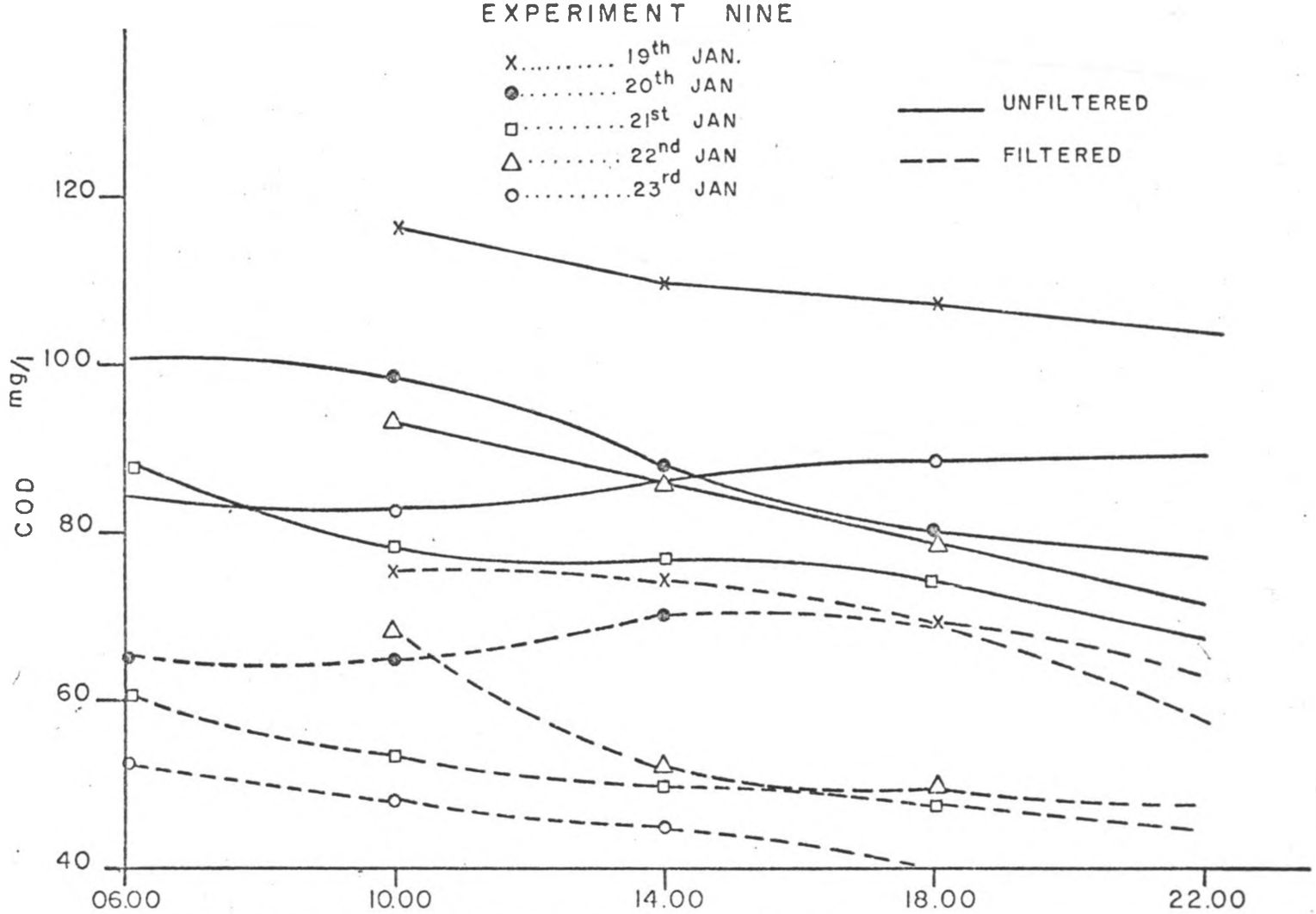


FIG. 5.43: CHEMICAL OXYGEN DEMAND VERSUS TIME
3 litre feeds added on 19th and 22nd Jan., 1976

Experiment six:— 2-9-75 to 8-9-75

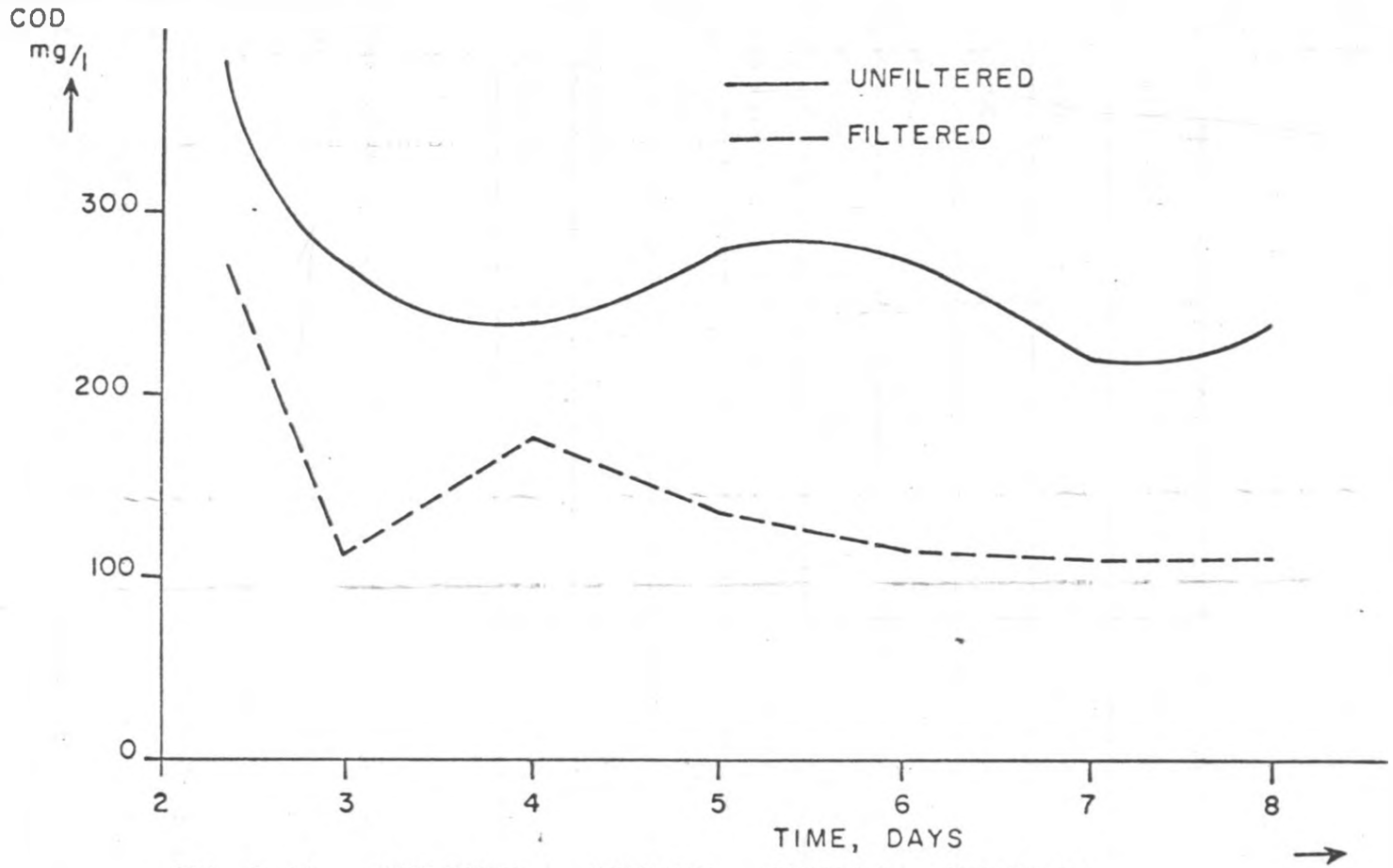


FIG. 5.44. CHEMICAL OXYGEN DEMAND VARIATION

Table 5.23 Average Rates of Chemical Oxygen demand Reduction, in mg/l/hr, for the first two days

EXP. NO.	UNFILTERED	FILTERED
ONE	1.910	1.934
TWO	1.769	2.023
THREE	1.982	2.000
FOUR	2.066	3.182
FIVE	2.826	3.569
SIX	2.180	2.940
SEVEN	4.170	1.530
EIGHT	2.840	1.360

The rates of reduction for the filtered samples were generally higher than those for the unfiltered samples except in experiments seven and eight. Values of chemical oxygen demand for unfiltered samples reflect that portion of the growth medium which is susceptible to oxidation by dichromate whereas filtered samples represent chemical oxygen demand values of that part which passes through the filter papers. The filtered samples are intended to exclude algae only. In reality filtration conditions are subject to wide ranging variations. For example, pores of the filter papers

may vary in size and distribution, filter pressure may vary etc.

Slightly higher average rates of chemical oxygen demand reductions (unfiltered) were obtained in experiments three to nine. In these experiments a multi-component substrate was used as the growth medium. In experiments one and two, in which a single component substrate was used as a growth medium, lower average rates of chemical oxygen demand reductions (unfiltered) were obtained. It may not be valid to compare the rates of chemical oxygen demand reductions for single and multi-component substrates as their initial chemical oxygen demand values differed greatly. For the single component substrate experiments the initial chemical oxygen demand values were 140 mg/l whereas for the multi-component substrate experiments, initial chemical oxygen demand values were about 340 mg/l. In addition initial bacterial seed composition, species variety and predominance, concentrations and biokinetic properties may have differed and were difficult to assess experimentally.

The initial reduction in chemical oxygen demand is followed by a period of fluctuations in the COD values. The overall trend, however, is a steady reduction in the chemical oxygen demand of the filtered samples towards a lower value; and a slight increase in the COD of the unfiltered samples as the biomass progresses from log growth to stationery growth and declining growth. The COD of the growth medium is, at this stage, a result of several factors. There is a

build-up of complex organics not oxidised by the dichromate used in determining the chemical oxygen demand. These organics may be made up of substances contained in the synthetic sewage, products of excretion and lysis of the biota. In addition algal growth and the fixation and conversion of carbon dioxide, released by the bacterial oxidation of the substrate, into new organic matter may contribute to the increase in chemical oxygen demand.

In experiment six (tables 5.8 to 5.12 and figure 5.44) the initial reduction in chemical oxygen demand was fairly rapid. This period was then followed by a steady rise in the chemical oxygen demand of the growth medium. Since, in this experiment, the biomass growth biota was unable to restore aerobic conditions, the increase in the chemical oxygen demand of the reactor may be attributed to the conversion of the substrate by the predominantly anaerobic and facultative bacteria, to organics more susceptible to oxidation by the dichromate. For example, short-chain acids such as acetic acid etc. The second decrease in chemical oxygen demand (fig. 5.44) may be as a result of conversion of the acids to carbon dioxide. An increase in the chemical oxygen demand towards the end of the experimental run is probably brought about by lysis and decay of the algal detritus in the growth reactor.

In the fill and draw experiment nine, introduction of fresh substrate at different times and places on the growth phases of the biomass resulted in different substrate consumption rates. Addition of a feed on the

19th January (Fig. 5.43) resulted in average chemical oxygen reduction of 5.5% in four hours and 8.8% in eight hours for the unfiltered chemical oxygen demand. On the 22nd, addition of substrate gave COD reductions of 6.6% and 14.22% for 4 and 8 hours respectively. The rates of chemical oxygen demand reductions were probably influenced by the state of growth of the bacteria and bacterial, algal, protozoal and zooplankton decay.

The rates of chemical oxygen demand reduction is complex and does not follow a defined function. It depends on the type of substrate i.e. its composition, concentration and oxidation state. It also depends on the bacterial activity, their numbers and the consumption patterns set up during multi-component substrate assimilation and on the prey-predator relationships established in the biomass growth unit. It is further greatly depended on physical conditions such as temperature, mixing conditions etc.

5.2.4 pH AND TEMPERATURE

Temperature and hydrogen ion concentration measurements were taken at daily intervals in experiments one to five (tables 5.3 to 5.7), and at hourly intervals in experiments six to nine (tables 5.8 to 5.13 and appendix III tables 1 to 20). The temperatures were lowest at the beginning of each experimental run, but quickly attained an equilibrium temperature. Then slight variations in temperature occured thereafter. For example in experiment four (table 5.6) the initial

growth medium temperature was 15.5°C. The temperature rose rapidly and by the second day a temperature of 25°C was recorded. There was then a slight drop in temperature on the third day to 24.6°C. The lowest temperature recorded being 24.2°C on the fourth day. In general in most experiments growth reactor temperatures tended to equilibrate around 24.5°C although temperatures as high as 27°C were recorded (Table 5.5). Room day-temperatures during the experiments ranged between 20°C and 27°C. Maximum temperatures recorded were 25°C. to 27°C and minimum temperature were 15°C to 19°C.

The pH of the biomass growth reactor varied in general between the pH ranges 7.40 and 6.20. The pH was usually high at the beginning of the experiment. During the phase of intensive chemical oxygen demand reduction the pH, in most experiments dropped slightly. For example in experiments seven and eight the pH dropped to 6.00 and 6.20, respectively. With the commencement and progression of algal growth, the pH rose to 6.50 in both experiments. It then remained steady at about this value for the duration of the experiments.

In experiments three to five (tables 5.5 to 5.7) lower pH values were recorded for the unilluminated reactors. This was probably due to production of acids in the unilluminated reactors.

5.2.5 BACTERIAL CONCENTRATIONS AND CARBON DIOXIDE

Bacterial numbers or concentrations were determined by the method described in section 4.4 Chapter 4, with the aid of agar dip slides. Results of the bacterial counts are given in table 5.24 for experiments seven and eight. Estimates of bacterial numbers, by the method of agar dip slides, gives

Table 5.24 BACTERIAL CONCENTRATIONS

TIME DAYS	EXP. SEVEN NUMBERS/ML	EXP. EIGHT NUMBERS/ML
0	10^3	10^3
1	10^5	10^5
2	10^5	10^5
3	10^6	10^6
4	10^6	10^6
5	10^7	10^7
6	10^8	10^7
7	10^7	10^7
8	10^6	10^6
9	10^6	10^6
10	10^5	10^5
11	10^5	10^5
12	10^6	10^7
13	10^5	10^5
14	-	10^6

bacterial counts to the nearest order and was therefore not sensitive enough to differentiate concentrations lying between two orders e.g. 10^3 and 10^4. In general, in all experiments, bacterial concentrations increased rapidly soon

after start-up of the biomass growth reactor. At this time, there was also a rapid decrease in the chemical oxygen demand of the growth medium. Then a period of fluctuating growth followed. During this period factors such as protozoal predation, bacterial decay, and regeneration of biodegradable and non-biodegradable organic matter were probably responsible for the fluctuations.

During the study, unsuccessful attempts were made to measure the oxidative capacity of the bacteria, by analysing the reactor contents for dehydrogenase activity. The method tried was a modification of the triphenyl tetrazolium chloride test (TTC - test) proposed by Bucksteeg and Thiele (1959) for measuring the activity of activated sludges. Several investigators have reported on this method (Lenhard et al, 1964; Ford et al, 1966, Patterson et al 1969, Jones and Prasad, 1969, Klapwijk et al, 1974; Rysson-Niessen, 1975). In this test the dehydrogenase enzyme activity is measured by using a tetrazolium salt (triphenyltetrazolium chloride - TTC) as hydrogen acceptor. The oxidation of a substrate is coupled with the reduction of TTC to a red coloured triphenyl formazan (TF). The intensity of the red colour is taken as a measure of the dehydrogenase activity.

In applying the TTC test to measure the bacterial activity of the growth reactor contents, difficulties were encountered in separating the bacteria from the algae without loss of bacteria, and in obtaining a more concentrated culture of bacteria from the diffuse growth medium. Results

obtained were unreliable and inconsistent and procedures tried failed to produce a sensitive enough test.

Carbon dioxide results are shown in Tables 5.5 and 5.9 for experiments three, four, five and six.

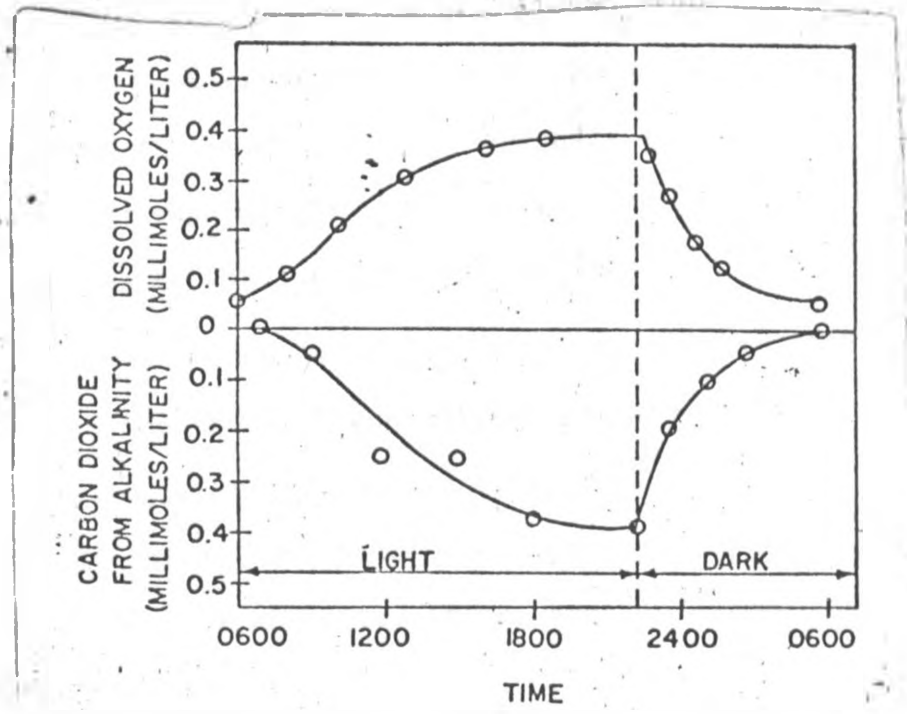


Fig. 5.45 —Diurnal variation in dissolved oxygen concentration and carbon dioxide extraction from the carbonate alkalinity associated with active algal photosynthesis.

(after King, 1970)

6. DISCUSSION

The dynamics of algal growth, oxygen production/consumption and substrate utilisation in batch culture growth systems are governed by complex chemical, biological and physical processes.

During the study, two types of batch reactors were used. For experiments one to five 250 ml measuring cylinders were employed as completely mixed batch reactors. A specially designed closed reactor (see appendix II) was the reactor of choice in experiments six to nine. The mixing characteristics of the batch reactor are shown in appendix IV.

In the biomass growth reactors, changes take place with time in the chemical characteristics and composition of the substrate, physical characteristics of the synthetic sewage i.e. dissolved, colloidal and suspended states etc, and in the biological characteristics, species composition and variety of the biota.

The characteristics of the single-component (glucose) and multi-component (glucose, starch and yeast) substrates change as a result of bacterial oxidation of the organic matter contained in the substrates. The synthetic sewage characteristics also change as a result of the release of the by-products of the metabolic activities of the bacteria, algae, protozoa and zooplankton, to the growth medium.

The above-mentioned changes in turn, induce changes in the metabolic processes of the biota and the rates at which these processes occur. Conceptually, the biomass growth reactor may be represented by the schematic diagram shown below.

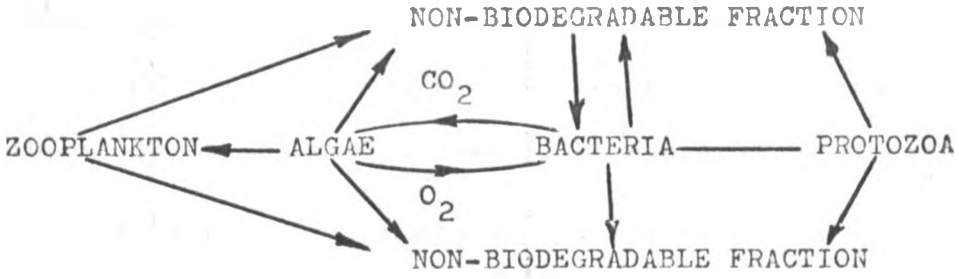


Figure 6.1 Microbial interactions and substrate components of Batch Reactor.(Extension of model by Canale R.P. et al, 1974)

In general, most substrates have bio-degradable and non-biodegradable fractions. The ratio of the biodegradable to the non-biodegradable fractions can be determined by substrate removal analysis and it is a characteristic of the medium.

Figure 6.1 shows that bacteria utilise the biodegradable fraction of the sewage for growth. The losses of living cells of bacteria result from protozoan predation, endogenous respiration bacterial death and decay. Algae utilise for growth the carbon dioxide released by the respiratory activities of the community. The algae increase by growth depending on light, temperature, carbon dioxide and nutrient availability. They decrease as a result of continuous respiration during long periods of darkness and grazing by zooplankton.

Zooplankton and protozoa increase in number proportional to the concentrations of available algae and bacteria respectively. Bacterial, protozoan, zooplankton and algal respiration and decay processes expel waste products that may contribute to the increase of either the biodegradable or the non-biodegradable fractions of the synthetic sewage. All the above-mentioned processes may be affected by toxic substances contained in the synthetic sewage, or excreted by the biota.

At the beginning of each experiment during the study, the organic constituents of the substrate were rapidly oxidised by the bacteria. This was evidenced by reductions in the dissolved oxygen content of the growth medium, increase in bacterial numbers and reduction in the chemical oxygen demand of the synthetic sewage (Table 5.3 to 5.19). For example, the COD reductions were 62.6% and 57.8% in experiments seven and eight respectively in the course of two days after the start of the experiments (figs 5.41 and 5.42 and Tables 5.14 and 5.18) Bacterial numbers increased from 10^3 to 10^5 in both experiments Table 5.24). Bacteria utilise the substrate for growth. Bacterial increase or decrease in the reactor is the result of three factors. These may be expressed as:-

$$\begin{matrix}\text{change in} \\ \text{Bacterial} \\ \text{concentration}\end{matrix} = \begin{matrix}\text{Bacterial} \\ \text{growth}\end{matrix} - \begin{matrix}\text{Bacterial} \\ \text{decay}\end{matrix} - \begin{matrix}\text{Protozoal} \\ \text{grazing}\end{matrix}$$

According to Ghosh et al (1972), in the presence of competing multiple sources of carbon and energy, the substrate

assimilation pattern that is adopted e.g. sequential or concurrent, should enable the organisms to multiply at the fastest attainable specific growth rate and at the same time achieve the maximum possible yield of biomass. The growth yields and the specific growth rates are controlled by that substrate which permits:-

1. The highest maximum specific growth rate
2. The highest yield coefficient.
3. The lowest saturation constant when provided as the sole carbon and energy source.

In addition bio-kinetic properties of the initial seed population and the initial concentrations of the individual organic components of the substrate (and therefore the growth rates they permit) are important factors in determining whether the uptake pattern would be sequential or concurrent. This would mean that the faster growing bacteria i.e. the more efficient substrate utilising bacteria would predominate. This may, however be modified by protozoal selective grazing resulting, in some cases, in the survival of a less efficient bacteria having lower rates of substrate utilisation. Illustrations of phasic utilisation of substrate are shown in figures 6.15 to 6.20.

At the start-up of each experiment a complexity of factors contributed to determining the rates of the various growth reactions taking place in the reactors. The nature of the substrate, bacterial acclimation to the substrate, bacterial competition, biokinetic properties of the seed population determined the substrate assimilation patterns

and the growth rates achieved. In all the experiments bacterial seeds were not necessarily of the same concentrations, species variety, protozoal populations and biokinetic properties. Of importance at this stage, are the bacteria. Their composition, concentrations, species variety and biokinetic properties, as well as their response to the substrate determine the patterns and rates of substrate utilisation. Ghosh et al, 1972 states that there is little opportunity for population dynamics in batch cultures and that the specific growth rates are at all times defined by the growth parameters of the slow or the fast growers depending on which organisms remain dominant during the duration of the experiment.

The initial rapid oxidation of the substrates in experiments seven and eight was followed by fluctuations of the chemical oxygen demand for both filtered and unfiltered samples. The fluctuations were probably caused by changes in the biodegradable and non-biodegradable fractions of the synthetic sewage. More correctly by changes in the fractions amenable to oxidation by dichromate. This phenomenon is a result of a number of factors and can be illustrated with the help of the following expressions:-

$$\text{change in Biodegradable fraction} = -\text{Bacterial uptake} + \text{Bacterial decay} + \text{algal decay} + \text{Protozoan decay} + \text{Zooplankton decay}$$

change in Non-biodegradable fraction = Bacterial decay + algal decay + Protozoan Decay + Zooplankton Decay

The initial reduction of the chemical oxygen demand resulted in the rapid increase of the bacterial population. The bacterial oxidation of the substrate provided carbon dioxide and nutrients for algal growth. Protozoal and zooplankton growth soon followed the rapid growths of bacteria and algae. The growth of the entire biota was accompanied by natural death of the organisms and release of excretory by-products. These processes occured at different times and rates, but their total effect was to contribute to the increase or decrease of the biodegradable and non-biodegradable fraction of the substrate.

Algal concentrations in the batch growth reactor is a result of the following factors:-

change in algal concentrations = algal growth - Algal decay - Zooplankton grazing

Seeding of the biomass growth reactors resulted in a drop in the concentration of algae. During this period the algae were adjusting to the new environment. Growth reactions by the algae ceased or were greatly reduced in

in rate, and the algae went into a state of continuous respiration. This algal respiration caused a decrease in their numbers. The new environment probably did not have readily available nutrients essential for algal growth. In addition, the characteristics of the new environment might have imposed a selective pressure, inducing a shift in algal species predominance and composition. Zooplankton grazing at a time when algal growth had slowed down or ceased and algal decay increased, would, in certain instances, be a contributory factor in reducing algal concentrations.

Algal growth as measured by chlorophyll concentration show that the initial decrease in the concentration of the algae was followed by lag phase, log phase, stationery phase and declining growth phase. Examination of figures5.9 shows that for two days, on the 1st and 2nd October 1975, there was no change in the chlorophyll concentration of the algal biomass. This apparent pause in the production of chlorophyll was followed by a rapid growth or increase in the chlorophyll content of the biomass. The normal stationery phase of algal growth comes just before the declining growth phase in batch cultures. It can be explained by the fact that at the stationery phase, the sum of the rates of algal decay and the rates of zooplankton grazing equal the rate of algal growth. The first stationery phase in figure 5.9 cannot be reasonably explained as above, since the pause was followed by increased growth. The first stationery phase

seemed to have been caused by some inhibitory mechanism or by a lack of one or more essential nutrients to algal growth. The lack of essential nutrients can only come about as a result of a temporary pause in bacterial oxidation of the synthetic substrate. This pause in the bacterial oxidation of multi-component substrates has been shown to result from diauxic or triauxic metabolism figure 6.15. (Ghosh et al, 1972). In the three-component substrate used, the following patterns of substrate utilisation are possible

1. Sequential utilisation of glucose starch and Yeast Extract
2. Concurrent utilisation of two components e.g. Glucose and starch and sequential utilisation with regard to the third component e.g. yeast.
3. Concurrent utilisation of all three components.

Patterns one and two can be used to explain the pause in algal growth as determined by chlorophyll concentration. In the case of the first pattern of utilisation of substrates, there are two pauses during which, the bacterial culture is deploying the necessary enzymes for the oxidation of the next component e.g.

Glucose $\xrightarrow{\text{Pause}}$ Starch $\xrightarrow{\text{Pause}}$ Yeast Extract

If the algal utilisation of the essential nutrients to growth is not lagging far behind the bacterial substrate utilisation and release of nutrients essential to algal growth, then a

pause such as the one shown above may result in a temporary lack of essential nutrient. The same reasoning would apply to the second pattern of bacterial utilisation of substrate, e.g.

Glucose

Pause

Starch ——————► Yeast Extract

The length of the period of bacterial enzymatic deployment may vary and may be dependent on the state of the substrate i.e. dissolved, colloidal or particulate.

Though the above stated may be a plausible explanation for the phenomenon observed it may not be the only explanation A possibility exists that a change in the species of the algae accompanied by release of toxic extracellular substances may have increased the decay rate of the species being replaced, to the same extent as the increased growth rate of the new algal species.

Oxygen accumulation in the batch growth reactor is a function of several factors. The most positive of these factors is algal oxygen production. Dissolved oxygen in the biomass reactor is a result of the interaction of the following:-

Oxygen Accumulation = Algal Oxygen Production − Bacterial Respiration − Bacterial Growth − Protozoan synthesis − Protozoan Active Metabolism

- Protozoan Respiration - Zooplankton Synthesis -

- Zooplankton Active Metabolism - Algal Night Respiration

For example, in experiments seven and eight, rapid bacterial consumption of the substrate, through growth reactions, imposed a heavy demand on the oxygen resources of the reactor. On the other hand algal growth had slowed down and the rate of oxygen production had decreased. Other biota, which might have been present at this time imposed an additional demand on the oxygen resources of the biomass growth reactor. The result was a rapid depletion of the dissolved oxygen in the growth medium. The oxygen consumption rate is determined by the requirements of the biota for cell synthesis, growth and energy for maintenance. Initial seed population compositions and concentrations, biokinetic properties of the biota and substrate induced assimilation patterns will affect the rates of oxygen consumption. With algal growth, algal oxygen production increases to such an extent that it is able to meet the oxygen requirements of the community. It is interesting to note that, although experiment seven had a higher standing crop of algae and a higher dissolved oxygen content of the biomass growth reactor was recorded during the experiment, (Table 5.14 to 5.17) experiment eight had an active oxygen producing algal crop for a longer period. (Figs. 5.15, 5.16). From tables 5.17 and 5.19 it

will be seen that the chlorophyll content was higher in experiment seven than in experiment eight during the last days of the experimental runs. Oxygen production during these days was comparable in both experiments. However, there was a higher oxygen utilisation rate in experiment seven than in experiment eight and hence a greater decrease in the dissolved oxygen content of the biomass growth reactor. In addition, it is probable that the oxygen producing ability of the algae decreased more rapidly in experiment seven than in experiment eight, due to unknown toxic effects or physiological conditions, or a higher proportion of dead algae. The technique used for determining algal biomass, i.e. analysis for chlorophyll content, suffers from the fact that inactive chlorophyll and degradation products are determined along with the active chlorophyll of the living algae. (Gloosch-nko et al, 1972). This makes it difficult to establish the proportion of productive algae. Further the activity of the algae and its chlorophyll content per cell varies among species and as a function of cell growth state, nutritional status and light conditions (Breezonik et al, 1975). Shading of the algae at higher algal concentrations has also an adverse effect on oxygen production.

The relationship between oxygen production/utilisation and algal biomass chlorophyll concentration are graphically shown in figures 6.3 to 6.13. Cumulative oxygen production, utilisation, measured dissolved oxygen and nett oxygen production are shown as functions of chlorophyll concentration of the algal biomass in figures 6.3 to 6.9 and figures 6.11

to 6.13 for experiments seven and eight respectively. Figures 6.9 and 6.10 graphically depict the daily average oxygen production, utilisation as functions of the average chlorophyll concentrations.

In figure 6.3 the oxygen production varied exponentially with algal concentration. The curve can be described by the equation.

$$y = 0.0032x^{88.68} \qquad (6.1)$$

where y = Cumulative oxygen production mg/l/t

x = Chlorophyll concentration mg/l

The curves shown, depict the variations at different stages of the growth cycle. The form varies from exponential relationship, through quadratic to linear. Under the experimental conditions employed a linear relationship between cumulative oxygen production and chlorophyll concentration was established only during the algal logarithmic growth phase. Luebbers et al, (1966) in studies of oxygen production at different concentrations of algae, found that oxygen production was a linear function of chlorophyll concentration (Figure 6.14). Deviations from linearity were postulated as caused by mutual shading of the algae at higher algal concentrations. Under the experimental conditions employed in this study, deviations from linearity may be accounted for by factors such as:-

1. The state of growth of algae.
2. Chlorophyll content and composition (active or dead)
3. Nutritional conditions.

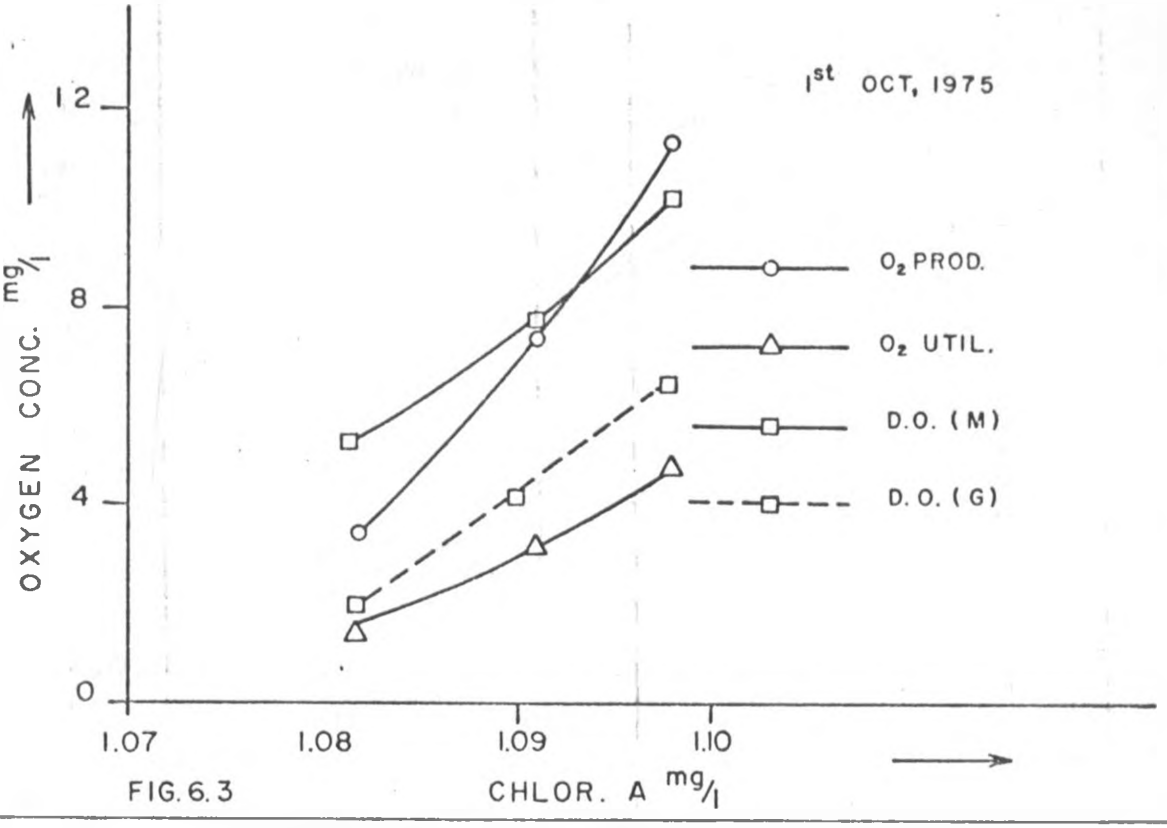


FIG. 6.3

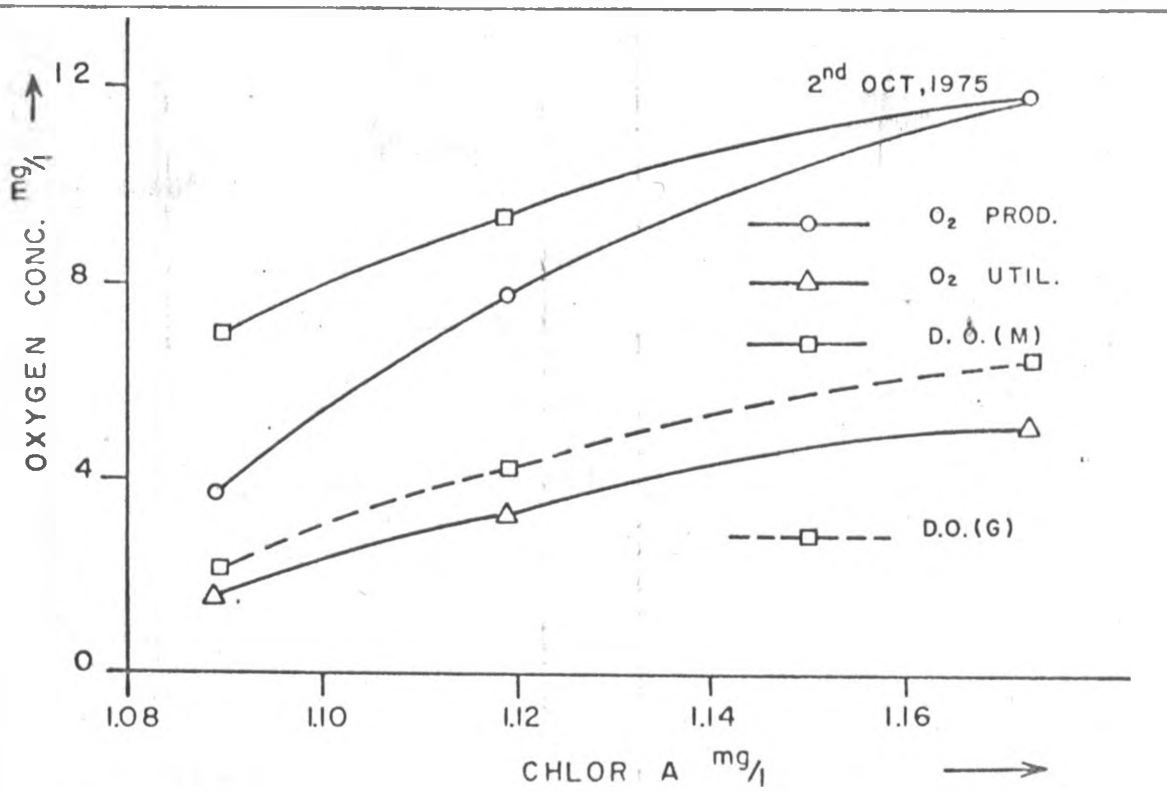


FIG. 6.4 : CUMULATIVE OXYGEN / PRODUCTION AND DISSOLVED OXYGEN VERSUS CHLOROPHYLL CONCENTRATION

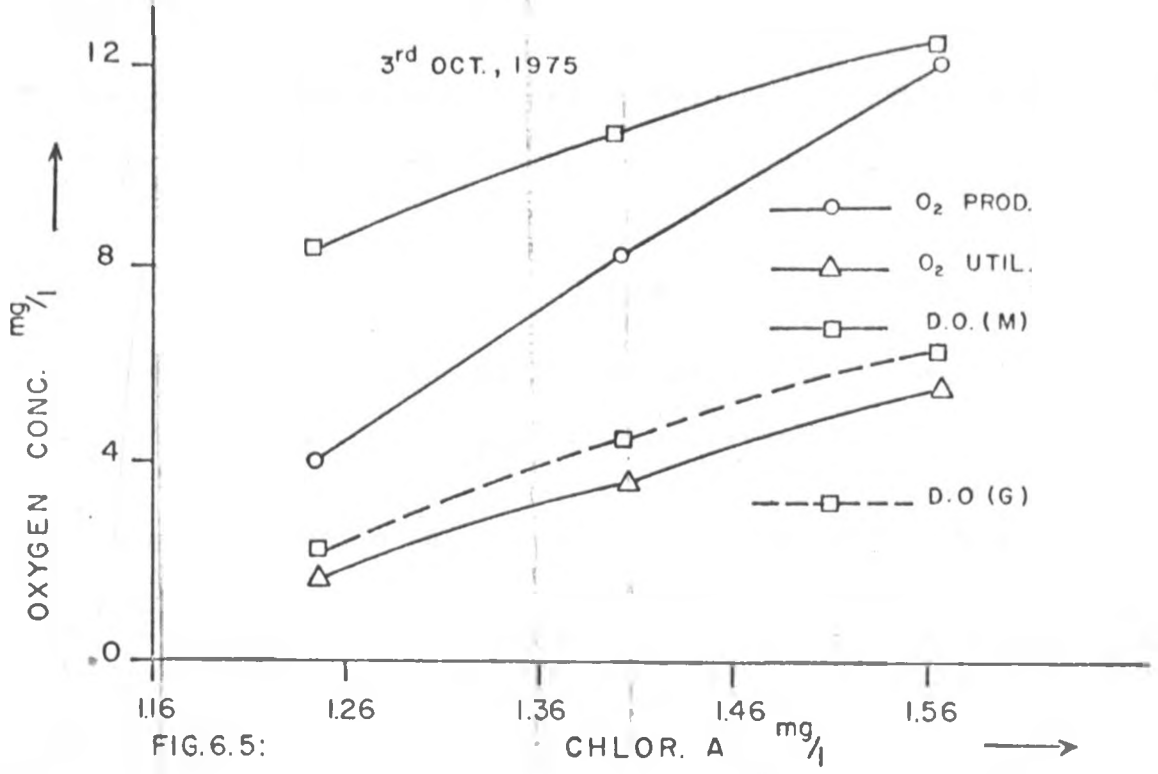


FIG. 6.5:

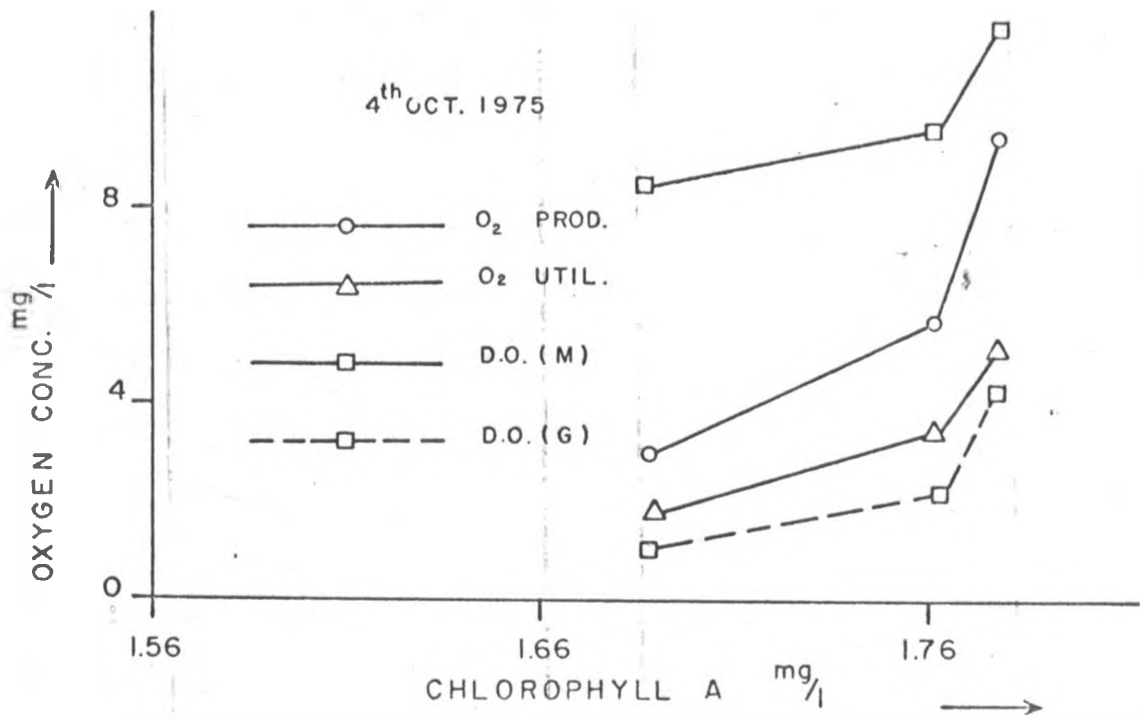


FIG. 6.6: CUMULATIVE OXYGEN PRODUCTION UTILISATION AND DISSOLVED OXYGEN VERSUS CHLOROPHYLL CONCENTRATION

The oxygen production versus chlorophyll concentration curve in figure 6.5 can be represented by the equation.

$$y = 26.996x - 29.542 \qquad (6.2)$$

where y = Cumulative oxygen production mg/l/t

x = Chlorophyll concentration mg/l

The oxygen production curve in figure 6.6 is made of two straight lines with slopes of 37.6 and 209.9 respectively. This suggests that there was a qualitative change in the oxygen production mechanism, probably a change in algal species or algal species predominance. For the fill and draw experiment eight, figures 6.12 and 6.13 depict algal oxygen production in the log growth phase and in the declining growth phase respectively.

The experimental conditions employed in the study did not permit the establishment of precise relationship between the COD reductions per unit algal growth or oxygen production. The greatest COD reduction took place at the beginning of the experiments. At this time algal growth was decreasing. The sequence of the processes taking place in the biomass growth reactor were bacterial growth accompanied by COD reduction. Algal growth and measurable oxygen production occured at a time when COD values were low and at times fluctuating due to substrate regenerative processes. However, it has been shown that COD reductions affect algal growth rates and the maximum algal standing crop produced.

In general, the behaviour of the studied completely

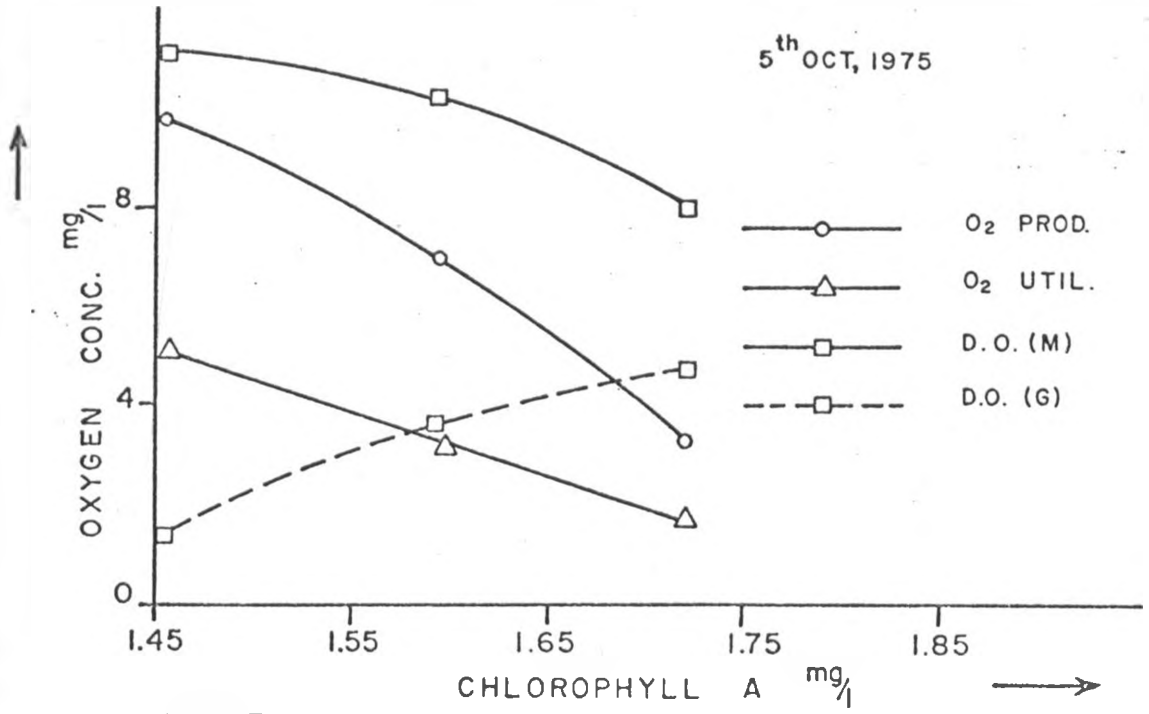


FIG. 6.7:

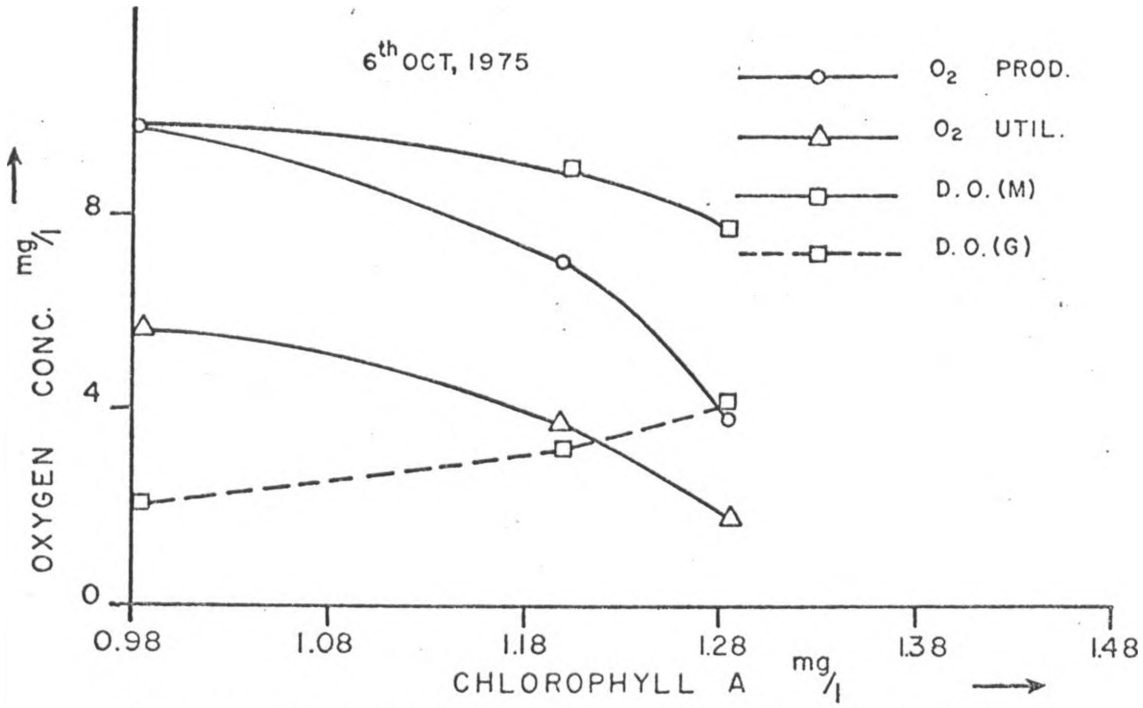
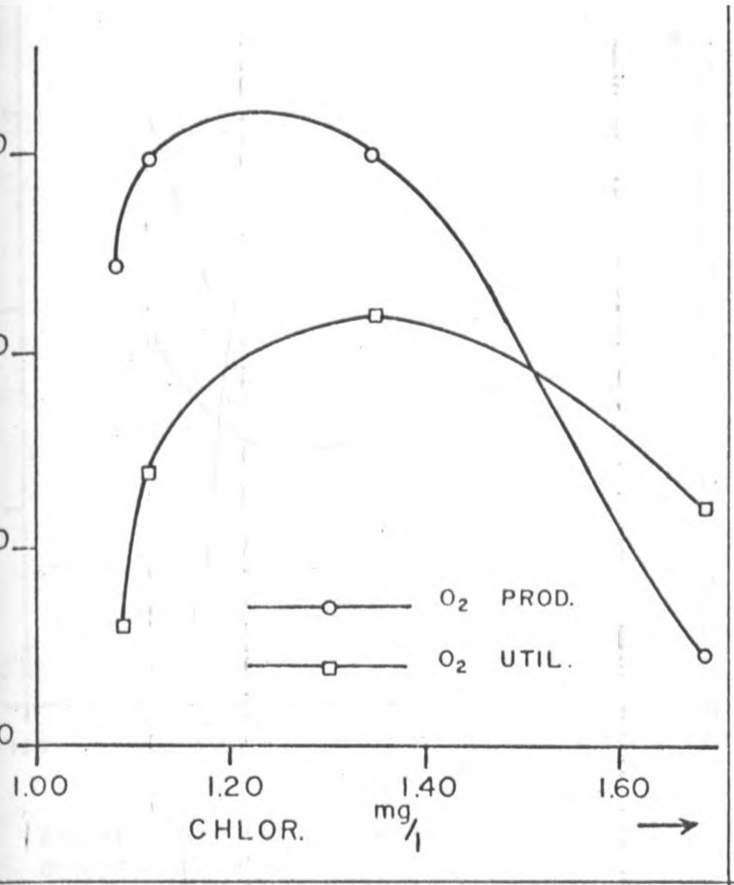
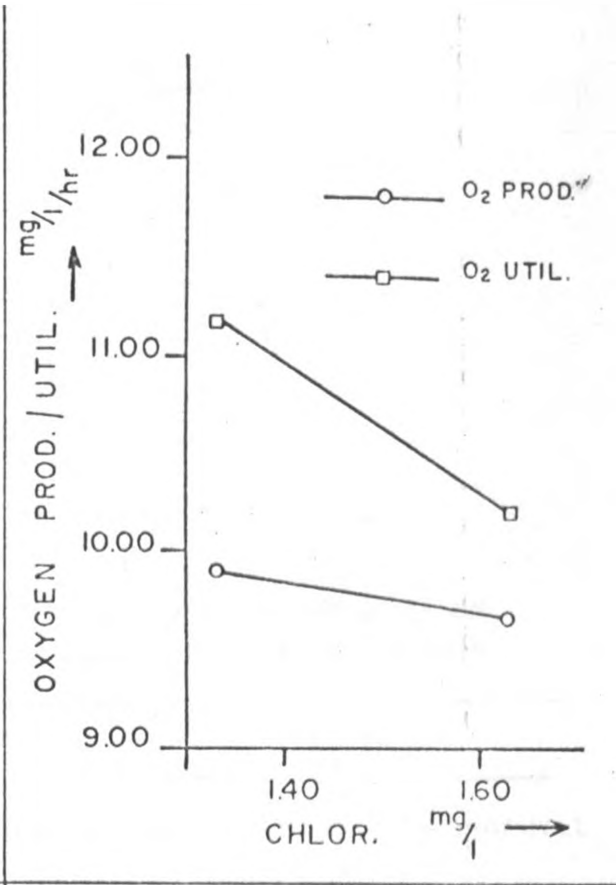
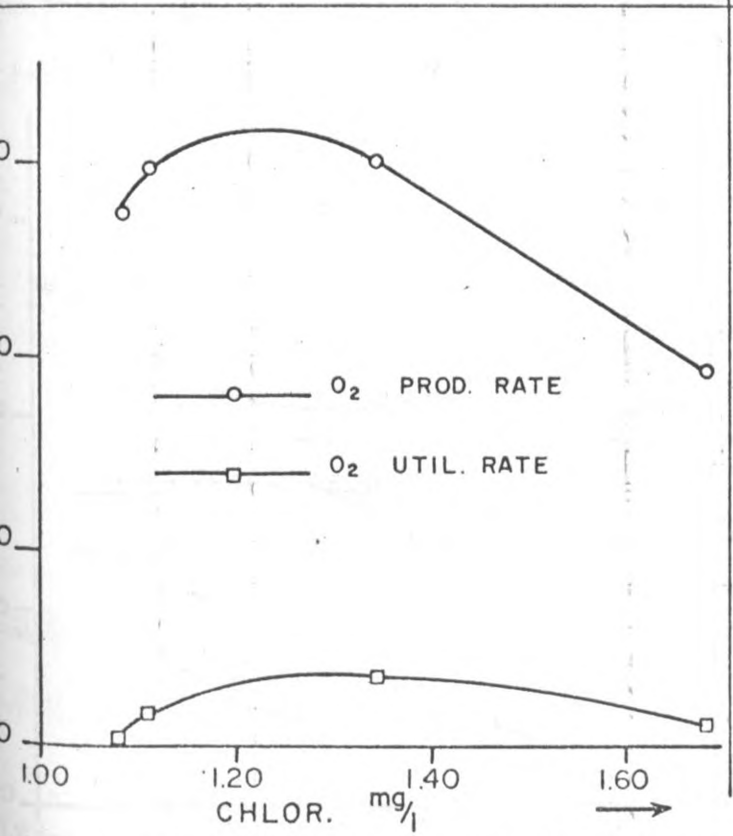
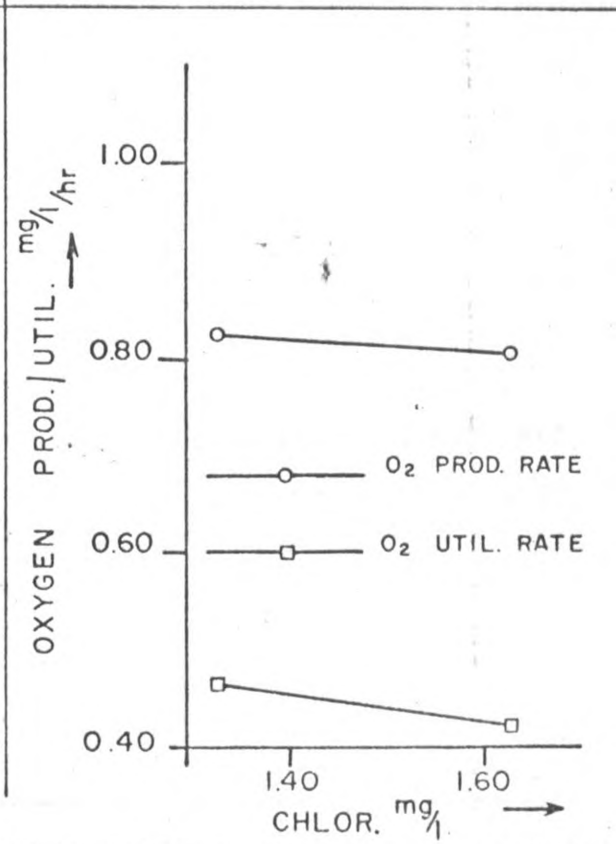


FIG. 6.8: CUMULATIVE OXYGEN PRODUCTION/UTILISATION AND DISSOLVED OXYGEN VERSUS CHLOROPHYLL CONCENTRATION

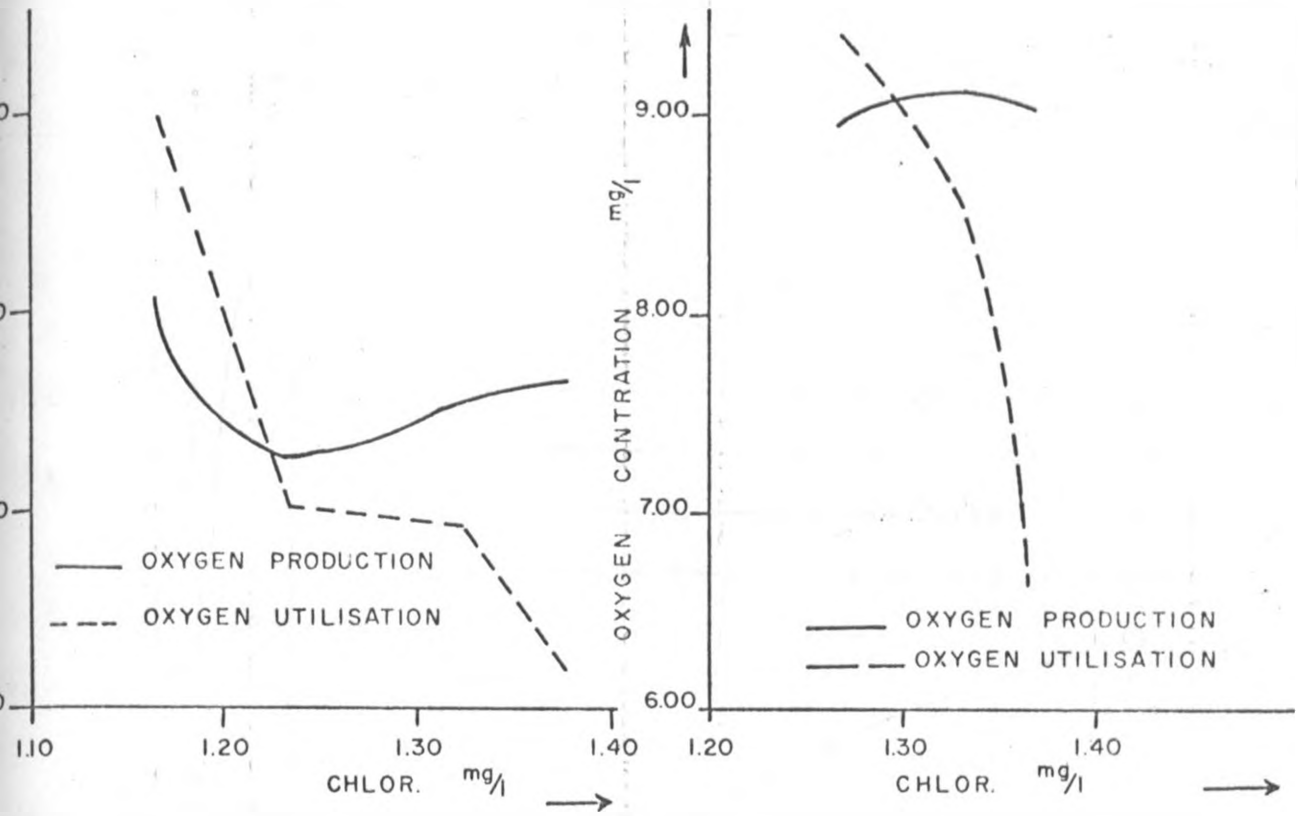








6.9: AVERAGE OXYGEN PRODUCTION/UTILISATION VERSUS AVERAGE CHLOROPHYL CONCENTRATION



AVERAGE OXYGEN PRODUCTION / UTILISATION VERSUS AVERAGE CHLOROPHYLL CONCENTRATION

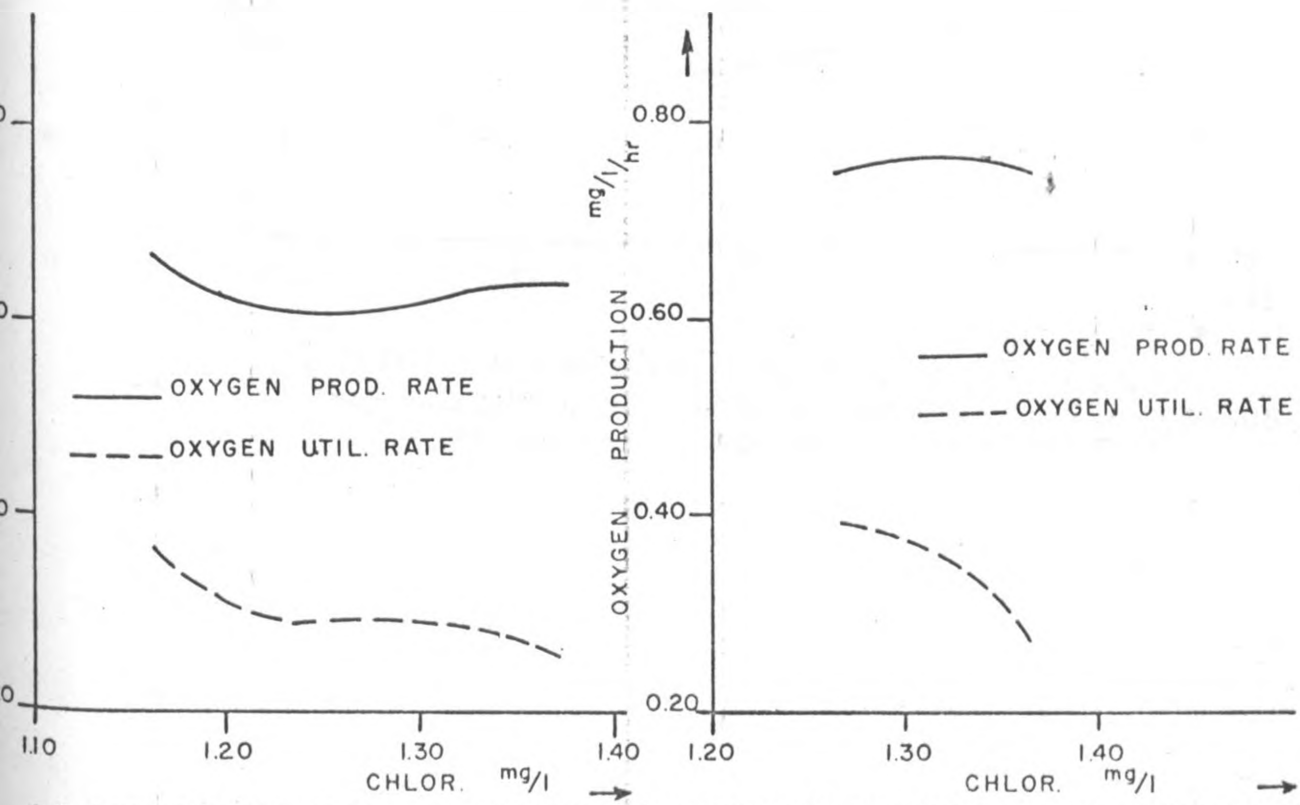


FIG. 6.10: AVERAGE OXYGEN PRODUCTION / UTILISATION VERSUS AVERAGE CHLOROPHYLL CONCENTRATION

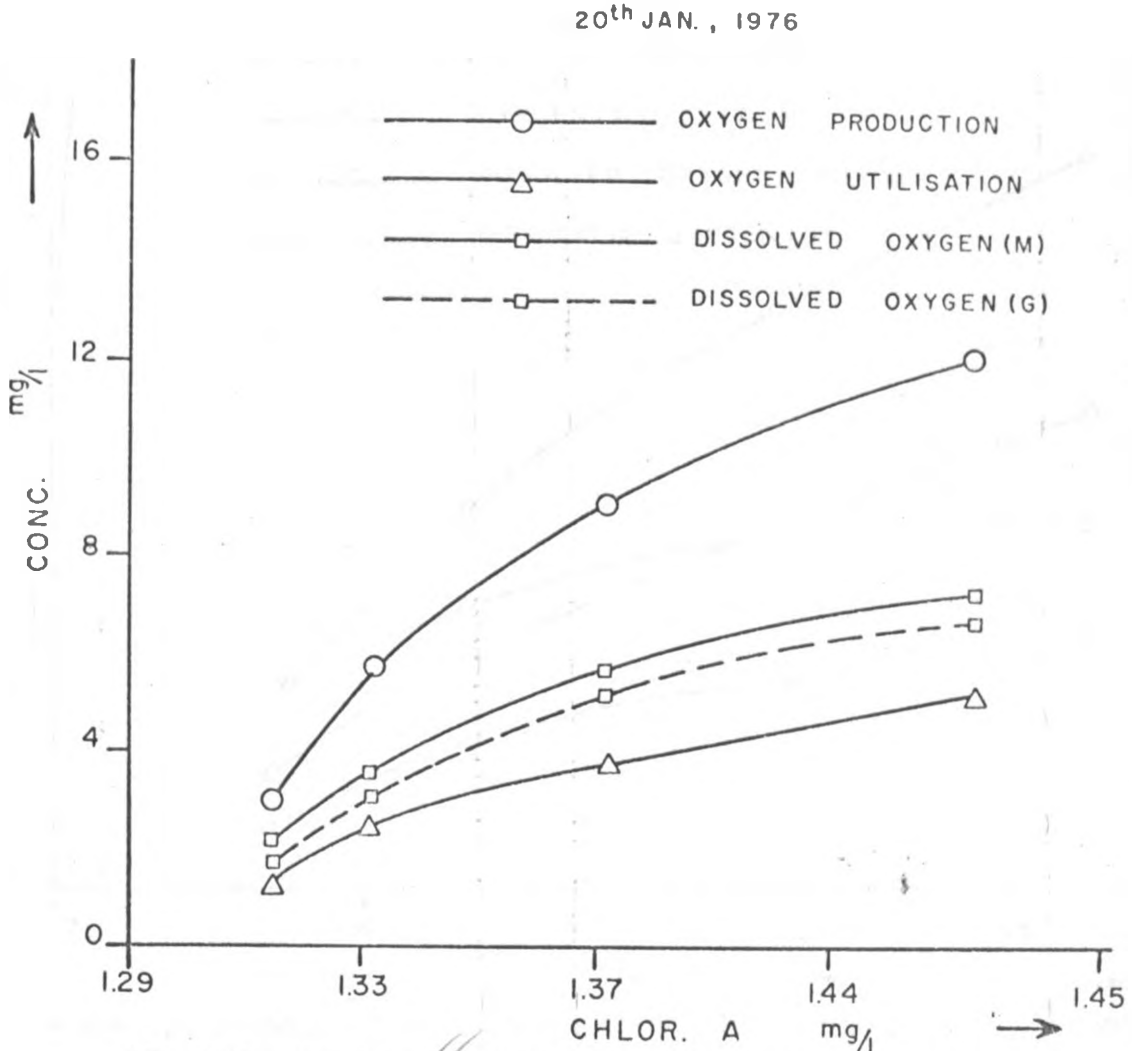


FIG. 6.11 : CUMULALATIVE OXYGEN PRODUCTION / UTILISATION AND DISSOLVED OXYGEN VERSUS AVERAGE CHLOROPHYLL CONCENTRATION

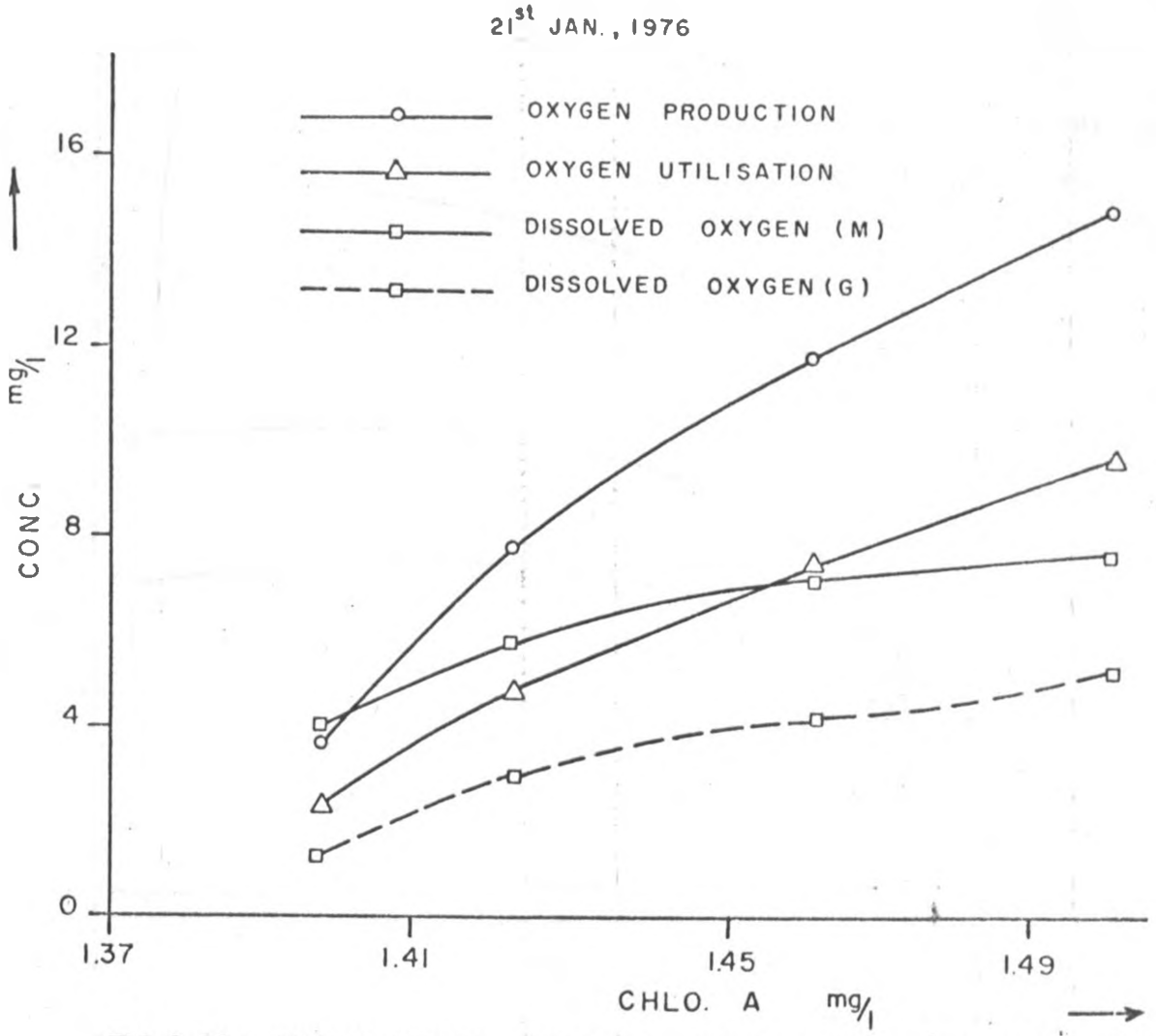


FIG. 6.12 : CUMULATIVE OXYGEN PRODUCTION / UTILISATION AND DISSOLVED OXYGEN VERSUS AVERAGE CHLOROPHYLL CONCENTRATION

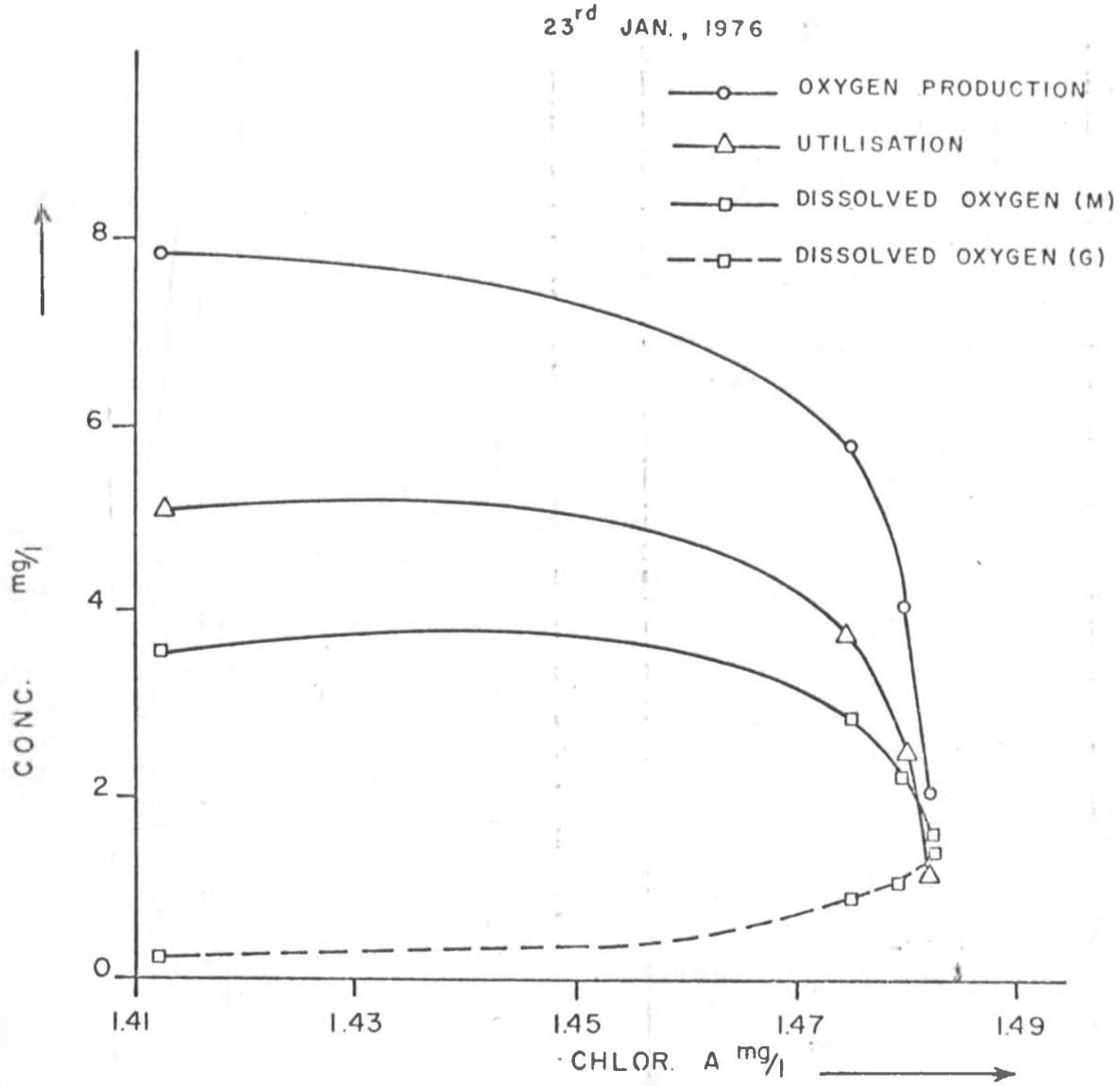


FIG. 6.13 : CUMULATIVE OXYGEN PRODUCTION / UTILISATION AND DISSOLVED OXYGEN VERSUS AVERAGE CHLOROPHYLL CONCENTRATION

EXPERIMENT SEVEN

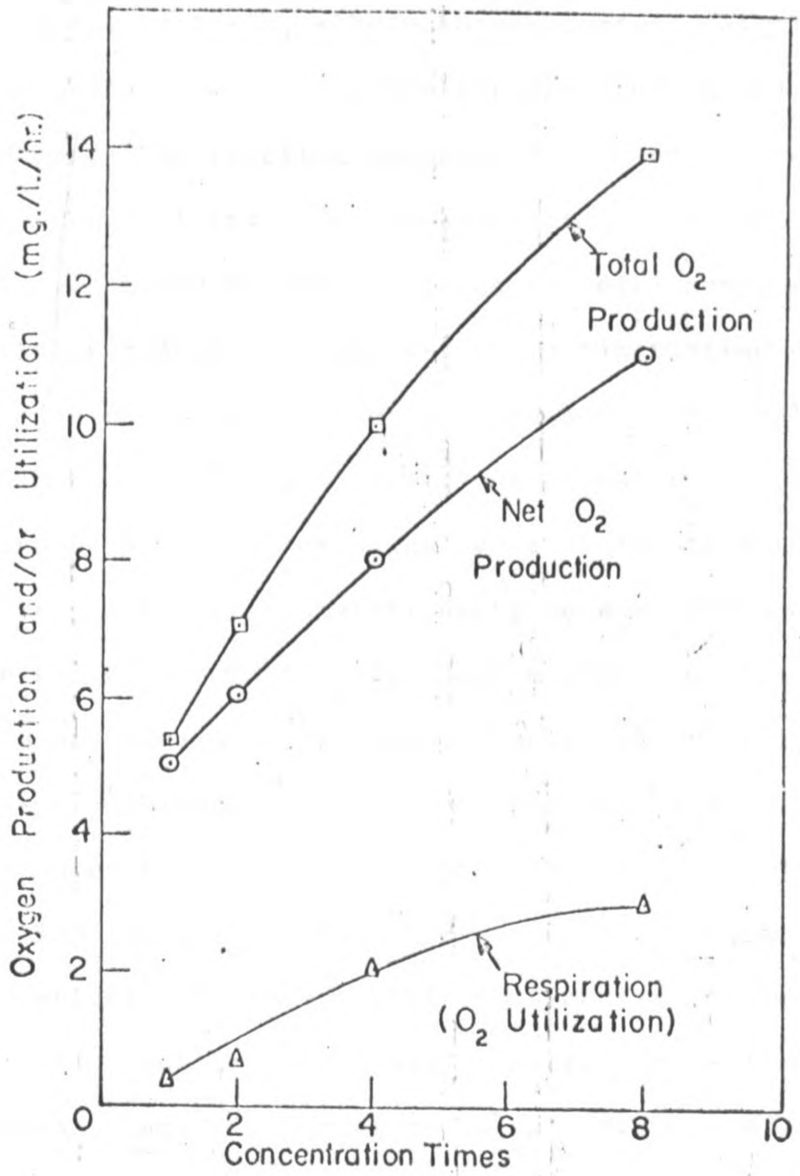


Figure 6.14 · *Effect of algae concentration on oxygen production.*

(AFTER LUEBBERS, 1966)

mixed biomass growth reactor shows that the relationships between the bacteria, substrate consumption, algal growth, oxygen production and consumption are coupled and oscillatory in nature. The reaction sequence of bacterial growth - substrate consumption-oxygen consumption on the one hand and algal growth oxygen production on the otherhand, may be characterised as a linearly-coupled fluctuating control system in which the intensity of each response will oscillate and will be slightly out of phase with the preceding component of the system. The above stated is attained only when a symbiotic relationship between the bacteria and algae has been established. Such a state is of a temporary nature as there are alternating bacterial and algal active phases brought about by food and nutrient availability, light intensity and periodicity, toxicity etc. Waste stabilisation pond operation experience has shown that the symbiotic algal-bacterial relationship may be thrown out of dynamic equilibrium by many factors. Moawad et al (1969) reported a periodic variation in the effluent BOD of a waste stabilisation pond. He postulated that the periodic variation was caused by the fact that the symbiotic relationship between algae and bacteria could not reach dynamic equilibrium of a continuous symbiotic nature. He states that:-

> "In certain instances the conditions are such that instead of mutual interdependence, the development of one partner proceeds at the expense of the other leading to a disturbance of the acquired balance."

They further stated that one of the major effects which follow the death and autolysis of bacterial cells is the liberation of endotoxins and metabolite antagonists which render the medium unfavourable for microbial activity.

When a new waste stabilisation pond is commissioned, waste water is allowed to fill the pond up to a certain depth and left to stand. According to Oswald et al (1953), oxidation of the soluble organic matter by bacterial action takes place during the first two weeks or so. This phase is soon succeeded by the algal phase when, as a result of photosynthesis, molecular oxygen is produced in amounts permitting bacterial oxidation to proceed unhindered. These two phases are not necessarily sharply divided; rather they may overlap to some extent. After adequate development of the algal phase, the waste stabilisation pond is operated as a continuous flow reactor. Amin and Ganapati (1972), conducted laboratory studies of the first stage of commissioning of waste stabilisation ponds. They reported on the bacteria concerned in the algal bacterial symbiosis and the pattern of degradation of the soluble organic constituents. Changes taking place in the bacterial populations, in the soluble organic constituents, in the physicochemical and biological condition, in the algae and protozoan populations, along with the interrelationships existing among them were studied. Some of their experimental findings are shown in figures 6.21 to 6.24 and there is a similarity between their results and those reported herein.

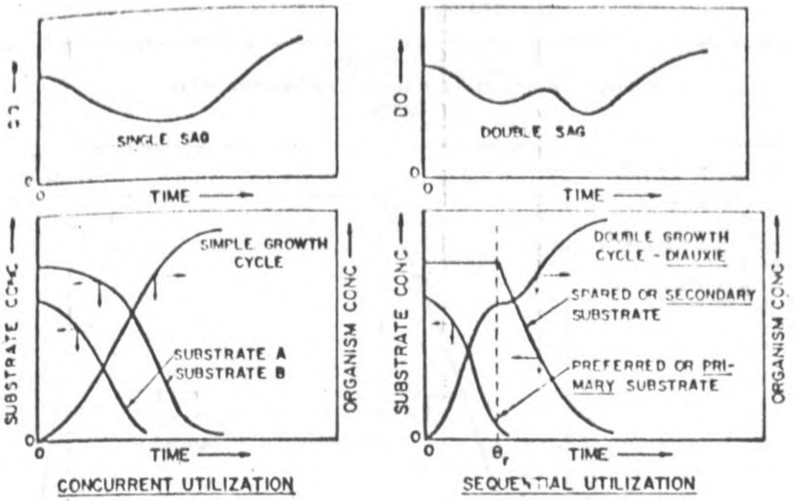


Fig. 6.15.—Schematic representation of concurrent and diauxic assimilation of substrates in batch or plug-flow processes.

(AFTER GHOSH ET AL, 1972)

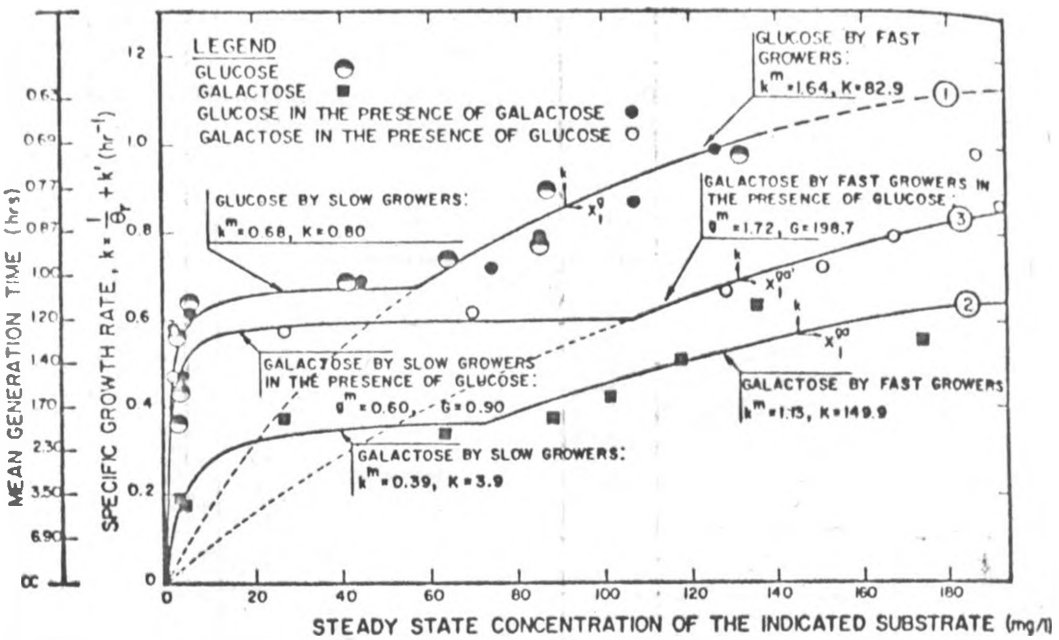


Fig. 6.16.—Steady-state concentrations of indicated substrates at various specific growth rates. (AFTER GHOSH ET AL, 1972)

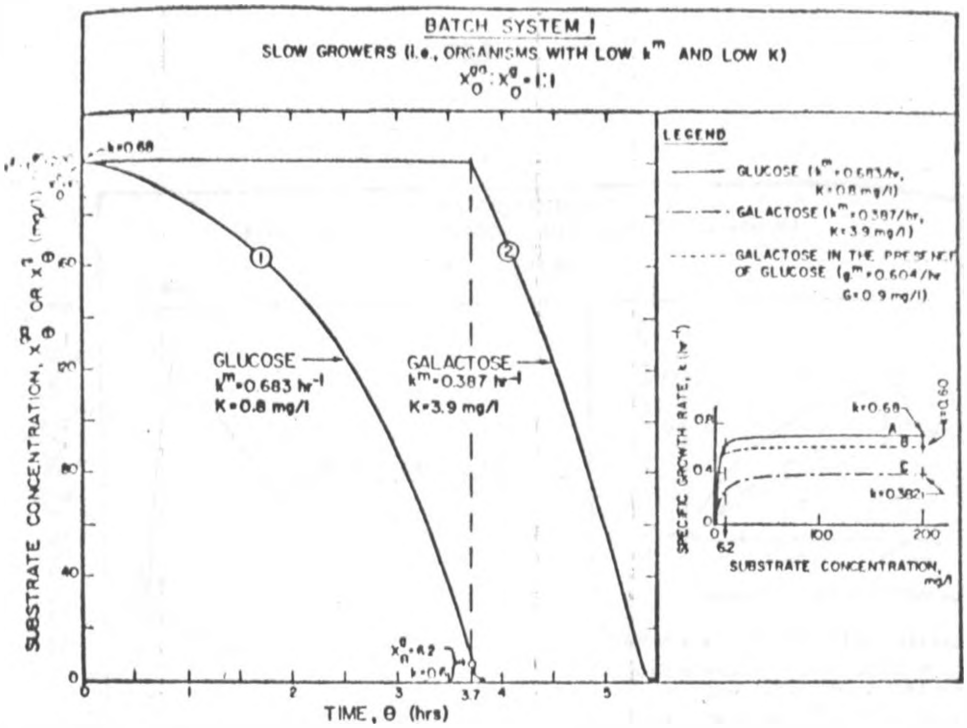


Fig 6.17 —Theoretical curves of sequential utilization of glucose and galactose by a batch culture of slow growers. (AFTER GHOSH ET AL 1972)

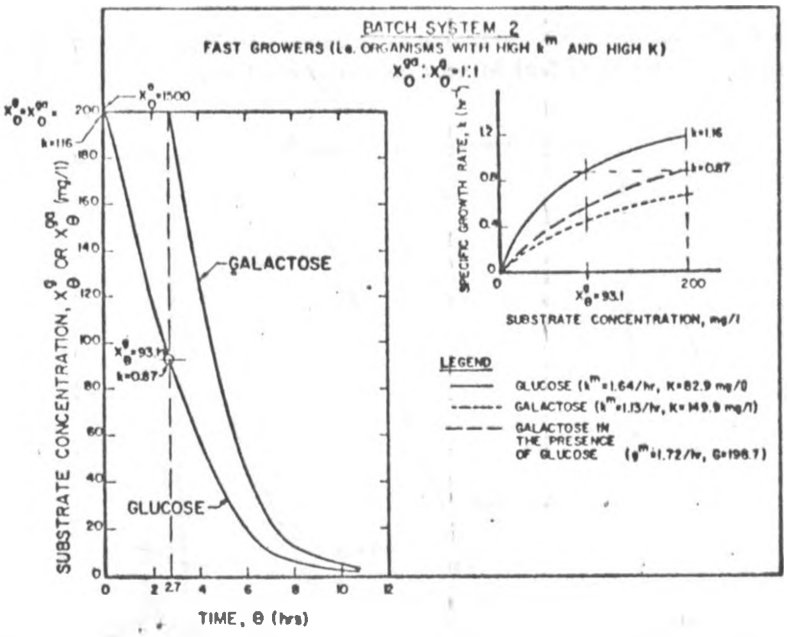


FIG. 6.18 —Theoretical curves of sequential utilization of glucose and galactose by a batch culture of fast growers. (AFTER GHOSH ET AL, 1972)

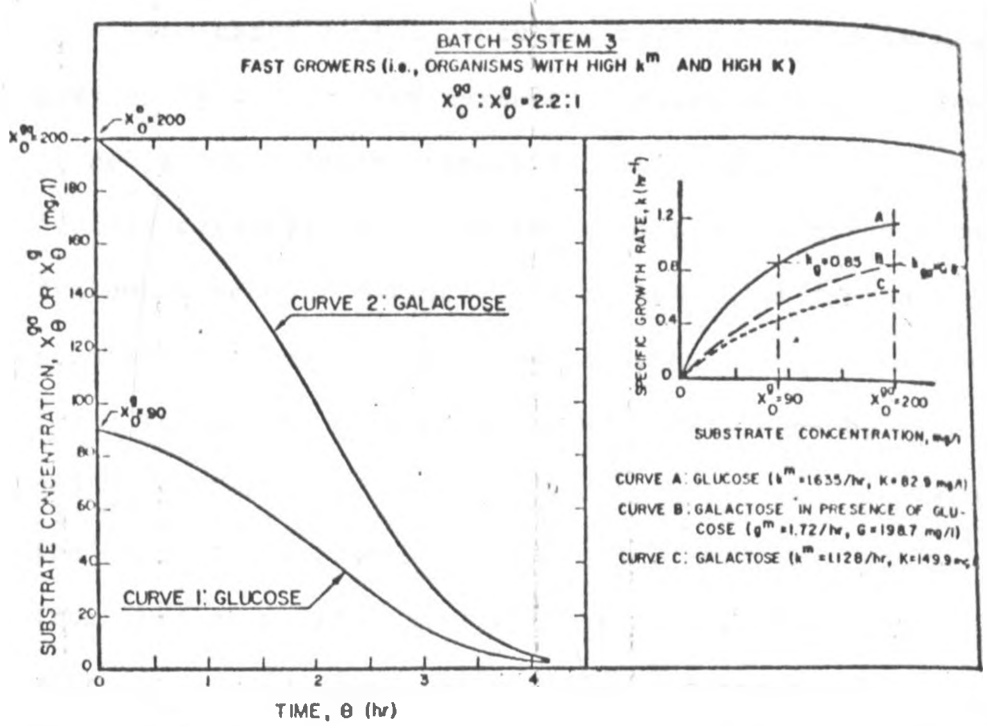


Fig. 6.19—Theoretical curves of concurrent utilization of glucose and galactose by a batch culture of fast growers. (AFTER GHOSH ET AL 1972)

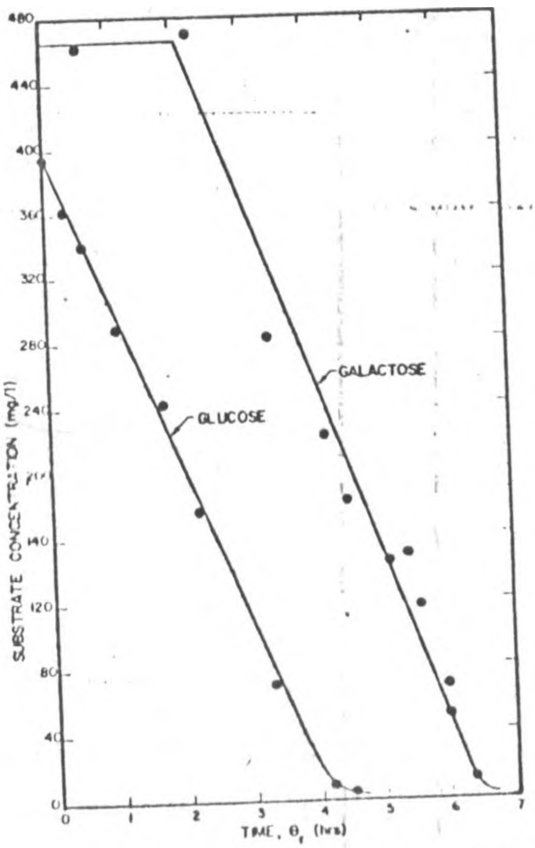


Fig. 6.20—Sequential utilization of glucose and galactose at 20°C by a seed population obtained from a galactose-fed, continuous-flow reactor operated at a detention time of 12.2 hr. (AFTER GHOSH ET AL, 1972)

The experimental study has been conducted in a closed, completely mixed batch systems in which oxygen was derived from algae photosynthesis (except for the originally present atmospheric oxygen). Although the condition in these batch systems were well defined, the experimental findings can be related to natural ecosystems behaviour.

The aquatic environments of lakes, rivers, impoundments and estuaries are different from each other. For example, the aquatic environment of a flowing river is vastly different from that of an impounded body of water. However, factors affecting production, assimilation and recycling of organic matter and nutrients in these water bodies are basically the same (Rawson, 1939). Therefore, under certain conditions, the behaviour of one water body may be comparable to that of another.

In the batch reactor, the organic input undergoes physical, chemical and biological changes with time as a result of bacterial and algal growth. The bacteria and algae go through changing states of growth, representing different phases on their respective growth curves. An organic input is followed by substrate consumption, bacterial growth and oxygen consumption. After some time or at the same time in certain cases, algal growth takes place. The rate and amount of algal growth are limited to the availability of carbon dioxide and nutrients released by bacterial oxidation of the organic input.

In water bodies, the discharge of organic and inorganic substances into them provides conditions which enhance, in the majority of cases, the growth of algal bacterial ecosystems.

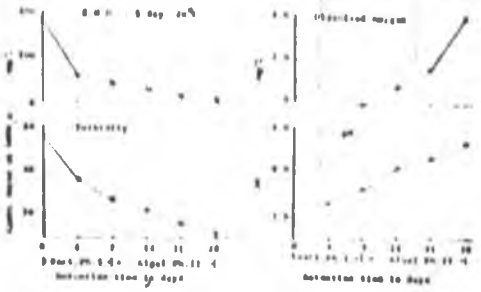


Fig. 6.21 —Changes in physico-chemical variables.

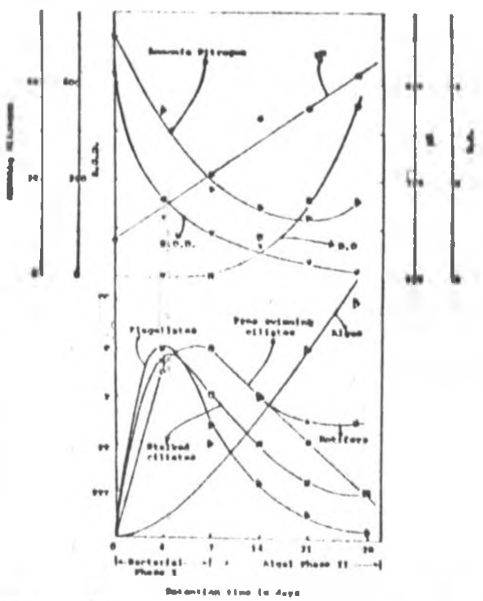


Fig. 6.22—Relation between important physicochemical variables and the protozoan population.

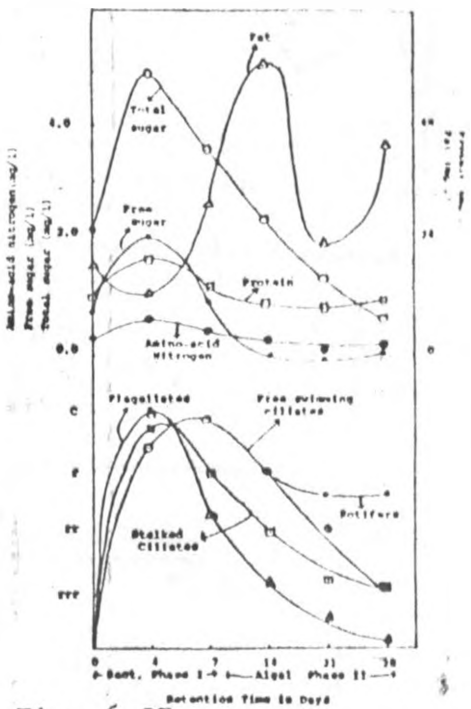


Fig. 6.23—Relation between the biochemical constituents and the protozoan population.

(AFTER AMIN ET AL, 1972)

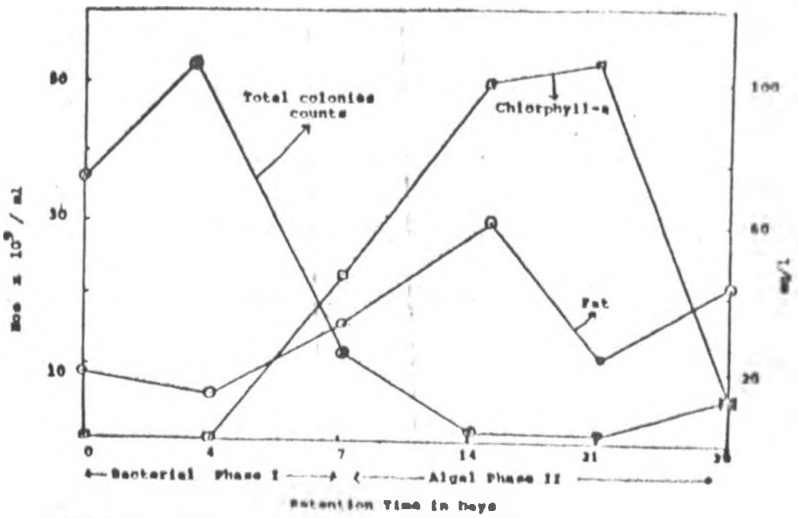


Fig. 6.24.--Relation between total colonies count, chlorophyll a, and fat content.

(AFTER AMIN ET AL, 1972)

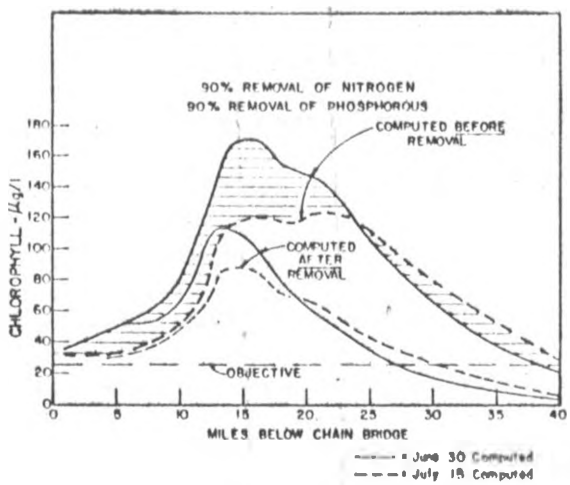


Fig. 6.25.--1969 Simulation of Chlorophyll: Dashed Line, June 30 Profile; Solid Line, July 15 Profile (1 mile = 1.61 km) (AFTER THOMANN ET AL, 1974)

The organic input may be constant or variable but continuous or it may be constant or variable but intermittent. The organic input may be into standing, running waters or into oscillating flows as occurs in the tidal reaches of estuaries. Discharge into any of these water bodies causes a space-time variability in the physical, chemical and biological characteristics of the water. Bacterial, algal and zooplankton growth processes, chlorophyll concentrations, dissolved oxygen, and oxygen production/consumption are among the processes and parameters that exhibit a space-time variability. The algal-bacterial growth processes, substrate consumptive and oxygen productive processes in these water bodies are complex. They are controlled by interdependent physical, chemical, biological and environmental factors. In general, organic inputs into any water body result in bacterial growth and consumption of the dissolved oxygen for aerobic breakdown. Bacterial growth may be associated with algal growth depending on such factors as availability of nutrients, light conditions, hydrodynamic conditions, temperature and toxic substances. Photosynthetic oxygenation may play a major role in providing the oxygen required by the bacteria for stabilisation of the organic input.

Dresnack and Metzger 1968 studied the response of oxygen to inputs of pollutants into streams. They focussed their attention on dissolved oxygen concentration changes due to atmospheric aeration and photosynthetic oxygen production. They found that bacterial oxidation of the organic

input was the predominant factor causing changes in dissolved oxygen concentrations at the source and for some distance from the source. The effect of the bacterial oxidation of the organic input decreased with distance. They also found that at some distance from the source of pollution, variations in dissolved oxygen became dependent upon the algae. With algal growth oxygen production increased but also there was a greater respiratory algal oxygen demand at night. The substantial effect of algae was demonstrated by experiments in which the algae effect was taken into account and others in which this effect was not present. Values of dissolved oxygen concentrations taken along the stream at 6 a.m. and 6 p.m. are shown in figure 6.26. The algae effect is shown to be substantial.

Thomann R.V, et al, 1974 presented a preliminary model of the dynamic behaviour of phytoplankton in the upper Potomac Estuary. The model incorporated the space time variability of chlorophyll as a water quality parameter indicative of a eutrophied environment. Chlorophyll variation with distance is shown in figure 6.25. The basic model is built on spacial considerations. The potomac estuary is divided into segments using data on depth, cross-sectional areas and segment volumes. Effects of tidal bays, tidal flat areas and latteral effects of the shallow water areas are taken into account. Each segment is assumed to be completely mixed. Interactions between nine variables which are space and time dependent are investigated. Among the nine variables are phytoplankton chlorophyll, dissolved oxygen and zooplankton.

Edwards R.L. et al 1968 investigated the ecology of the Trent Watershed (figure 6.27) samples of algae and zooplankton as well as the chemical and physical properties of the water were surveyed. The results of the survey are shown in figure 6.27 under the categories primary producers, primary consumers and primary predators. Some of the lakes had high levels of both producers and consumers. Others had high levels of producers and low levels of consumers and vice versa. The results suggested that different biological control mechanisms were at work in different parts of the system.

The applicability of controlled laboratory experiments concerning biological processes to aquatic ecosystems is limited by the greater complexity (in nature) of the water quality (physical, chemical and biological properties), its hydrodynamics and the environment. Sources from which organic/ inorganic inputs emanate are many and varied. Much of the inputs are obtained through the inflow of streams (e.g. to lakes, estuaries, impoundments) and point sources (sewage outfalls), A very substantial quantity may be added directly from the shores of lakes and banks of rivers. The organic inputs may undergo stabilisation by bacterial action immediately or be partially or wholly stored within the system through sedimentation,, to be degraded later under more favourable environmental and/or chemical conditions. In general, build-up and displacement of the inputs in the water-body may vary between two extremes, i.e. fast, purging displacement

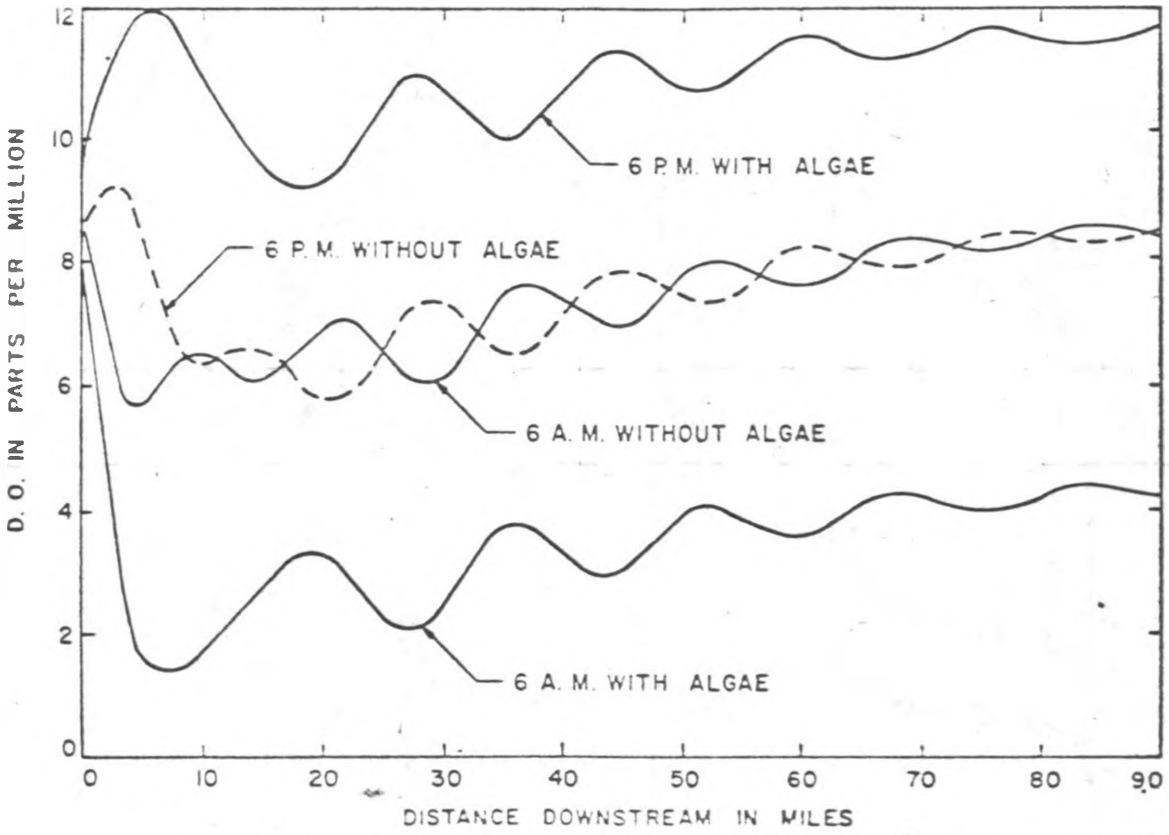
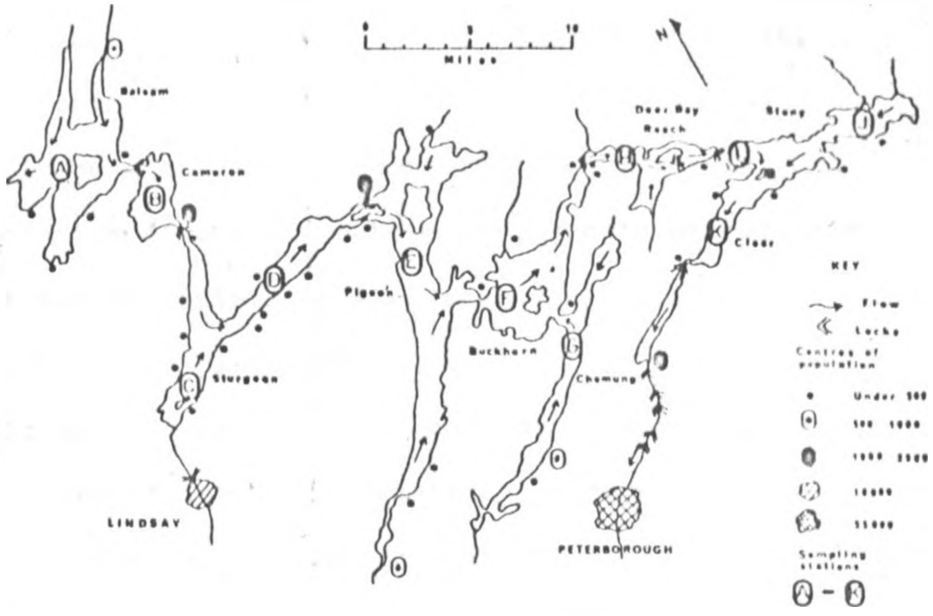


Fig. 6.26 Comparison of algae effects on DO profiles (AFTER DRESNACK ET AL, 1968)



The Kawartha Lakes showing centers of permanent (winter) population, direction of flow of water in the lakes, location of dams and locks, and the sampling stations used during the 1967 ecological survey.

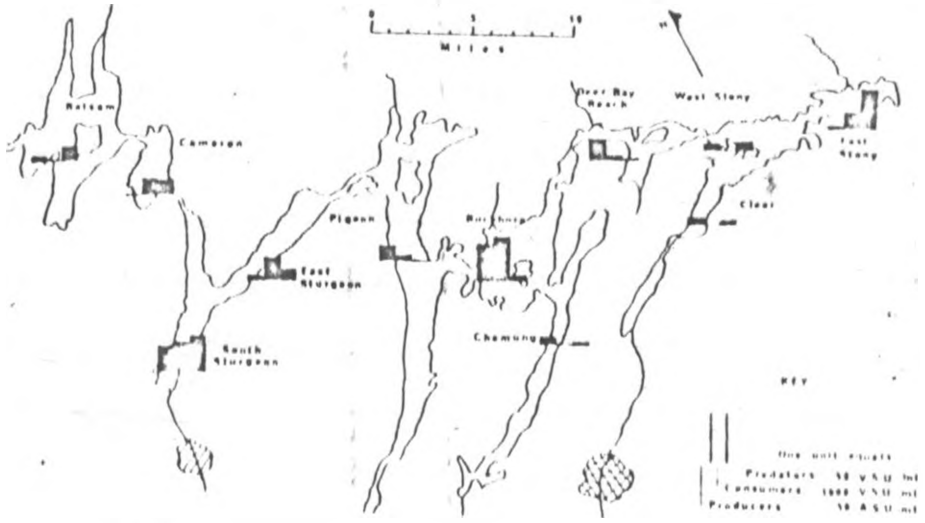


Fig. 6.27 - *Relative quantities of primary producers, primary consumers, and primary predators collected at each sampling station in the Kawartha Lakes during the period August 3-9, 1967. Primary producers expressed as ASU ml, primary consumers and primary predators expressed as VSU/liter. (See bottom page 679 for ASU and VSU methods.)*

(AFTER EDWARDS ET AL 1968)

and a slow diffusion. This may vary from point to point, locality to locality, reach to reach in the water body. However, it is possible that characteristics of inputs, and biota, degradation of organic substances, growth of bacteria and algae at various points in the water body exhibit a certain stability and predictability.

Dynamic changes in substrate consumption, bacterial growth, algal growth, oxygen production - consumption as found in batch systems, may occur in a more or less established pattern or range at points, localities or reaches in the water body. Food inputs, under favourable conditions, may set off the sequence of processes, bacterial growth-substrate consumption- - oxygen consumption, algal growth - oxygen production/ consumption, - zooplankton growth etc. These may take place, with time, at a single stationery locality or with time and distance from the source of inputs. Such changes have been demonstrated by a number of investigators (Bailey T.E., 1970; Edwards et al, 1968, Thoman et al, 1974, Dresnack et al 1968). They have been shown to be governed by complex and interrelated physical, chemical, biological and environmental conditions.

7. CONCLUSION

1. An aquatic ecosystem is a network of delicately balanced interactions between physical, chemical and biological forces. For the improvement of our knowledge of such systems, consideration of their dynamic behaviour is necessary because their process characteristics have large temporal variations. Laboratory batch reactor systems may form useful tools for investigating the dynamic behaviour of such ecosystems under certain conditions. The process characteristics of aquatic ecosystems are usually oscillating around some steady state condition or changing to some new equilibrium or undergoing a complete change in the characteristics of the process itself.

2. The present study has shown that the relationships between bacterial growth, substrate consumption, algal growth, oxygen production/consumption may be characterised as coupled and fluctuating in nature. The processes bacterial growth-substrate consumption - oxygen consumption on the one hand and algal growth-oxygen production/consumption on the otherhand are sequential and the intensity of one may be dependent on the intensity of the preceding component of the systems.

3. The substrate, its composition and concentration, all other factors being equal, affects the rates and extent of both bacterial and algal growth. A single component substrate such as glucose requires a less complex deployment of enzymes for bacterial degradation of the substrate than a multi-component

substrate (glucose, yeast extract, starch). The ratio of biodegradable to non-biodegradable fraction of the substrate may also influence the pattern and rates of degradation. The above may determine the extent and rates of carbon dioxide availability to the algae for growth.

4. Bacterial oxidation of the substrate reduces the chemical oxygen demand of the growth medium and releases carbon dioxide and other nutrients to the algae for growth. The growth of algae and bacteria is eventually followed by zooplankton and protozoan growth. Algal population dynamics was characterised by the initial growth and abundance of non-motile algae (ankistrodesmus). Later when food was not so plentiful - motile algae were the predominant types (Euglena). In general, seeding of the reactor resulted in reduction in the overall numbers of the algae (dilution effect)as well as the variety of species. A lag phase followed and then growth of non-motile algae.

5. The greatest reduction in chemical oxygen demand occured within the first two days after start-up of the biomass growth reactor. The rates of chemical oxygen demand reduction were generally higher for the filtered samples than for the unfiltered samples. Slightly higher average rates of chemical oxygen demand reductions (unfiltered) were obtained for the experiments in which a multi-component substrate was used as a growth medium. After the initial rapid reduction in the COD, the COD values tended to fluctuate around some minimum value.

6. The oxygen production by algae is determined by the activity of the algae and its active chlorophyll content per unit cell. It is also a function of cell growth state, nutritional

status and light conditions. Shading at higher algal densities affects oxygen production. Oxygen consumption rate is determined by the requirements of the biota for cell synthesis, growth and energy for maintenance. At start-up, bacterial oxidation of the substrate was the predominant factor utilising oxygen. With algal growth, algal respiration during dark period made heavy demands on the oxygen resources of the reactor.

7. It is difficult to establish precise relationships between substrate consumption, algal growth and oxygen production/ consumption because of the complexity of the processes taking place in the reactors and the variability of the experimental conditions in terms of initial seed concentrations, physiological properties of the biota etc. In practise, mathematical models are used to simulate the behaviour of parameters of significant importance in eutrophication studies, and self-purification studies of streams. The models have to be adapted to each particular case and may change with changes in the types and quantities of inputs, factors affecting oxygenation, phytoplankton growth etc.

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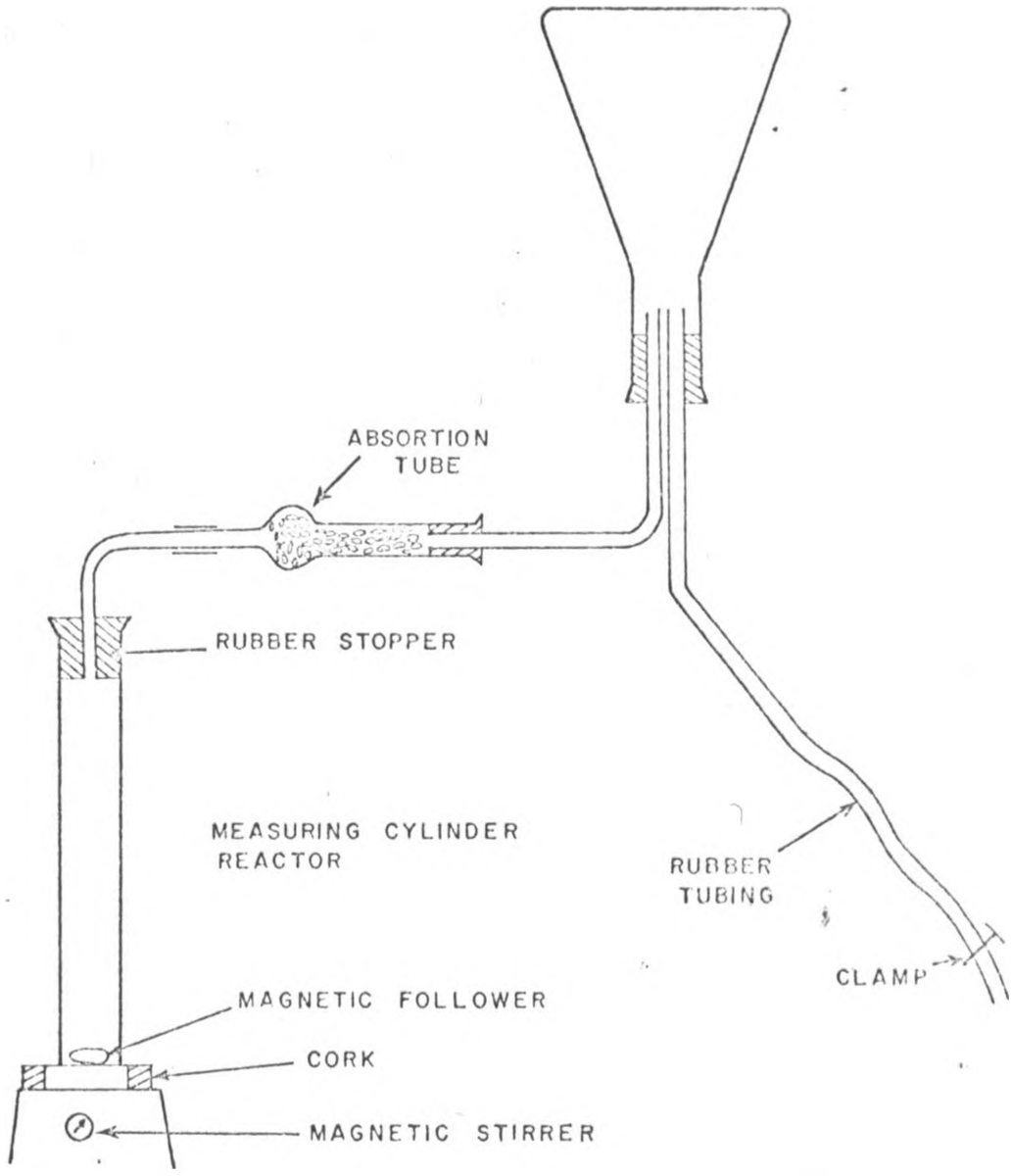
APPENDIX, I



BATCH REACTOR - EXPERIMENTS ONE TO FIVE

APPENDIX II

BIOLOGICAL GROWTH REACTOR

1. Reactor Material Perspex

   Thickness - 6 mm

2. Reactor Components

   A. Base plate

      a. shape - hexagonal

         thickness - 18 mm (Central part - 6 mm)

      b. Diameter of circumscribed

         circle - 418 mm

      c. Area 0.1764 m^2

   B. Cover Plate

      a. shape - hexagonal

      b. Thickness - 6 mm

      c. Diameter - 418 mm

         (circumscribed circle)

      d. Holes for eight - 4 mm Bolts and Nuts

   C. Side Plates

      a. Shape - Rectangular - 500 x 130

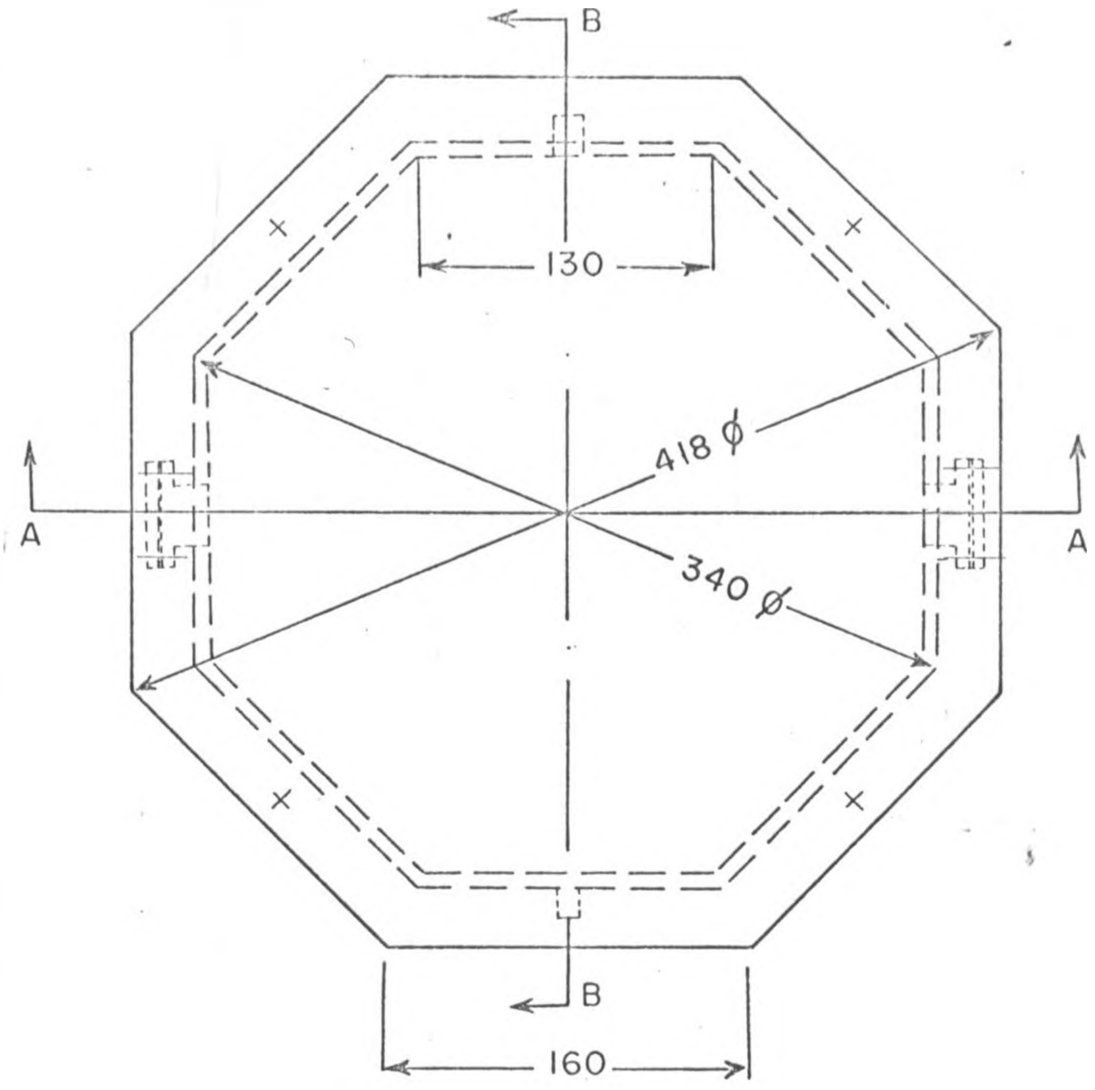
      b. Number of Plates - Eight

   D. Flange (Top of Reactor)

   E. DO and pH Probes

      a. Number - 3

APPENDIX II

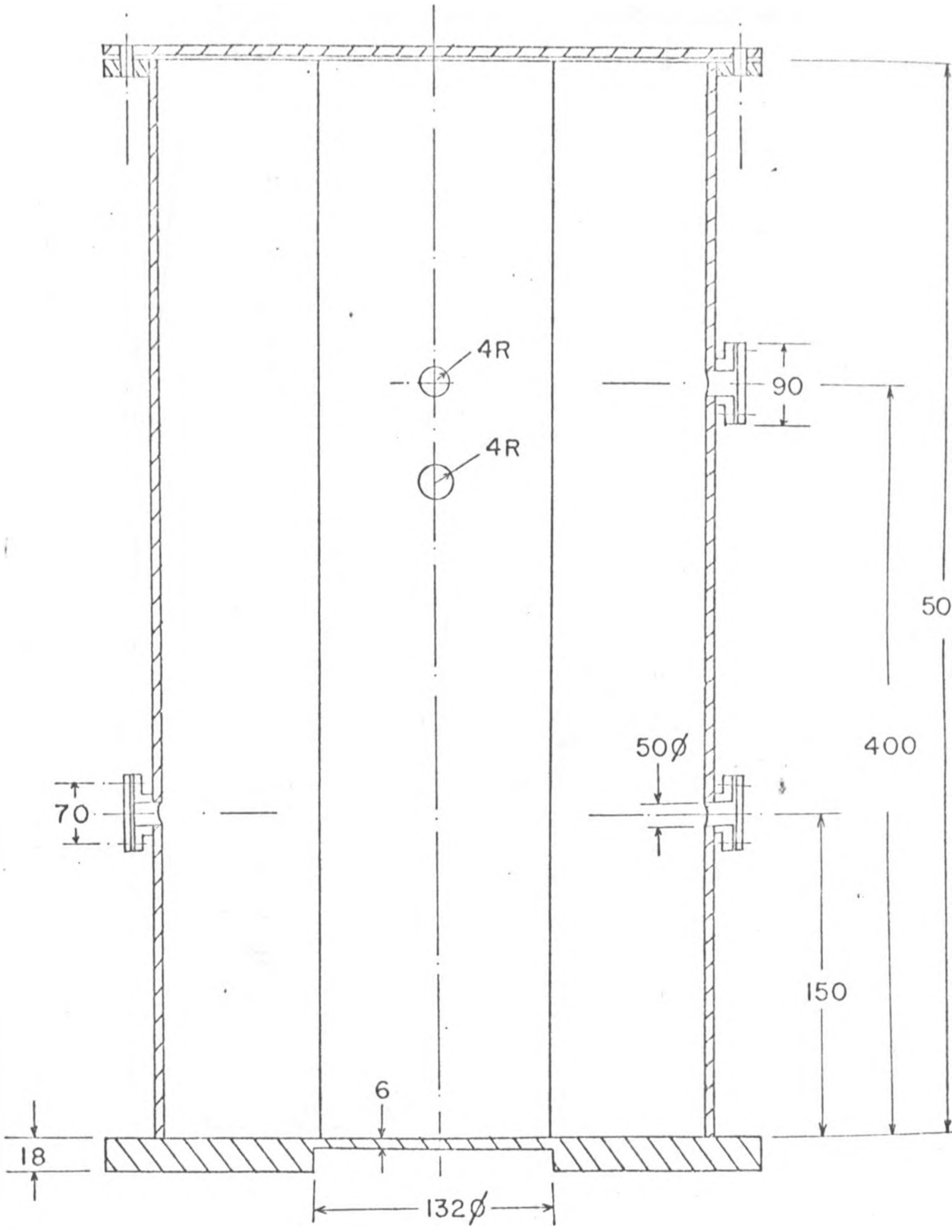


BATCH REACTOR

APPENDIX II

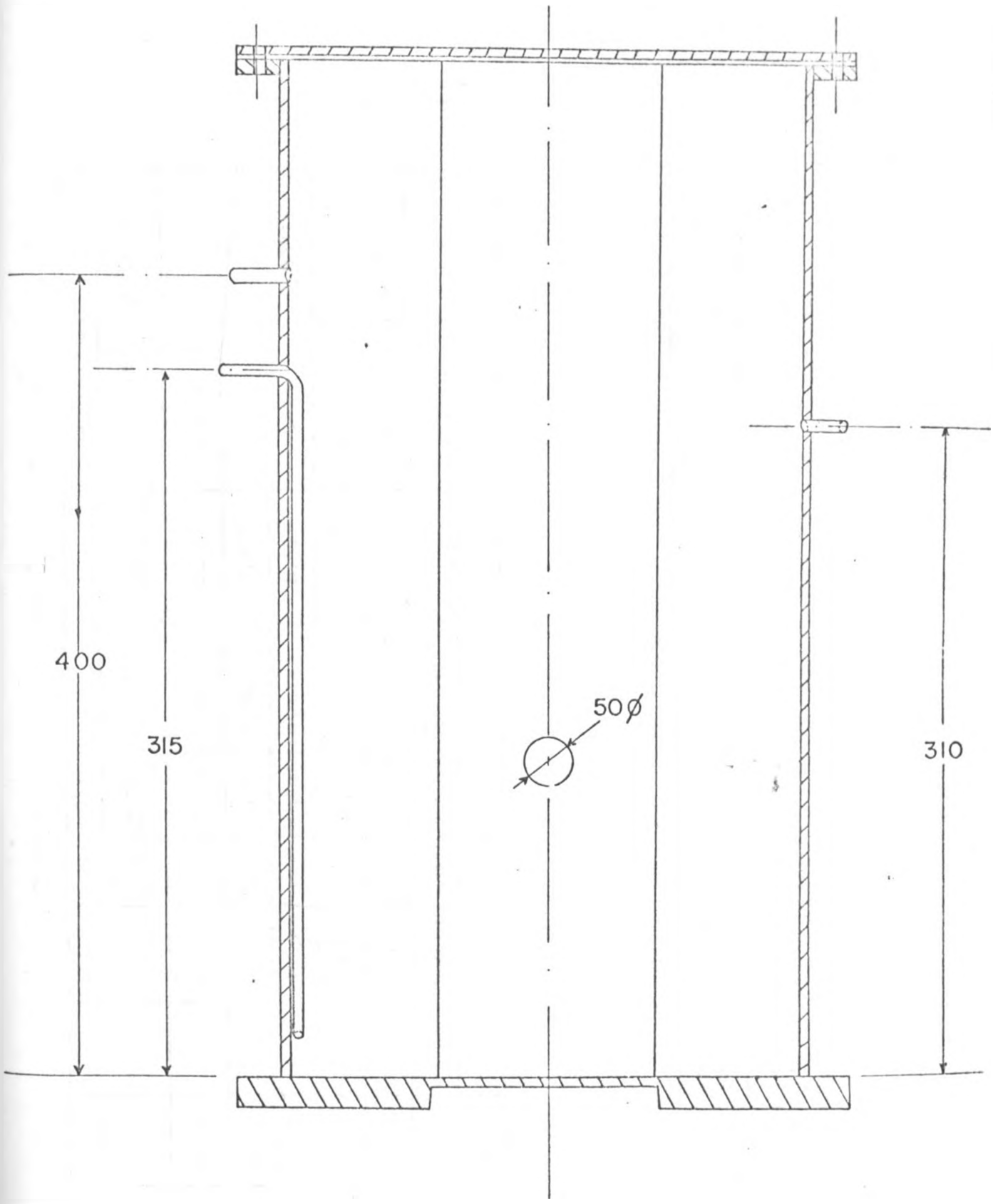
BATCH REACTOR

SECTION ON A – A



APPENDIX II

SECTION ON B — B



BATCH REACTOR

TABLE 1 EXPERIMENT SEVEN 30/9/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO	Resp. R mg/l/hr	O_2Prod. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.40	25.10	5.69	0.100	0.400	0.500	0.9982	
10.00	6.40	25.00	5.89	0.050	0.400	0.450		
12.00	6.40	25.00	5.99	0.115	0.400	0.515	1.0323	0.0341
14.00	6.40	25.00	6.22	0.340	0.400	0.740		
16.00	6.40	25.00	6.90	0.350	0.400	0.750	1.0482	0.0159
18.00	6.40	25.00	7.60	0.400	0.400	0.800		
20.00	6.40	25.00	8.40				1.0874	0.0392

Av. RR mg/l/hr	Av. Δ DO mg/l/hr	Av. O_2 PR. mg/l/hr	O_2PR. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas. Δ DO mg/l/day
0.400	0.2258	0.6258	7.5096	9.6000	2.0904	

TABLE 2 EXPERIMENT SEVEN - 1/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO	Resp.R. mg/l/hr	O_2Prod. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.40	24.00	3.60	0.450	0.400	0.850	1.0755	
10.00	6.40	24.30	4.50	0.500	0.400	0.900		
12.00	6.40	24.40	5.50	0.650	0.400	1.050	1.0819	0.0054
14.00	6.40	24.40	6.80	0.525	0.400	0.925		
16.00	6.45	24.60	7.85	0.575	0.400	0.975	1.0912	0.0093
18.00	6.49	24.60	9.00	0.600	0.400	1.000		
20.00	6.50	24.90	10.20				1.0980	0.0068

Av. R.R. mg/l/hr	Av.Δ DO mg/l/hr	Av.O_2PR. mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	ΔDO mg/l/day	Meas.ΔDO mg/l/day
0.4000	0.5500	0.9500	11.4000	9.6000	1.8000	1.8000
0.4165	0.5500	0.9665	11.5980	9.9960	1.6020	1.8000

TABLE 3 EXPERIMENT SEVEN 2/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO	Resp.R mg/l/hr	O_2 Prod. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.40	24.50	5.00	0.425	0.433	0.8580	1.0796	
10.00	6.40	24.50	5.85	0.600	0.433	1.0330		
12.00	6.44	24.50	7.05	0.475	0.433	0.9080	1.0897	0.0101
14.00	6.50	24.50	8.00	0.700	0.433	1.1330		
16.00	6.55	24.60	9.40	0.600	0.433	1.0330	1.1189	0.0292
18.00	6.55	24.60	10.60	0.600	0.433	1.0330		
20.00	6.55	24.60	11.80				1.1729	0.0540

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av.O_2PR. mg/l/hr	O_2 PROD. mg/l/day	O_2 Util. mg/l/day	Δ DO mg/l/day	Meas.Δ DO mg/l/day
0.4330	0.5667	0.9997	11.9964	10.3920	1.6044	1.6000
0.4500	0.5667	1.0167	12.2004	10.8000	1.4004	1.6000

TABLE 4 EXPERIMENT SEVEN - 3/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Res.Rate mg/l/hr	O_2 PROD. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.55	24.20	6.20	0.500	0.467	0.9670	1.1600	
10.00	6.55	24.20	7.20	0.600	0.467	1.0670		
12.00	6.70	24.20	8.40	0.500	0.467	0.9670	1.2450	0.0850
14.00	6.75	24.20	9.40	0.700	0.467	1.1670		
16.00	6.75	24.20	10.30	0.500	0.467	0.9670	1.4068	0.1618
18.00	6.75	24.20	11.80	0.400	0.467	0.8670		
20.00	6.75	24.20	12.60				1.5192	0.1124

Av. R.R. mg/l/hr	Av.Δ DO mg/l/hr	Av. O_2Pr. mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	ΔDO mg/l/day	Meas.ΔDO mg/l/day
0.4667	0.5333	1.0000	12.0000	11.2008	0.7992	0.8000
0.4459	0.5333	0.9792	11.7504	10.7016	1.0488	0.8000

TABLE 5 EXPERIMENT SEVEN - 4/10/76

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DC mg/l	Δ DO mg/l/hr	Res.Rate mg/l/hr	O_2 PROD. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.89	24.00	7.40	0.200	0.425	0.625	1.5608	
10.00	6.89	24.20	7.80	0.400	0.425	0.825		
12.00	7.00	24.20	8.60	0.410	0.425	0.835	1.6891	0.1283
14.00	7.00	24.20	9.42	0.090	0.425	0.515		
16.00	7.10	24.40	9.60	0.850	0.425	1.275	1.7608	0.0717
18.00	7.20	24.40	11.30	0.225	0.425	0.650		
20.00	7.20	24.40	11.75				1.7789	0.0181

Av. R.R. mg/l/hr	Av. Δ DO mg/l/hr	Av. O_2 PROD. mg/l/hr	O_2 PROD. mg/l/day	O_2 UTIL. mg/l/day	ΔDO mg/l/day	Meas. ΔDO mg/l/day
0.4250	0.3625	0.7875	9.4500	10.2000	0.7500	0.7500
0.4250	0.3625	0.7875	9.4500	10.2000	0.7500	0.7500

TABLE 6 EXPERIMENT SEVEN - 5/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	ΔDO mg/l/hr	Resp.Rate mg/l/hr	O_2PROD. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.88	23.80	6.65	0.275	0.425	0.700	1.7805	
10.00	7.00	23.90	7.20	0.500	0.425	0.925		
12.00	7.10	24.00	8.20	0.700	0.425	1.125	1.7209	-0.0596
14.00	7.20	24.00	9.60	0.350	0.425	0.775		
16.00	7.25	24.20	10.30	0.325	0.425	0.750	1.5998	-0.1211
18.00	7.30	24.30	10.95	0.125	0.425	0.550	1.4532	-0.1466
20.00	7.50	24.40	11.20					

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av.O_2PR. mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	ΔDO mg/l/day	Meas.ΔDO mg/l/day
0.4250	0.3792	0.8042	9.6500	10.2000	0.5500	0.5500
0.4459	0.3792	0.8251	9.9012	10.7016	0.8004	0.5500

TABLE 7 EXPERIMENT SEVEN 6/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	RES.RATE mg/l/hr	O_2PROD. mg/l/hr	CHLOR. mg/l	Δ CHLOR.
08.00	7.05	24.00	5.60	0.550	0.4667	1.0167	1.4409	
10.00	7.10	24.00	6.70	0.475	0.4667	0.9417		
12.00	7.20	24.00	7.65	0.325	0.4667	0.7917	1.2873	-0.1536
14.00	7.30	24.20	8.30	0.400	0.4667	0.8667		
16.00	7.40	24.40	9.10	0.150	0.4667	0.6167	1.2053	-0.0820
18.00	7.50	24.40	9.40	0.250	0.4667	0.7167		
20.00	7.60	24.50	9.90				1.1250	-0.0803

AV.R.R. mg/l/hr	Av.Δ DO mg/l/hr	Av.O_2PR. mg/l/hr	O_2PROD. mg/l/day	O_2UTIL. mg/l/day	Δ DO mg/l/day	Meas.ΔDO mg/l/day
0.4667	0.3583	0.8250	9.9004	11.2008	1.3004	1.3000
0.4975	0.3583	0.8558	10.2696	11.9400	1.6704	1.3000

TABLE 8 EXPERIMENT EIGHT - 29/10/75

TABLE 8 EXPERIMENT EIGHT - 29/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2UTIL. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.30	26.50	6.45	0.650	0.650	1.3000	1.1108	
10.00	6.29	26.50	5.15	0.475	0.475	0.9500		
12.00	6.20	26.40	4.20	0.425	0.425	0.8500	1.1174	0.0066
14.00	6.20	26.20	3.35	0.300	0.300	0.6000		
16.00	6.20	26.00	2.75	0.200	0.200	0.4000	1.1159	0.0051
18.00	6.20	26.00	2.35	0.215	0.215	0.4300		
20.00	6.20	25.90	1.92				1.1221	0.0113

Av. R.R. mg/l/hr	Av.Δ DO mg/l/hr	Av. O_2PR. mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas.ΔDO mg/l/day
0.3775	0.294	0.6715	8.0580	9.0600	1.0020	1.0000
0.3367	0.294	0.6307	7.5678	8.5698	1.0020	1.0000

TABLE 9 EXPERIMENT EIGHT - 30/10/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2 UTIL. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.29	26.00	5.45	0.5000	0.500	1.000	1.2116	
10.00	6.25	25.90	4.45	0.375	0.3750	0.750		
12.00	6.20	25.90	3.70	0.325	0.325	0.650	1.2052	0.0064
14.00	6.23	25.90	3.05	0.200	0.200	0.400		
16.00	6.22	25.90	2.65	0.205	0.205	0.410	1.1962	0.0154
18.00	6.22	25.50	2.24	0.170	0.170	0.340		
20.00	6.20	25.20	1.90	...			1.1911	0.0205

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av. O_2PR mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas.ΔDO mg/l/day
0.2958	0.3083	0.6041	7.2492	7.0992	0.1500	0.1500
0.2917	0.3083	0.6000	7.2000	7.0500	0.1500	0.1500

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	ΔDO mg/l/hr	Resp.Rate mg/l/hr	O_2UTIL. mg/l/hr	Chlor. mg/l	ΔChlor.
08.00	6.30	25.90	5.60	0.440	0.440	0.880	1.2744	
10.00	6.30	25.50	4.72	0.370	0.370	0.740		
12.00	6.30	25.60	3.98	0.290	0.290	0.580	1.2510	0.0234
14.00	6.30	25.60	3.40	0.350	0.250	0.500		
16.00	6.30	25.50	2.90	0.175	0.175	0.350	1.2709	0.0035
18.00	6.30	25.30	2.55	0.200	0.200	0.400		
20.00	6.25	25.00	2.15				1.2812	-0.0068

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av. O_2PR mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	ΔDO mg/l/day	Meas.ΔDO mg/l/day
0.2875	0.3415	0.6272	7.5504	6.9000	0.6504	0.65000
0.2725	0.3415	0.6142	7.3704	6.7200	0.6500	0.6500

TABLE 11 EXPERIMENT EIGHT - 1/11/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Res.Rate mg/l/hr	O_2 UTIL. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.35	25.60	6.25	0.410	0.410	0.820	1.3686	
10.00	6.30	25.60	5.43	0.355	0.355	0.710		
12.00	6.30	25.60	4.72	0.295	0.295	0.590	1.3559	0.0127
14.00	6.30	25.60	4.13	0.240	0.240	0.480		
16.00	6.30	25.60	3.65	0.120	0.120	0.240	1.3623	0.0063
18.00	6.30	25.60	3.41	0.125	0.125	0.250		
20.00	6.30	25.60	3.16				1.3780	-0.0094

Av. R.R. mg/l/hr	Av. Δ DO mg/l/hr	Av. O_2 PROD mg/l/hr	O_2 PROD. mg/l/day	O_2 Util. mg/l/day	Δ DO mg/l/day	Meas. Δ DO mg/l/day
0.2575	0.3767	0.6342	7.6104	6.1800	1.4304	1.4300
0.2671	0.3767	0.6438	7.7256	6.4104	1.3152	1.4300

TABLE 12 EXPERIMENT EIGHT 2/11/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2 UTIL. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.50	25.60	7.68	0.510	0.510	1.020	1.3709	
10.00	6.50	25.70	6.65	0.380	0.380	0.760		
12.00	6.45	25.90	5.88	0.220	0.220	0.440	1.3791	-0.8882
14.00	6.45	25.90	5.00	0.250	0.250	0.500		
16.00	6.42	25.90	4.50	0.200	0.200	0.400	1.3780	-0.0071
18.00	6.40	25.90	4.10	0.100	0.100	0.200		
20.00	6.40	25.90	3.90				1.3892	-0.0183

Av. R.R. mg/l/hr	Av. Δ DC mg/l/hr	Av. O_2 PR mg/l/hr	O_2 PROD. mg/l/day	O_2 Util. mg/l/day	Δ DO mg/l/day	Meas Δ DO mg/l/day
0.2767	0.4750	0.7517	9.0204	6.6408	2.3790	1.9200
0.3175	0.4750	0.7925	9.5100	7.6200	1.8900	1.9200

TABLE 13 EXPERIMENT EIGHT 3/11/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2 UTIL mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.70	26.40	9.60	0.520	0.520	1.040	1.3429	
10.00	6.80	26.30	8.56	0.480	0.480	0.960		
12.00	6.60	26.20	7.60	0.355	0.355	0.710	1.3406	0.0023
14.00	6.60	25.90	6.89	0.350	0.350	0.700		
16.00	6.60	25.90	6.19	0.250	0.250	0.500	1.3432	-0.0003
18.00	6.60	25.90	5.69	0.195	0.195	0.390		
20.00	6.60	25.90	5.30				1.3444	-0.0015

Av. R.R. mg/l/hr	Av ΔDO mg/l/hr	Av. O_2 PROD. mg/l/hr	O_2 PROD. mg/l/day	O_2 Util. mg/l/day	Δ DO mg/l/day	Meas. ΔDO mg/l/day
0.3583	0.4042	0.7626	9.1500	8.5992	0.5508	0.5500
0.3771	0.4042	0.7813	9.3750	9.0492	0.3258	0.5500

TABLE 14 EXPERIMENT EIGHT 4/11/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2 UTIL. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	6.95	25.60	10.15	0.625	0.625	1.250	1.3159	
10.00	6.90	25.60	8.90	0.600	0.600	1.200		
12.00	6.89	25.50	7.70	0.500	0.500	1.000	1.3132	0.0027
14.00	6.90	25.60	6.70	0.350	0.350	0.700		
16.00	6.90	25.50	6.00	0.260	0.260	0.520	1.3148	0.0011
18.00	6.90	25.50	5.48	0.040	0.040	0.080		
20.00	6.80	25.60	5.40				1.3162	-0.0003

Av. R.R. mg/l/hr	Av.Δ DO mg/l/hr	Av.O_2PR. mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas Δ DO mg/l/day
0.3958	0.3500	0.7458	8.9496	9.4992	0.5496	0.5500
0.3729	0.3500	0.7229	8.6748	8.9496	0.2748	0.5500

TABLE 15 EXPERIMENT EIGHT 5/11/75

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2 UTIL. mg/l/hr	Chlor. mg/l	Δ Chlor.
08.00	7.20	25.90	9.60	0.450	0.450	0.900	1.2123	
10.00	7.10	25.90	8.70	0.490	0.490	0.980		
12.00	7.00	25.90	7.72	0.485	0.485	0.970	1.2098	0.0025
14.00	7.00	25.90	6.75	0.230	0.230	0.460		
16.00	6.95	25.90	6.29	0.195	0.195	0.390	1.2107	0.0016
18.00	6.95	25.40	5.90	0.250	0.250	0.500		
20.00	6.90	25.40					1.2120	0.0003

Av. R.R. mg/l/hr	Av. ΔDO mg/l/hr	Av. O_2 PR. mg/l/hr	O_2 PROD. mg/l/day	O_2 Util. mg/l/day	Δ DO mg/l/day	Meas. ΔDO mg/l/day
0.3500	0.3333	0.6833	8.1996	8.4000	0.2000	0.1800

TABLE 16 EXPERIMENT NINE - 19/1/76

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	DO mg/l/hr	RESP.RATE mg/l/hr	O_2PROD. mg/l/hr	CHLOR. mg/l	CHLOR.
06.00	6.45	24.00	1.92	0.190	-	-		
08.00	6.45	24.00	2.30	0.175	-	-		
10.00	6.50	24.20	2.65 2.82			-	1.1722	
12.00	6.50	24.30	1.80	-0.15	-	-		
14.00	6.40	24.60	1.10	-0.35	-	-	1.2095	0.0373
16.00	6.40	25.00	1.25	0.075	-	-		
18.00	6.40	25.00	1.80	0.275	-	-	1.2320	0.0225
20.00	6.40	25.00	2.40	0.300	-	-		
22.00	6.40	25.50	3.20	0.400	-	-	1.2980	0.0660

TABLE 17 EXPERIMENT NINE - 20-1-76

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr.	O_2 PROD. mg/l/hr	Chlor. mg/l/hr	Δ Chlor.
06.00	6.35	25.50	0.60	0.35	0.325	0.675	1.2901	
08.00	6.40	25.00	1.30	0.45	0.325	0.775		
10.00	6.50	25.20	2.20	0.55	0.325	0.875	1.3158	0.0064
12.00	6.50	25.00	3.30	0.20	0.325	0.525		
14.00	6.50	25.40	3.70	0.60	0.325	0.925	1.3321	0.0041
16.00	6.50	25.50	4.90	0.45	0.325	0.775		
18.00	6.50	25.50	5.80	0.40	0.325	0.725	1.3720	0.0100
20.00	6.50	25.00	6.60	0.410	0.325	0.735		
22.00	6.50	25.00	7.42				1.4341	0.0155

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av.O_2PROD mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas.ΔDO mg/l/day
0.3250	0.4263	0.7513	12.0200	7.8000	4.2200	4.2200
0.4638	0.4263	0.8901	14.2408	11.1312	3.1095	4.2200

TABLE 18 EXPERIMENT NINE 21/1/76

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2PROD. mg/l/hr	Chlor. mg/l	Δ Chlor.
06.00	6.50	25.60	2.60	0.300	0.6025	0.9025	1.3750	
08.00	6.50	25.40	3.20	0.350	0.6025	0.9525		
10.00	6.50	25.80	3.90	0.425	0.6025	1.0275	1.3976	0.0057
12.00	6.50	25.02	4.75	0.525	0.6025	1.1275		
14.00	6.60	25.00	5.80	0.350	0.6025	0.9525	1.4235	0.0065
16.00	6.60	25.20	6.50	0.350	0.6025	0.9525		
18.00	6.70	25.20	7.20	0.300	0.6025	0.9025	1.4602	0.0092
20.00	6.70	25.20	7.80	0.075	0.6025	0.6775		
22.00	6.80	25.20	7.95				1.5059	0.0114

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av.O_2PR. mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas.ΔDO mg/l/day
0.6025	0.3344	0.9369	14.9904	14.4600	0.5304	0.5300
0.5238	0.3344	0.8582	13.7312	12.5712	1.1600	0.5300

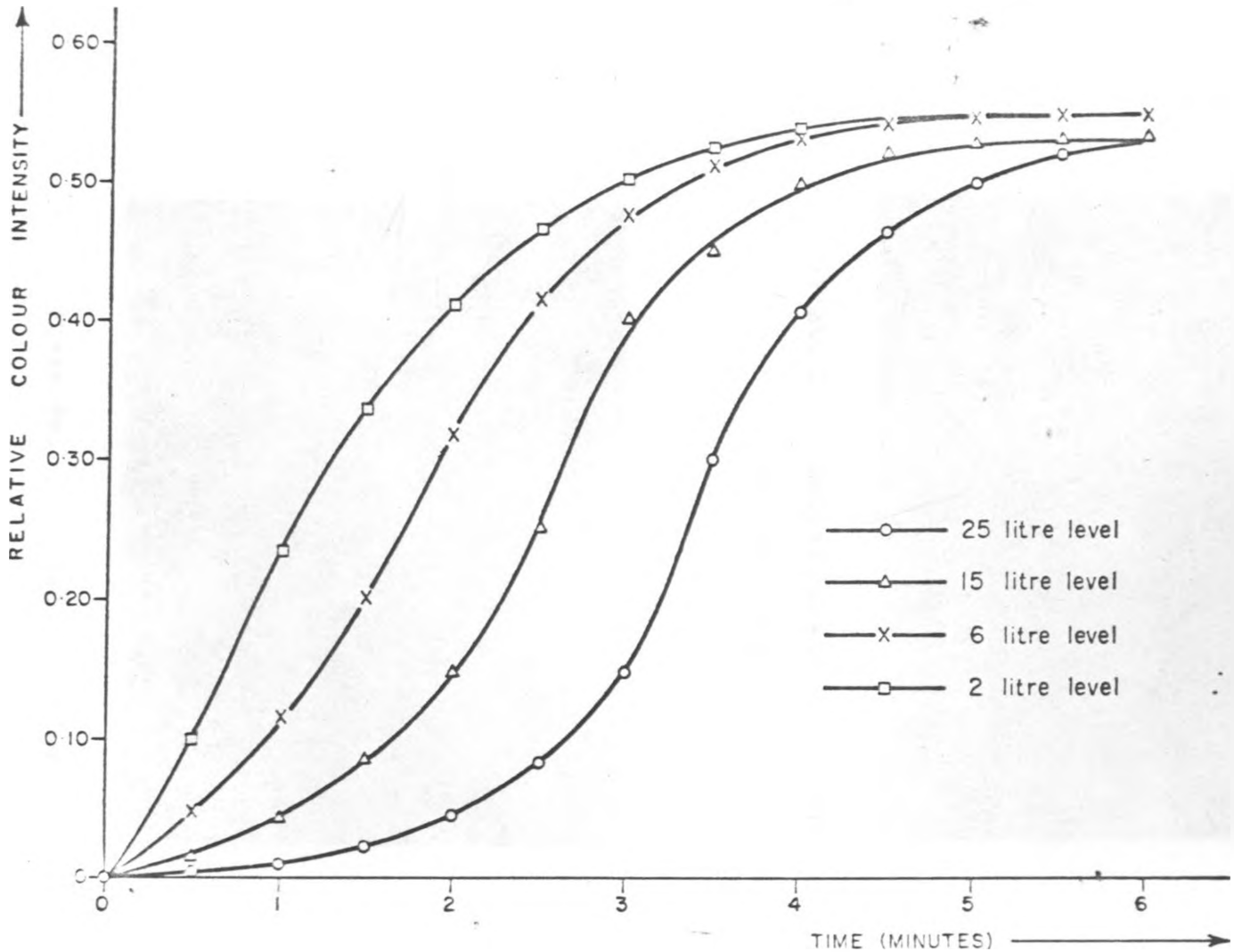
TABLE 19 EXPERIMENT NINE - 22/1/76

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	DO mg/l/hr	RESP.RATE mg/l/hr	O2 PROD. mg/l/hr	CHLOR. mg/l	CHLOR.
06.00	6.50	25.10	4.35	0.185	0.450	0.735		
08.00	6.50	25.10	4.72	0.115	0.450	0.565		
10.00	6.40	25.00	4.95 5.35				1.4500	
12.00	6.55	25.00	3.61	-	-			
14.00	6.55	25.00	2.39	-0.610	-	-	1.4598	
16.00	6.50	25.00	2.30	-0.045	-	-		
18.00	6.50	25.00	2.55	0.125	-	-	1.4698	
20.00	6.50	25.00	2.90	0.175	-	-		
22.00	6.50	25.00	3.40	0.25	-	-	1.4803	

TABLE 20 EXPERIMENT NINE - 23/1/76

TIME HRS	pH	T°C	OXYGEN				CHLOROPHYLL	
			DO mg/l	Δ DO mg/l/hr	Resp.Rate mg/l/hr	O_2PROD. mg/l/hr	Chlor. mg/l	Δ Chlor.
06.00	6.40	24.90	0.850	0.175	0.319	0.4940	1.4782	
08.00	6.40	25.00	1.200	0.200	0.319	0.5190		
10.00	6.45	25.00	1.600	0.175	0.319	0.4940	1.4820	0.0038
12.00	6.50	25.00	1.950	0.185	0.319	0.5040		
14.00	6.50	25.00	2.320	0.140	0.319	0.4590	1.4809	-0.0011
16.00	6.50	25.00	2.600	0.175	0.319	0.4940		
18.00	6.50	25.00	2.950	0.175	0.319	0.4940	1.4750	-0.0059
20.00	6.50	25.00	3.300	0.125	0.319	0.4440		
22.00	6.50	25.00	3.550				1.4120	-0.0630

Av. R.R. mg/l/hr	Av.ΔDO mg/l/hr	Av. O_2PR mg/l/hr	O_2PROD. mg/l/day	O_2Util. mg/l/day	Δ DO mg/l/day	Meas.Δ DO mg/l/day
0.3190	0.1688	0.4878	7.8048	7.6560	0.1488	0.1500
0.3552	0.1688	0.5240	8.3840	8.5248	0.1488	0.1500



BATCH REACTOR MIXING CONDITIONS

Investigated at 25, 15, & 6 litre levels (near periphery).
and 2 litre level (centre).

APPENDIX V

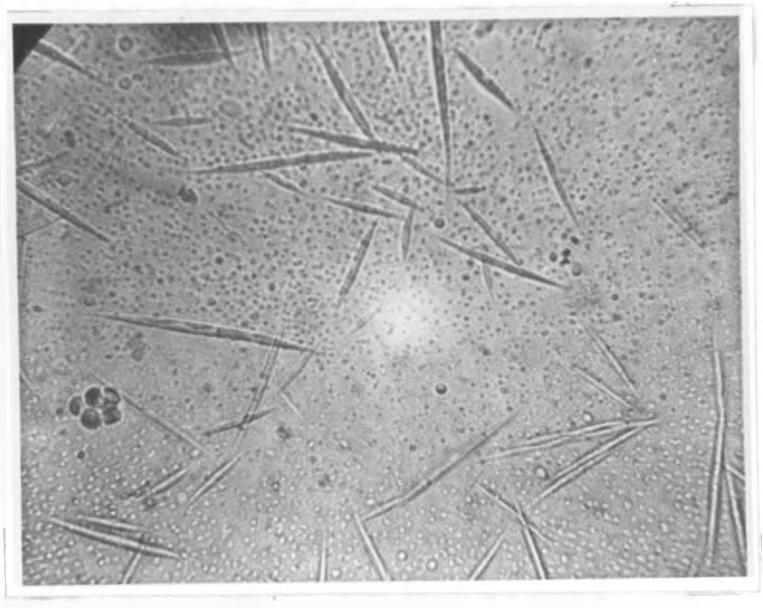


Plate 1 - Ankistrodesmus, chlorella and/or westella

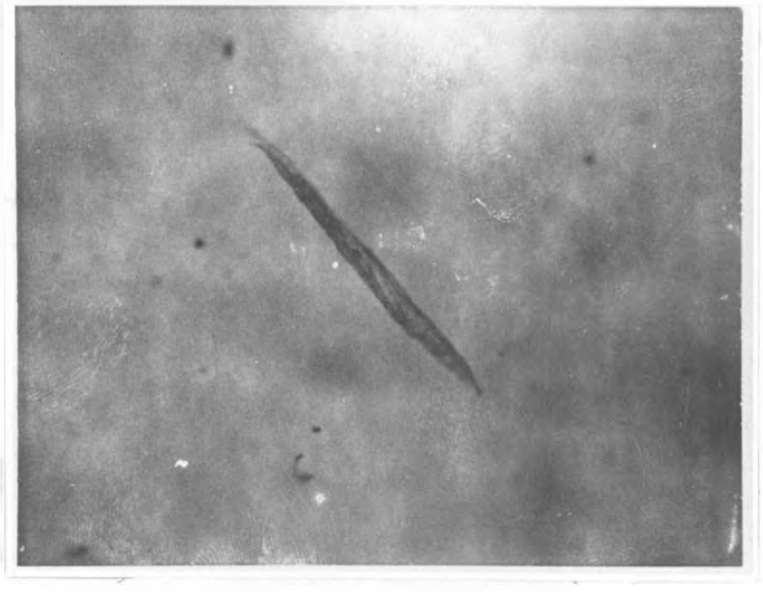


Plate No. 2 - Enlarged view of Ankistrodesmus

APPENDIX V

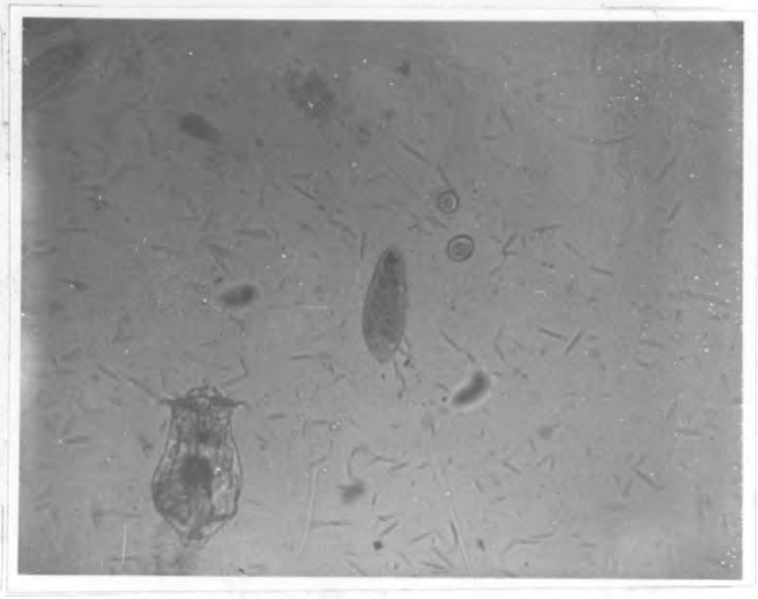


Plate No. 3 Ankistrodesmus, chlorella and/or westella, Euglena and Rotifer

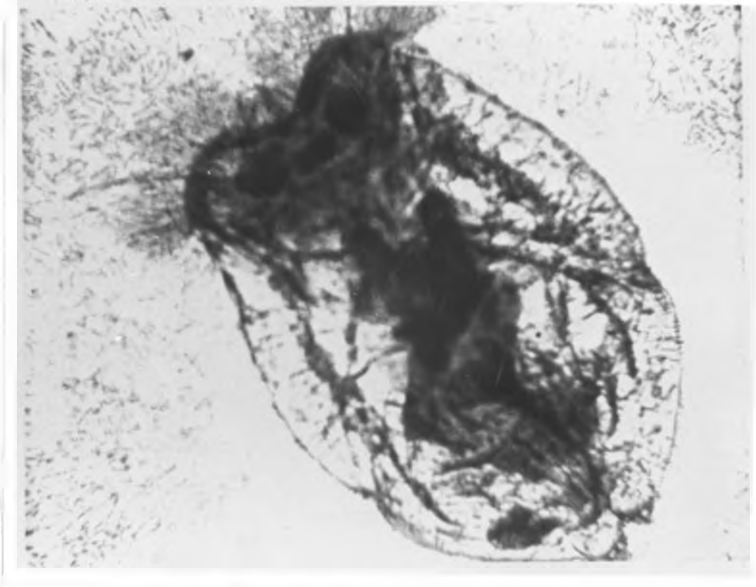


Plate No. 4 - Enlarged view of Rotifer

APPENDIX V

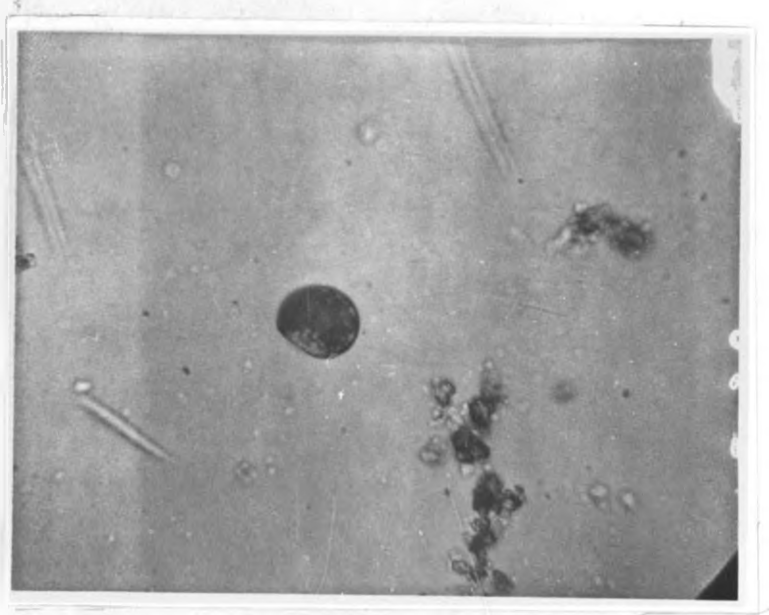


Plate No. 5 - Lepocinclis

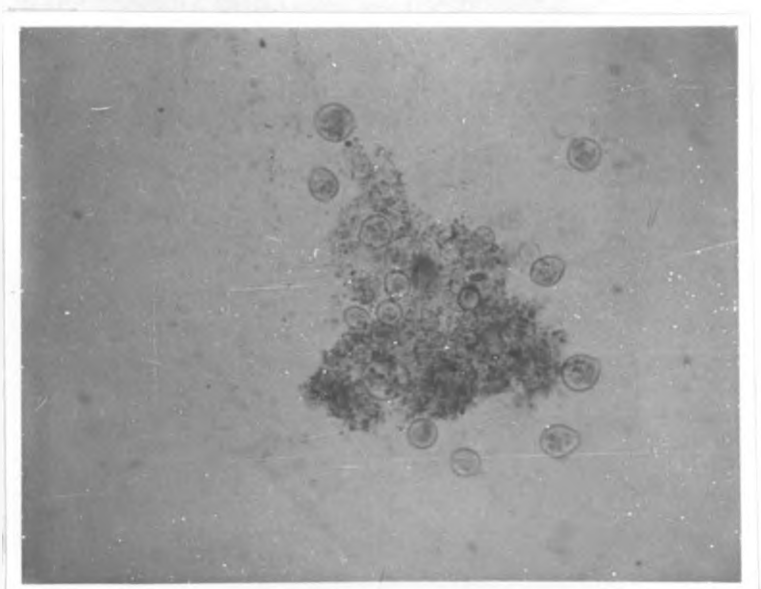


Plate No. 6 - algal debris chlorella and/or westella.