PARAMETRIC STUDIES OF BIOCHEMICAL OXYGEN DEMAND THROUGH IMPROVISED RESPIROMETRIC SYSTEMS

A THESIS

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By

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CERTIFICATE

Certified that the thesis entitled **"Parametric Studies of Biochemical Oxygen Demand through Improvised Respirometric Systems"** which is being submitted by Mr. R.P. Garg, in fulfilment of the requirements for the award of the Degree of Doctor of Philosophy in Civil Engineering, Thapar Institute of Engineering & Technology, Patiala (Deemed University), is a record of candidate's own work carried out by him under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.

This is further to certify that the candidate has worked during the period March, 1983 to January 1987, for preparing the thesis.

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SYNOPSIS

To overcome the limitation of the dilution technique for BOD, a respirometer* and disposable respirometric BOD bottles* were fabricated. In the improvised respirometric BOD systems, the wastewater sample was surrounded by a polyethylene membrane permeable to oxygen. Contents of the respirometer were continously stirred and D.O. was determined with a D.O. probe whereas the contents of respirometric BOD bottles were not stirred and D.O. was determined by Winkler's Method. Mass transfer co-efficients were estimated for the two respirometric BOD systems.

BOD exertion of 50 to 200 mg/l of standard glucose, glutamic acid synthetic waste was studied in the two respirometric BOD systems. The BOD exertion curve was divided into bacterial growth phase and endogenous phase. Logistic curve was fitted to bacterial growth phase. BOD exerted, till the stoichiometric end point of the BOD reaction, was termed as limiting growth BOD (L_1). Co-efficient of correlation between L_1 and TOD was 0.98. Relationship between 2-day respirometric BOD and 5-day standard BOD gave a co-efficient of correlation of 0.99.

The respirometric systems, developed in the present investigations, show a lot of promise in conventional, research and modelling of BOD.

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* Patent pending.

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LIST OF SYMBOLS

SYMBOL	MEANING	DIMENSIONS
A	Area of membrane/area of interface.	L^2
a	Ratio of the area of the membrane liquid interface per unit volume.	L^{-1}
a'	Constant.	--
BOD_5	Biochemical oxygen demand, 5-day, (dilution method).	ML^{-3}
$(BOD_2)_R$	Respirometric Biochemical Oxygen Demand, 2-day.	ML^{-3}
b'	Constant	--
c_i	Interface Dissolved Oxygen Concentration.	ML^{-3}
c_o	Initial Dissolved oxygen concentration.	ML^{-3}
C_s	Dissolved oxygen saturation concentration.	ML^{-3}
D	Dissolved oxygen saturation deficit.	ML^{-3}
D_{AV}	Average Dissolved oxygen saturation deficit.	ML^{-3}
D_i	Interface Dissolved Oxygen saturation deficit.	ML^{-3}
D_L	Diffusivity of the liquid.	L^2T^{-1}
D_o	Initial Dissolved Oxygen saturation deficit.	ML^{-3}

SYMBOL	MEANING	DIMENSIONS
D.O.	Dissolved Oxygen Concentration.	ML^{-3}
D_p	Diffusivity of the polymer.	L^2T^{-1}
D_p'	Diffusion constant of the polymer in the homogenous amorphous phase.	L^2T^{-1}
K_1	Deoxygenation rate constant.	T^{-1}
K_2	Reoxygenation rate constant.	T^{-1}
K_L	Oxygen transfer coefficient of the liquid.	LT^{-1}
K_L°	Overall oxygen transfer coefficient.	LT^{-1}
K_m	Oxygen transfer coefficient of the membrane.	LT^{-1}
K_m'	Oxygen Transfer Coeffiecient of the membrane per unit area.	$L^{-1}T^{-1}$
K_s	Rate constant of the logistic curve (Differential form).	$L^3M^{-1}T^{-1}$
K_s'	Rate constant.	L^3T^{-1}
L	Ultimate BOD.	ML^{-3}
L_1	Limiting Growth BOD.	ML^{-3}
ln	Log Natural	--

SYMBOL	MEANING	DIMENSIONS
L_o	Total oxidisability of the organic matter initially present in the sample at zero time or ultimate BOD.	ML^{-3}
L_t	Amount of oxidisability remaining to be expressed at the corresponding time, t.	ML^{-3}
m	$= (L_1-y_o)/y_o$ and $y=y_o$ when t=o	Dimensionless
n	Rate constant of the logistic growth curve.	T^{-1}
$n_1, n_2, n3$ etc	Stoichiometric numbers of the BOD reaction.	--
P	Permeability of the gas in the semicrystalline polymer.	L^2T^{-1}
q	Bacterial count per unit volume.	L^{-3}
q_o	Initial bacterial count per unit volume.	L^{-3}
r	Respirometer constant.	T^{-1}
r_1	Respirometer constant of Respirometric BOD bottle, Rectangular, Single Compartment.	T^{-1}
r_2	Respirometer constant of Respirometric BOD bottle, Rectangular, Two Compartments.	T^{-1}

SYMBOL	MEANING	DIMENSIONS
r_3	Respirometer constant of Respirometric BOD bottle, Rectangular, Three Compartments.	T^{-1}
r_c	Respirometer constant of Respirometric BOD bottle, circular.	T^{-1}
(r')	Coefficient of correlation.	Dimensionless
r_b	Respirometer constant of R.BOD bottle when the contents of the bottle are not stirred.	T^{-1}
r_b'	Respirometer constant of R.BOD bottle when the contents of the bottle are stirred.	T^{-1}
S	Solubility of the gas in the semicrystalline polymer.	Dimensionless
S'	Solubility of the gas in a hypothetical completely amorphous polymer.	Dimensionless
t	time	T
TOD	Theoretical oxygen demand.	ML^{-3}
t_L	Tranfer Lag.	T

SYMBOL	MEANING	DIMENSIONS
V	Volume of the liquid.	L^3
x	Depth of penetration.	L
x_L	Thickness of laminar layer.	L
y	BOD exerted.	ML^{-3}
y_c	Yield coefficient.	Dimensionless
y_1	BOD exerted at time t_1.	ML^{-3}
y_2	BOD exerted at time t_2.	ML^{-3}
y'	Computed value of y.	ML^{-3}
Z	Relative rate of growth.	T^{-1}
α	Fractional Crystallinity of the polymer.	Dimensionless
α_o	Rate constant of growth equations.	--
$(1/\beta)$	Fractional reduction in diffusivity due to reduction in amorphous chain segment mobility.	Dimensionless
$(1/\tau)$	Fractional reduction in diffusivity due to the necessity of diffusing molecules to bypass crystallites & move through nonuniform cross sectional area of the amorphous phase.	Dimensionless

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Water is a multipurpose resource. It is able to carry dissolved oxygen in a limited way. Sufficient dissolved oxygen levels are to be maintained in the streams for maintaining ecological balance and optimum use. Measurement of the pollutional potential of an aqueous waste, in accordance with its potential drain on the dissolved oxygen resource, is logical. In this context, the concept of biochemical oxygen demand is vitally important.

The 'BOD test', which employs the concept of carbonaceous BOD, was developed on the premise that the oxygen utilizing biochemical reactions can be 'assayed' in the laboratory. The standard test procedure requires several dilutions of the sample with specially prepared dilution water. The need for variable dilutions arises due to the limited capacity of water to hold oxygen in solution. The standard five day 20°C BOD test is an integral part of dissolved oxygen sag curve analysis and also forms a major basis for regulatory action for the prevention and control of water pollution. The test results are regarded as the best available criteria for design and operation of biological waste treatment units.

Since its inception, the BOD test has been the subject of much controversy and continuing research. The attitude of many workers towards the standard BOD test has been one of disenchantment, as the "bottled" system is 100% accurate in representing

itself but is relatively unreliable to represent the system from which the sample was drawn. The statement, of Hoover, Jacewicz, and Porges (1953), concerning the BOD test is note-worthy, "the BOD test is paradoxical. It is the basis of all regulatory actions. It has been the subject of tremendous amount of research, yet no one appears to consider it adequately understood or well adapted to his own work". This statement according to Gaudy (1972), "is in large measure applicable even today, even though we now have a more adequate understanding of the biological events which can take place in the BOD bottle". Stack (1972) stated that, "for historical record and postmortem examination, the information from the BOD test is useful, but as direct input to water quality control, it is useless". Today many seem willing to discard the BOD concept, because they see inadequacies in the BOD test, specially in the dilution technique, BOD model and its application to water quality control.

1.2 DRAWBACKS OF THE BOD DILUTION TECHNIQUE

Dilution of the wastewater sample is an objectionable feature of the test procedure as the results of different dilutions of the sample do not agree, and an arbitrary selection of the most correct result has to be made.

At high dilutions of the wastewater sample, seed correction is important. Organic matter in the dilution water is oxidized and is subjected to the action of small numbers of attenuated bacteria; in the dilutions, however, this organic matter is subjected to the action of a greater variety of less attenuated

bacteria. Seeded dilution water having BOD of its own results in increasing BOD values with increasing dilutions.

Even if the dilution water is free of organic materials, seed correction is to be applied by determining the oxygen depletion of the seed through separate series of seed dilutions. Nitrification will occur early in the lower concentrations of biological seed. Observed oxygen depletion due to oxidation of biological seed cannot be interpreted as a seed correction.

1.3 DEFECTS OF BOD MODEL

Major defect of the traditional BOD model is the assumption that BOD exertion in the BOD bottle is a first order decreasing rate reaction and that the kinetics can be represented by a first order rate constant (K_1) and an ultimate BOD value (L).

Today, the concept of BOD has been refined and the diphasic nature of the exertion of BOD has been recognised. Accumulated oxygen uptake in the BOD bottle consists of two sequential autocatalytic curves. Sequential oxygen uptake has been attributed to sequential growth cycles, first of bacteria which remove the original external carbon source in the waste sample, and second, of either protozoa or other bacteria using the cells which grew during the first cycle as a carbon source. When the plateau is reached, the maximum concentration of bacteria has been attained and the soluble substrate has been exhausted.

Oxygen uptake during bacterial growth phase is exerted initially at first order increasing rate (logarithmic growth) followed by a decreasing rate separated by the inflection point.

However, during the second sequential growth cycle, oxygen uptake is registered at an ever declining rate. Thus the reaction rates during sequential growth cycles are entirely different since the nature of reactants is different. During active growth cycle of bacteria, cell concentration is a parameter in the reaction rate law. Instead, the two sequential autocatalytic oxygen uptake curves are approximated by a first order decreasing rate kinetics. In this exercise, the logarithmic growth phase for the bacteria (which is usually considered as a lag phase in oxygen uptake) and also the pause between the bacterial and protozoan phases of oxygen uptake are neglected. Approximating the two entirely different rate laws of bacterial and protozoan oxygen uptake by one rate law with a first order rate constant (K_1) and an ultimate BOD value (L) is neither acceptable nor necessary in assessing the kinetics of BOD exertion.

The second major defect in the BOD model is that the 5-day evaluation period is inconveniently long and arbitrary. The 5-day 20°C BOD gives a single numerical answer of the complex ecosystem in the BOD bottle and has no fixed relationship with the ultimate carbonaceous BOD (L).

To judge the true worth of the standard BOD test, we see whether the analytical information available from it is correlatable with conditions in the actual biological system being investigated.

1.4 BOD IN DISSOLVED OXYGEN SAG CURVE ANALYSIS

Sag curve of Streeter and Phelps (1935), as ref.by Gaudy(1972), is

one in which two opposing first order decreasing rate kinetic processes are combined. The equation is written as

$$(dD/dt) = K_1L - K_2D \qquad \ldots.(1.1)$$

D represents the dissolved oxygen saturation deficit, K_2 reaeration constant of the stream, K_1 and L are the first order rate constant and ultimate BOD of the waste and are derived from the standard BOD test. Issacs and Gaudy (1967) determined the oxygen sag curve in a 670 litre simulated receiving stream. The maximum DO deficit in the sag curve occured immediately before the plateau in the BOD exertion curve and the downward leg of the DO profile formed an S-curve. Using the standard BOD technique, K_1 and L were estimated for the waste under study. DO sag, calculated using K_1, L and the sag equation, were in serious disagreement with the observed DO profile in the simulated stream. Maximum DO deficit, calculated in the standard way, was only about one third the actual maximum deficit. Bacterial growth in the simulated stream leads to a rapidly increasing oxygen uptake during logarithmic growth and a rapidly decreasing downward leg of the actual DO profile. The large difference between the actual first order increasing kinetics of oxygen uptake during logarithmic growth and the assumed first order decreasing kinetics can lead to a serious discrepancy between the actual DO profile and that calculated by the sag equation. The large difference in the rate constants also leads to a high estimate of the assimilation capacity of the stream by the sag equation using the analytical information from standard BOD model.

1.5 BOD IN DESIGN AND OPERATION OF BIOLOGICAL TREATMENT

There is no justifiable design criteria to employ the 5-day BOD test as a functional loading parameter in the design of a biological treatment unit. Sizing of a biological treatment unit should be based on specific growth rate, energy oxygen and cell yield. Amount of biochemical oxygen demanding material which can be removed in a reasonable aeration time, capacity of aeration equipment and the size of the tank to handle suspended solids can be worked out on a rational basis if oxygen consumption of the undiluted waste is measured continuously.

Oxygen uptake rate and energy oxygen provide more insight into the biochemical and overall efficiency of the plant.

1.6 BOD AS AN AID IN REGULATION OF WATER QUALITY

Looking to the future, all used water containing significant amount of biodegradable organic matter will be subjected to some sort of treatment before reentry into the water resource. Thus the standard BOD test will become less and less significant for raw wastes.

It is, however, important to keep some record of estimates concerning the pollutional oxygen demand of the receiving stream from the effluent from the wastewater treatment plant. The best way would be to generate the DO sag curve in the laboratory by determinations on the effluent of each treatment plant, preferably using water from the receiving body as diluent and a dilution factor approximating that which exists in the receiving body. This will help the regulatory agency and treatment plant manager

to pin point the causes for the DO situation and could serve as a basis for regulatory actions.

1.7 THE NEED

A more meaningful and quantitative representation of the concept of BOD can be developed, through a suitable BOD testing technique, if the concept of first order decreasing rate is discarded and the concept of actual occurences in the BOD bottle is substituted. The BOD bottle is a batch operation in which oxygen consumption involves

(a) Oxygen utilized in energy reactions (energy oxygen) which oxidize part of the organic material to carbon dioxide, water and other stable end products, and the energy produced is used in the synthesis of the remaining organic materials into new biological cells; and

(b) Oxygen consumed in subsequent endogenous reactions to bring about reduction of the cell mass to stable end products.

The need is great for perfection of BOD procedures to determine specific growth rate, energy oxygen and endogenous oxygen as a replacement for the standard BOD procedure.

Manometric and other respirometric techniques which permit the use of less dilute samples that can be permitted in the BOD dilution technique, retain the basic concept, that is measurement of biochemical oxygen demand. Such techniques are employed to assess the oxygen requirements of the microorganisms in utilizing an organic waste substrate, but they provide little information about the behaviour of the sample in the receiving stream.

Although manometric methods for the determination of oxygen demand have been known for some time, the technique has been slow to establish itself as an alternative to the standard dilution procedure. This is presumably due to the uncertainty of the relationship between the two methods, and to the greater complexity of the apparatus involved. Respirometric BOD determination methods were introduced in the 11th edition of "Standard Methods" and dropped from the 12th edition because of poor correlations with the standard 5-day BOD.

There is a basic need for a biological oxidation parameter(s) to be applied in the monitoring and control of water quality, rational design of biological wastewater treatment units, operation of waste treatment plants and biodegradability studies of organic compounds. The standard BOD test does not satisfy this need because it has limited accuracy as the results are greatly affected by the dilution employed.

A great needwas felt for a simple BOD procedure, in which the wastewater is tested without dilution and also capable of automatic recording of the measured parameter so that the BOD curve is developed and exact course of oxygen uptake is observed directly.

Garg (1965) in an earlier study developed a lead silver and lead antimony galvanic cell for the measurement of dissolved oxygen in water. The galvanic cell was covered with polyethylene film permeable to oxygen.

The idea that such membranes, having permeability to oxygen,

can be utilized to overcome a serious limitation of the BOD dilution tehnique, led to the present investigations. Liquid samples were visualized to be surrounded with a membrane, permeable to oxygen, to replenish oxygen through the membrane as it is used due to biochemical activity.

Low density polyethylene films were utilized for the fabrication of respirometer and disposable respirometric BOD bottles.

Results presented in the thesis show a lot of promise in utilising these disposable units in conventional, research and modelling of BOD.

CHAPTER II

LITERATURE REVIEW

2.1 INTRODCTION

The study of BOD can be distinguished between the concept of BOD and the BOD test as brought out by Gaudy (1972). There cannot be two opinions about the importance and utility of the idea or concept, of assessing the pollutional potential of wastewaters in terms of some general property that is susceptible to measurement and the most outstanding property is the consumption of oxygen for the biological oxidation of the organic matter present in the wastewater. The BOD test, originally proposed for sewage plant effluents, has been universally employed for assessing the strength of sewage and the loading and performance of sewage treatment plants (Le Blanc, 1974). The discrepancies and inadequacies of the test itself and its kinetics help to explain why it cannot serve adequately all the purposes for which it has been put. In many investigations, to replace the test, its drawbacks became more and more clear. Today many seem willing to discard the BOD test and, in effect, are compelled not only to discard the test itself but the universally accepted BOD concept too.

2.2 CONCEPT OF BOD

2.2.1 Origin

In the nineteenth century the performance, of sewage treatment plants, was measured mainly by the chemical analysis related to the determination of various forms of nitrogen, as an index of the

state and progress of the oxidation of organic matter. These determinations gave little clue, if any, to the further course of oxidation of organic matter in the receiving stream..

Frankland (1868), as ref. by Shrivastava (1982), first observed depletion of dissolved oxygen upon storage of a sample of water containing organic matter. He concluded that the depletion of dissolved oxygen was dependent on the time of storage and was the result of chemical reactions.

The root of the biochemical nature of the BOD concept was, however, found in the work of Dupre (1884), as ref. by Shrivastava (1982), who recognised that observed oxygen depletions, upon storage of a sample of polluted water, were due to the activity of microorganisms which he called "microphytes".

Practical shape, to the concept of BOD, was given by the work done by various experts of the British Royal Commission on sewage disposal. Studies of Adeney (1908), as ref. by Jenkins (1960), pointed to the significance of the oxygen depletion values as measures of pollution. Later Adeney (1928), as ref. by Stones (1979), defined the absolute strength of sewage as the amount of dissolved oxygen required for its complete biochemical oxidation.

The outstanding characteristics of gross stream pollution viz. appearance of odours of putrefaction, blackening of water and destruction of fish life, result from the utilization of the stream's oxygen reserve and thus these effects of stream pollution can be scaled by the depletion of the stream's oxygen reserve.

2.2.2 Development Of The BOD Test

Phelps (1953) has presented the developmental history of BOD test and its kinetics. Gaudy (1972), Le Blanc (1974), Stones (1979) and Shrivastava (1982) have also reviewed the BOD test. In earlier studies, quality of sewage effluents was determined by putrescibility, or smell tests. Later, time required for decolouration of methylene blue was noted and effluent was said to be stable for so many days. Shortcoming of the stability test was purely its qualitative nature. Phelps (1909), as ref. by Phelps (1953), proposed a scale of relative stability and thus quantitative significance was attached to the stability test.

The work done by Royal Commission on sewage disposal pointed that the most satisfactory characteristic of the polluting index of sewage was determined by its potential to deplete the dissolved oxygen content of the receiving waters. Samples of effluent or of river water were examined for dissolved oxygen and duplicate samples, in sealed bottles, were incubated for 5 days at 65°F and the residual dissolved oxygen then determined. The loss was designated : "Dissolved oxygen absorbed in 5 days at 65°F". This dissolved oxygen absorption test is now generally known as the Biochemical Oxygen Demand (Lederer, 1914, as ref. by Stones, 1979), and for which the abbreviation **BOD** is universally employed.

The 5-day absorption test led to a considerable study along similar lines in the United States. Much work was undertaken to improve and standardize the technique. Today the term BOD refers to the results of a "standard" laboratory procedure laid down in "Standard Methods".

2.2.3 Methods Of Measuring BOD

Adeney and Letts (1908), as ref. by Jenkins (1960), described two methods to ensure an excess of dissolved oxygen during the fermentation of polluted waters. These two methods later formed the basis of the dilution method and manometric method of BOD measurement.

2.2.3.1 Dilution Method of BOD

The 5-day absorption test led to a considerable study along similar lines in the United States. Studies of the absorption test by Theriault and Hommon (1918), as ref. by Phelps (1953), clarified many details of the technique with respect to dilution water and the effect of varying degrees of dilution. Their technique was officially adopted in the Standard Methods for the examination of Water and Sewage.

Earlier work (as ref. by Phelps, 1953) of Phelps (1909), leading to the development of relative stability test, Streeter and Phelps (1925) and Theriault (1927), led to the establishment of the monomolecular equation for exertion of carbonaceous BOD and the adoption of the sag curve for estimating the assimilating capacity of a receiving stream. This led to the concentration of research on the dilution technique for BOD determination and the establishment of "optimum" procedures and conditions for the BOD test. Later, the interest centred around the BOD of industrial wastes and involved standardization of the dilution water.

Today the term **BOD** refers to the results of a standard laboratory procedure laid down in Standard Methods. The standard

conditions for the test, the growth medium and its pH are akin to a minimal growth medium. Complements of inorganic nutrients essential for microbial growth were added in the growth medium. The medium is very dilute one, called the dilution water and is designed to ensure that the carbon source is the limiting nutrient. A glucose-glutamic acid check is also described, in the standard test procedure, for checking the quality of the dilution water, the effectiveness of the seed and the technique of the analyst.

2.2.3.2 Respirometric Method of BOD

Jenkins (1960) has reviewed the application of manometric techniques to water pollution problems upto 1959. Montgomery (1967) has thoroughly reviewed respirometric methods for BOD determination and has classified respirometers into two categories,

(i) Small Volume Respirometers.

(ii) Large Respirometers.

2.2.3.2.1 Small Volume Respirometers

2.2.3.2.1.1 Small constant pressure respirometers

In a constant pressure respirometer, absorbed oxygen is measured by observing the change in volume of the gas phase in contact with the respiring liquid.

Gellman and Heukelekian (1951) improved the constant pressure respirometer of Sierp (1928) by introducing a cup containing alkali into the reaction vessel.

Popel et al. (1958), as ref. by Montgomery (1967), modified Sierp's apparatus by introuducing a alkali cup and a magnetic stirrer and determined BOD on a sample volume of 250-300 ml. Gillson respirometer was based on the construction of a Barcroft constant volume respirometer operated on the constant pressure principle.

2.2.3.2.1.2 Small constant volume respirometers

In a constant volume respirometer absorbed oxygen is measured by observing the change in pressure due to uptake of oxygen by the respiring liquid.

The Barcroft respirometer consists of two similar reaction flasks connected by a capillary manometer. Water is usually placed in one of the flasks called the compensation flask and the other forming the reaction vessel of the apparatus.

Wooldridge and Standfast (1936 a,b), as ref. by Montgomery (1967), applied Barcroft constant volume respirometer for the measurement of BOD of sewage and sewage effluents and suggested that "a much shorter period of examination than 5 days may allow a satisfactory judgement of a sewage or effluent". The main disadvantage, as noted by them, was the accumulation of drops of moisture in the manometric tubes after several days of working.

Murray (1955), as ref. by Montgomery (1967), increased the sample volume of the Barcroft respirometer and used a wick of filter paper to collect water droplets formed in the capillary above the flasks. Moisture was prevented from condensing beyond the wick by the use of an electric light bulb as a heater.

Dillingham et al. (1958), as ref. by Montgomery (1967), have investigated the use of manometric techniques for the prediction of 5-day BOD of pulp and paper mill wastes using Warburg and Barcroft respirometers and concluded that the manometric technique was more reproducible than the 5-day dilution technique.

Caldwell and Langelier (1948) used the Warburg constant volume respirometer for the measurement of BOD and concluded that "24 hour values of BOD at 25°C are approximately 75 per cent of standard 5 day BOD values". Manometric results for rate constant and ultimate BOD are appreciably higher. They have further summarized that present commercial equipment is expensive and too fragile for use by untrained analysts.

The Warburg and dilution methods have been compared by Lee and Oswald (1954) who found that the Warburg constant volume respirometer was most useful of the manometric methods for BOD determinations. They further stated that the same degree of skill was required for the two methods and that the expenditure of time per test was also about the same for the two methods. They concluded that values of ultimate BOD were 15 per cent higher for the Warburg technique but reaction rates were approximately the same for the two methods.

Dillingham and Jose (1960), as ref. by Montgomery (1967), compared the Warburg (18 hour at 37°C with a seed of Aerobacter Aerogenes) and dilution methods and concluded that the manometric method was more precise for pure solutions of glucose

but slightly less precise for river waters with added glucose. Myrick and Busch (1960) found manometric method to be less precise than the dilution method in studies of BOD of pure substrates in the presence of inhibitors; there were large variations in rates of oxidation and in lag periods. According to Wilson (1967), as ref. by Montgomery (1967), carbon dioxide transfer may be a source of error in the Warburg and other manometric methods. Gaffney (1965), as ref. by Montgomery (1967), pointed to the fact that carbon dioxide deficiency could have a large effect on the lag phase in the removal of glucose by sewage bacteria in the manometric BOD measurements.

A respirometer for water pollution work was manufactured by Hach Chemical Company (1973). It consisted of a closed leg manometer and drop in air pressure is read on the mercury manometer directly in mg / l BOD.

2.2.3.2.2 Large Respirometers

Large respirometers measuring gas exchange in which large volumes of test solutions may be added have been described by Snaddon and Harkness (1959) and Simpson (1966).

Respirometer of Snaddon and Harkness (1959) was improved and automated by Snaddon and Jenkins (1964). In this apparatus oxygen was added automatically by a system of pipettes and electromagnetic valves.

Clark (1960) introduced an electrolytic respirometer in which oxygen pressure was automatically maintained at a constant value by an electrolytic cell. The current passing through the cell is

proportional to the oxygen consumed and may be integrated and/or continuously recorded. The instrument was later modified by Young and Clark (1965). The maximum measurable rate of respiration is equal to the rating of the electrolytic cells.

Burchard (1966), as ref. by Montgomery (1967), has described an apparatus consisting of a screw topped reaction vessel in the lid of which is mounted an aneroid barometer. Oxygen exchange between the respiring liquid and the gas phase is effected by a vibrating table on which the reaction vessels are placed.

Arthur (1964) described a recording respirometer built around a vertical cylindrical reaction vessel. The gas phase and the sample liquid were circulated in the opposite direction. Oxygen uptake was measured by means of an oil manometer.

2.2.3.2.3 Techniques in which Dissolved Oxygen is determined by Electrometric Methods

Eye et al. (1961) determined the BOD of sewage by means of a solid oxygen electrode with intermittent or continuous magnetic stirring of the sample. Lamb et al. (1964), Mathews (1964), as ref. by Montgomery (1967), Eye and Ritchie (1966) and Vernimmen et al. (1967) have described apparatus for oxygen uptake measurements by the electrometric method.

Stack (1972) suggested reaeration as soon as the oxygen concentration in the BOD bottle approached depletion. The dissolved oxygen concentration established by reaeration was determined by a dissolved oxygen probe.

Karube et al. (1977) described microbial electrode BOD sensors. The method was rapid and provided an estimate of BOD in only one hour. A microbial electrode consisted of a bacteria collagen membrane and oxygen electrode. The electrode was inserted in wastewater sample and consumption of oxygen by the bacteria in the collagen membrane caused a decrease in dissolved oxygen around the membrane and current of the electrode decreased with time until steady state was reached. From the current BOD relationship, BOD of the sample was estimated. As stated by the authors, the relative error of measurement was less than 10% whereas in practice, the error inherent in the 5 day BOD test was more than 10%.

2.2.3.3 Method Based on Stoichiometry of BOD System

Busch (1952), as ref. by Schroeder (1977), assumed that the plateau in the oxygen demand curve established the end point for the conversion of organic matter to cellular mass. A mixed culture such as that used in the BOD test should also behave stoichiometrically (Schroeder, 1968). A mass balance would give an equation of the following form:

$$n_1 \text{ Substrate} + n_2 \text{ Nutrients} + n_3 O_2 \rightarrow n_4 \text{ Cells} + n_5 CO_2 + n_6 H_2O \quad \ldots(2.1)$$

For a given set of environmental conditions, the value of each stoichiometric number n_1, n_2, n_3, etc. should be constant. Physiological changes in the cells occur in going from the lag phase, through the log phase and into the stationary phase of growth which would be expected to affect the stoichiometric numbers (Schroeder, 1968).

Busch and Myrick (1961), Grady and Busch (1963) and Lewis and Busch (1964) concluded that the most practical method of

estimating BOD was to measure the plateau value, determine cell production gravimetrically and utilize an empirical cell formulation based on cell constituent ratios such as that of Porges et al. (1956) or of Luria (1960). Oxygen equivalent of the cells was added to the plateau value to estimate the total BOD. The same total oxygen demand was obtained with BOD bottles and with respirometers.

Introduction of the concept of stoichiometry into the BOD determination led to the development of a relatively fast method of determining the ultimate oxygen demand. Busch (1958), Hiser and Busch (1964) proposed a "total biological oxygen demand" (T_bOD) of a substrate. Various techniques for measuring the T_bOD were presented. The T_bOD test measures only the total soluble organic matter of a wastewater amenable to biological metabolism and that too includes the portion of organics measurable by the COD test.

Ultimate biological oxygen demand can be determined by the plateau test but the test is more difficult. McWhorter and Heukelekian (1962) and Gaudy et al. (1963) found that the plateau BOD value was not consistent and plateau was of no aid in determing BOD stoichiometry. Wuhrmann (1956), Sherrard and Schroeder (1972, 1973) inferred that cell yield and oxygen consumption was highly dependent upon the manner of process operation.

2.2.3.4 Carbon Parameter Methods

Total organic carbon (TOC) tests were based on oxidation of carbon of the organic matter to carbon dioxide and its determination either by absorption in KOH or by instrumental analysis. Busch

(1966), as ref. by Montgomery (1967), has suggested measuring the change in organic carbon content due to bacterial metabolism. Like the COD test, biodegradable organic matter was not determined in carbon parameter methods. In this sense, the carbon parameter (TOC) was not as useful as BOD. Total organic carbon merely established the presence of organic matter.

2.2.4 Course Of Carbonaceous BOD Exertion

The course of carbonaceous BOD exertion has been refined and the diphasic nature of the exertion of BOD was recognised by Hoover et al. (1953). Further investigations by Busch (1958, 1959), Wilson and Harrison (1960), Gaudy et al. (1963), McWhorter and Heukelekian (1964), Gaudy et al. (1965), Bhatla and Gaudy (1964, 1965a), Issacs and Gaudy (1967), Grewal (1969), Gates and Ghosh (1971) and Canale and Cheng (1974) confirmed the existence of diphasic carbonaceous BOD separated by a plateau in both heterogenous and pure culture populations of the seed.

Carbonaceous BOD consists of two biochemical reactions :

(i) the rapid growth of cells with synthesis of the nutrients into new cells. This phase is termed as bacterial growth phase abbreviated as BGP.

(ii) the subsequent slow endogenous respiration of these cells. The cyclic lysis-synthesis-lysis reactions continue until relatively stable organic materials remain and this phase is termed as cyclic degradation of biomass and abbreviated as CDBM.

Both the phases are illustrated in Fig. 2.1

2.2.4.1 BOD Exertion During Bacterial Growth Phase

During bacterial growth phase (BGP), the oxygen uptake curve is sigmoidal as shown in Fig. 2.1, the curve is similar to the familiar growth curve of bacteria in a batch reactor. General shape of both the curves is that of an autocatalytic process; the inflection point separates the first order increasing rate portion (logarithmic growth) and the decreasing rate portion. If either a metabolic acclimation or an adaptation (population selection) is required, there will be initial lag period before the onset of the log phase. At the levelling off of the growth curve, external sources of carbon are exhausted by the microbial population.

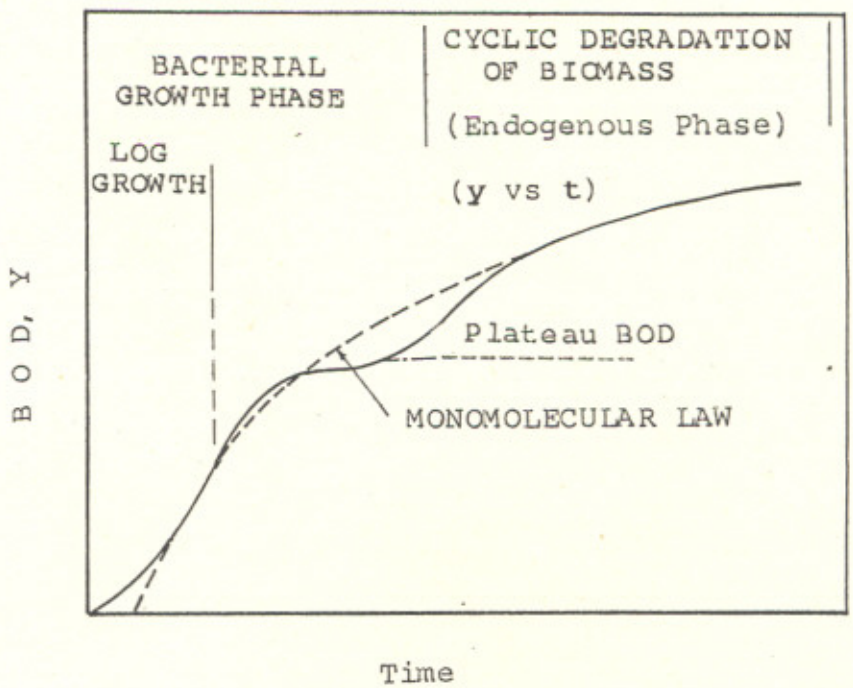


Fig. 2.1 Diphasic Exertion of Biochemical Oxygen Demand

2.2.4.2 BOD Exertion During Cyclic Degradation of Biomass (CDBM) or Endogenous Respiration

At the termination of the sigmoid curve, the general ecological situation has undergone a rather severe change, which existed at the start of the incubation period. At the start there was relatively a large external food supply in relation to the number of seeding organisms, whereas now the microbial population is large and the concentration of external food is practically nil. The biodegradable organic substrates in the wastewater sample have been converted to new cells, and a considerable portion of ultimate BOD (in the form of biomass) remains to be expressed.

The starvation conditions which exist in the ecosystem enhance competitive interactions including predation. The biomass can experience internally initiated and controlled reduction of its mass and/or a cyclic degradation of the biomass progressing through lysis-synthesis-lysis. During this period of cell stabilization, oxygen uptake is registered as carbonaceous BOD.

There is usually a time lag before some cells could synthesize the enzyme systems required to utilize the organic material of other cells for growth or before that portion of the predatory microorganisms, which can utilize the bacterial species, as food, would grow up to sufficient numbers to exert a discernible oxygen uptake. The pause which often occurs is known as the plateau in BOD exertion.

During the initial period of endogenous respiration, cellular storage products undergo rapid degradation upon exhaustion of

external food supply. Number of researchers have found storage products in laboratory activated sludge. Porges et al. (1956), Porges et al. (1963) and Walter et al. (1968) have reported on the conversion of external carbon sources as cellular storage products.

The energy evolved as a consequence of endogenous metabolism is utilized to maintain the integrity of the cell and culture viability.

2.2.4.3 Nitrogenous BOD Exertion

Buswell, Van Meter and Gereke (1950) concluded from their studies that nitrification does not occur until about eight days in the BOD bottle. Lack of agreement may thus be observed between actual measurement of 20-day BOD, usually equal to ultimate BOD, and actual calculation of ultimate BOD based on 5-day BOD data. Nitrification increases the 5-day BOD values of effluents from treatment plants. Most of the work on nitrogenous BOD has been well documeted by Downing (1966).

A number of chemical agents have been suggested for inhibition of nitrification : methylene blue, trichloromethyl pyridine, thiourea and allylthiourea (Young, 1973) and also ammonium ion (Siddiqi et al., 1967). The use of ammonium ion shows promise for convenient inhibition of nitrification.

Stack (1972) concluded that nitrification was a function of the concentration of organic material in a study of BOD exertion of monoethanolamine and that nitrification was inherent in the dilution procedure. Modification of the dilution procedure to

include reaeration has been suggested to increase the organic concentration in the BOD bottle and minimize interference due to nitrification.

2.3 FACTORS AFFECTING BOD EXERTION

Several factors affect the BOD exertion. Some of the important factors viz. seed, temperature and toxicants have been reviewed by Montgomery (1967). Eldridge (1933) has reported effect of seed on 5-day BOD value but ultimate BOD was not affected significantly. Rao and Gaudy (1966) observed that rate of oxidation of glucose by activated sludge varied by a factor of more than two, although the activated sludge was grown under constant conditions. Variable cell yields and rates of oxidation of fat free milk were obtained by acclimated bacteria. Eckenfelder (1970) has reported the effect of low seed concentration (0.1 per cent), as there is the existence of a zeroeth phase.

2.4 KINETICS OF BOD EXERTION

Studies of Streeter and Phelps (1925), as ref. by Gaudy (1972), led to the following generalizations concerning the course of biochemical oxidation of organic matter. "The rate of the biochemical oxidation of organic matter is proportional to the remaining concentration of unoxidized substance, measured in terms of oxidizability". Events which conform to this type of kinetic law can be described by first order kinetics.

Mathematically

$$-(dL/dt) = K_1 L \qquad \ldots.(2.2)$$

In integrated form

$$L_t = L_o\, e^{-K_1 t} \qquad \ldots.(2.3)$$

L_o represents the total oxidizability (BOD) of the organic matter initially present in the sample at zero time. This term is now known as ultimate BOD. L_t is the amount of oxidizability (BOD) remaining to be expressed at the corresponding time, t. K_1 is the velocity constant for the reaction. Data of Streeter and Phelps (1925), as ref. by Phelps (1953), could be fitted in the first order reaction; but it was also in line with the early work of Phelps (1909), as ref. by Phelps (1953) leading to the development of the relative stability test.

Theriault (1927), as ref. by Phelps (1953), also observed that the course of first stage BOD for Ohio River water conformed to the monomolecular law. Sag curve equation of Streeter and Phelps (1925), as ref. by Gaudy (1972), used for predicting the assimilating capacity of a receiving stream, has become one of the most widely quoted kinetic expressions in the water pollution control field.

Analysis of the first order equation indicates two variables, rate constant K_1 and ultimate BOD, L, dependent on each other. To find rate constant K_1 and ultimate BOD, L, least squares procedure was used. Fair (1936) proposed the log-difference method for the solution of the BOD equation but was difficult to be solved. Thomas (1937) developed the slope method (graphical solution) and for many years, this was the most used method for computing the constants of the BOD curve (K_1 and L). The method of moments developed by Moore, Thomas and Snow (1950) became the most used technique of

solving BOD constants. Thomas (1950) proposed a simple graphical approximation for the evaluation of the constant of the BOD curve.

2.4.1 Critical Reveiw Of First Order Kinetics

The first order rate law for BOD, although still used in the field of water quality control, has been severely criticised by various researchers. Thomas (1937), Phelps (1953), Orford and Ingram (1953), Woodward (1953), Bogan (1958), Schroepfer et al. (1960), Young and Clark (1965), Gaudy (1972) and scores of others have criticized the first order rate equation for various reasons as :

(a) Carbonaceous BOD exertion consists of two sequential autocatalytic curves separated by a plateau. In attempts to fit a monomolecular law to the BOD data, the log phase for bacterial growth and the plateau are neglected (Fig. 2.1). Cell concentration should be a parameter of the rate law during active bacterial growth.

(b) The sequential autocatalytic curves of bacterial and protozoan growth have entirely different rate constants and cannot be approximated with first order equation with a constant rate of reaction K_1.

(c) First order kinetics, at best, can represent the endogenous phase of the diphasic expression of BOD.

(d) There is no fundamental reason to assume that the complex ecosystem of the BOD bottle can be approximated by an ever declining rate law.

Other Formulations:

Young and Clark (1965) used a second order equation for the BOD data. Revella, Lynn and Rivera (1965) proposed the following second order equation,

$$(dy/dt) = K_s' q (L-y) \quad \ldots.(2.4)$$

in which K_s' is the rate constant, q is the bacterial count per unit volume. They also proposed a linear relationship between q and y:

$$q = q_o + b'y \quad \ldots.(2.5)$$

in which q_o is the initial value of q and b' is a constant.

Orford and Ingram (1953) have given the following equation for BOD exertion :

$$y = a' \ln t + b'$$

in which a' and b' are constants.

Hartman and Wilderer(1968) summarized that a close up view of the course of BOD reactions showed that it was impossible to describe the whole process by one mathematical formula. The process is composed of a set of single reactions, with each of them, under the proper load values, being the rate limiting one.

2.4.2 Simulated Stream Studies

Issacs and Gaudy (1968) determined the reaeration constant for a 670 litre simulated receiving stream and determined the resultant DO profile (sag curve) after adding organic matter to it. The BOD curve, observed in the simulated stream, consisted of two sequential autocatalytic curves (Issacs and Gaudy, 1967). The maximum DO

deficit in the sag curve occured immediately before the plateau in the BOD exertion curve, and the downward leg of the DO profile formed an S-curve.

Jennelle and Gaudy (1970), as ref. by Gaudy (1972), determined the course of BOD exertion in the simulated stream, an open stirred reactor and also in closed systems both stirred and quiescent. BOD curves obtained in the closed systems were not comparable to those obtained in the open systems. In the closed system, the quiescent and stirred systems yielded comparable oxygen uptake curves. When the waste concentration in the BOD bottle was the same as that in the open system, BOD curves were relatively comparable.

Jennelle and Gaudy (1970), as ref. by Gaudy (1972), have reported results of experiments of the BOD of standard substrate (glucose-glutamic acid) carried out under identical seeding conditions. In all cases a logarithmic phase of oxygen uptake was observed. The values of first order rate constant (K_1) increased with increasing concentration of the substrate. Thus the velocity constant was affected by the dilution factor.

2.5 BIOLOGICAL SYNTHESIS AND ENERGY YIELDS

A portion of the energy released during assimilation is made available for the synthesis of protoplasm. Biological growth is related to the free energy released from substrate oxidation and the efficiency of microorganisms in capturing the energy yields.

Studies by Placak and Ruchhoft (1947) showed that the proportion of substrate converted to cells varied within classes

of compounds. The highest yields (maximum, 85 per cent) were found with carbohydrates and the lowest (minimum, 10 per cent) with organic acids. Hoover et al. (1951), Helmers et al. (1951), Heukelekian et al. (1951), McKinney (1962), Servizi and Bogan (1963), using domestic, industrial and pure compounds have concluded that biological synthesis or solids production is related by a constant factor to the BOD or COD of the waste. Although ranges of yields were reported, the accepted yield constants are approximately 0.5 and 0.67 as fraction of BOD and COD loading respectively.

Heukelekian et al. (1951) and Sawyer (1956) showed that 0.5 g VSS were synthesized per gram of 5-day BOD removed from sewage and several other industrial wastes. Porges et al. (1956) showed 0.44 g VSS per gram of COD removed. Busch (1958) reported total synthesis from glucose to be 0.421 g cells per gram COD. McWhorter and Heukelekian (1964) found an average of 0.315 g VSS per gram COD from glucose when nitrate was used as source of nitrogen. These variations can be attributed to changes in environment, microbiological variations etc. Servizi and Bogan (1963) have shown that synthesis is proportional to the change in free energy of substrate oxidation.

McCarty (1964), Gaudy and Gaudy (1966) and Paynes (1970) have reviewed energy yields and growth of heterotrophs. Gunsalus and Shuster(1961) and McCarty(1964) have indicated that the energy using reactions of bacteria are linked by a common intermediate Adenosine triphosphate. Growth per mole of ATP generated should

generally be the same for most of the bacteria since once substrate energy is converted to biological energy or ATP, the growth for all organisms follow the same pathway.

2.5.1 Energy For Biological Synthesis

Based on ATP energy, McCarty (1964) has presented the energetics of microbial metabolism and illustrated the transfer of energy from the substrate or "food" to biological growth, Fig. 2.2. During energy metabolism, a portion of the substrate energy is transferred biochemically to ATP, the exact proportion depending on the efficiency of the particular process. The charged ATP then carries the biochemical energy to all parts of the cell where energy may be required for synthesis or for maintenance.

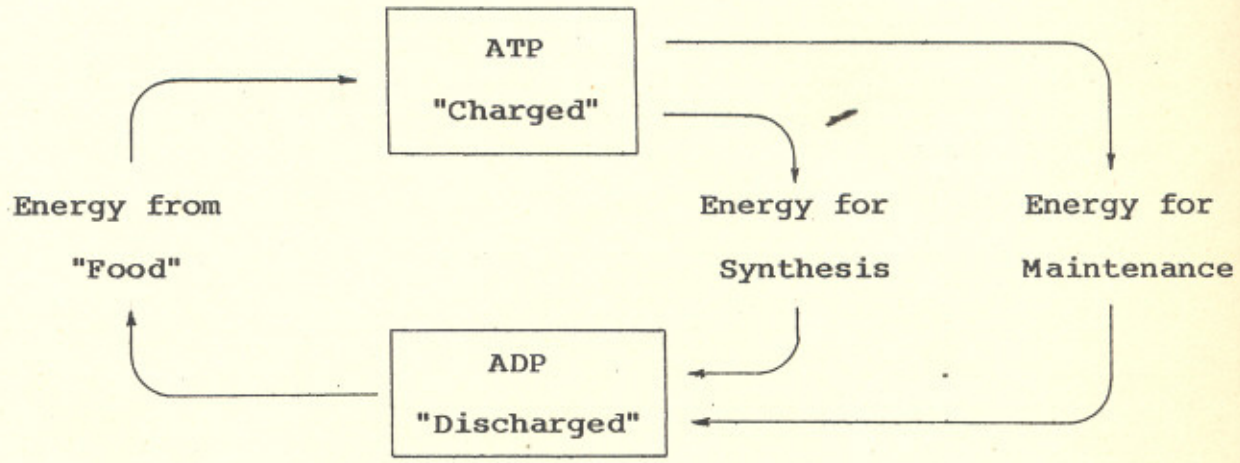


Fig. 2.2 Energy transfer from food source to biological growth through ATP.

Energy for net cell synthesis is composed of two energy requirements (McCarty, 1964). First, ATP energy is required to raise the cell carbon source to some intermediate level represented by pyruvate in the cell synthesis. However, energy

will be released during the growth of heterotrophic organisms on organic matter. Second, a certain quantity of ATP energy is then needed to convert the intermediate into cellular material.

2.5.2 Energy For Maintenance

Maintenance energy is required by the cell for such things as mobility, to prevent the undesirable flow of solutes either into or out of cell, or for resynthesis of proteins which are constantly degrading because of kinetic instability. According to Marr et al. (1963), as ref. by McCarty (1964), protein resynthesis accounts for a major portion of energy for maintenance. Maintenance energy is supplied by endogenous respiration or metabolism of intracellular components of the cell.

Energy for maintenance varies with temperature, environmental conditions and type of organism. Maintenance energy per gram COD of resultant cellular material varies from a minimum of zero with actively growing cultures upto some maximum value for very old cultures. Calculated transfer efficiencies from measured cell yields for aerobic heterotrophs were only 20 to 40 per cent as reported by McCarty (1964), since energy losses result from inefficiencies in energy transfer both from the substrate to the biological energy carrier ATP and from ATP to the cell for synthesis and maintenance.

Thus investigations carried out by various researchers were mainly concerned with the BOD dilution technique. 5-day BOD values are affected by the diluton employed.

Respirometric BOD determinations are more reproducible but efforts to replace the 5-day BOD test have not met with success.

First order decreasing rate law can, in no way, described the carbonaceous BOD exertion.

Variable cell yields are obtained. Calculation of growth is complicted due to maintenance energy requirements of microorganims.

CHAPTER III

THEORETICAL CONSIDERATIONS

3.1 INTRODUCTION

The idea that membranes, permeable to oxygen, can be utilized to overcome a limitation of the BOD dilution technique, was presented in Chapter I para 1.7. Liquid wastewater samples were visualized to be contained in a polyethylene film chamber. Considerations, which led to the development of workable pieces of equipment, are presented in this Chapter. Later part of the chapter deals with the theoretical considerations in the mathematical formulation of the diphasic BOD exertion curve.

3.2 POLYETHYLENE FILM CHAMBER

The purpose of surrounding the wastewater sample with a film, permeable to oxygen, is to replenish oxygen through the membrane as it is consumed, in the waste sample, due to biochemical activity. Rate of oxygen transfer, through the membrane and then to the liquid, is interphase mass transfer and it depends on many factors considered herein.

3.2.1 Rate Of Oxygen Transfer In The Chamber

Steady state transfer of oxygen in the chamber is defined by:

$$(dc/dt) = K_L^{\circ} a (C_s - c) \qquad \ldots.(3.1)$$

Where (dc/dt) = Steady state oxygen transfer rate mg/l/hr

K_L° = Overall oxygen transfer co-efficient Cm hr^{-1}

a = Ratio of the area (A) of the membrane liquid interface per unit volume (V) of the sample in the Chamber ($a = A/V$), Cm^{-1}.

C_s = Oxygen saturation concentration in the sample at test temperature and pressure, mg/l.

C = Oxygen concentration in the sample at time t, mg/l.

K_L° is composite oxygen transfer co-efficient (K_m) of the membrane and oxygen transfer co-efficient (K_L) of the liquid in the chamber and is given by

$$(1/K_L^\circ) = (1/K_m) + (1/K_L) \qquad \ldots.(3.2)$$

From equations (3.1) and (3.2), it is apparent that the rate of oxygen transfer in the chamber can be increased by

(i) increasing K_L, the oxygen transfer co-efficient of the liquid. However the value of K_L is fixed for the sample to be contained in the chamber.

(ii) increasing K_m, the oxygen transfer co-efficient of the membrane. This can be accomplished by suitably selecting a membrane with high permeability to oxygen.

(iii) increasing (a), ratio of the area (A) of the liquid membrane interface per unit volume (V) of the sample in the chamber. The value of (a) can be increased by increasing (A) and /or decreasing(V).

3.2.1.1 Selection of a Suitable Polymer Membrane

A good membrane for the purpose should have sufficient permeability for oxygen with insignificant change with temperature and capable of withstanding certain amount of stretching. The data in literature (Crank and Park, 1968) on diffusion co-efficients of

oxygen for various polymeric membranes reveal that silicone rubber has the maximum permeability to oxygen followed by natural rubber, polybutadiene, low density polyethylene and perbunan. Natural and silicone rubber will not withstand stretching and variable stretching will alter membrane thickness as well as rate of diffusion and hence will not be suitable for the purpose.

Low density polyethylene film was considered suitable as it has high permeability to oxygen and is easily available. Leak proof joints can also be made by heat sealing the membranes. Polyethylene is resistant to chemical and biological attack and diffusion properties do not change in the presence of these materials. Diffusion co-efficient of polyethylene varies with temperature. Since the BOD test is performed at constant temperature, change in diffusion properties with temperature will not be a limitation.

Consideration of the characteristics of polyethylene and various factors affecting membrane permeability of the gas will be in line with the topic for better understanding of the mechanism of gas permeation.

3.2.1.1.1 Characteristics of polyethylene membranes

Polyethylene is a homochain polymer of ethylene gas. The monomers are bonded together end to end in the polymerization reaction. The molecular weight of the polymer chain varies from 10,000-10,00,000. It has a specific gravity of less than one (low density sp. gr. 0.9 to 0.92 and high density sp. gr. 0.92 to 0.97).

3.2.1.1.2 Structure of polyethylene

Molecular architecture of the monomers and macromolecules is important. Consistent with the molecular ordering in the polymer, polyethylene exists in two phase states,

(a) crystalline phase (b) amorphous phase.

(a) Crystalline Phase

The crystalline phase is characterized by a three dimensional long range order of arrangement of monomers and macromolecules over distances hundred and thousand times greater than the size of the molecules. This long range order is exhibited in single crystals of polymer. These crystals are monolayer flat plane lamella with a thickness of 100 Å and size of the plate upto 1 μm. The conformation of a polymer chain in a crystal is characterized by the appearance of regularly repeating units.

In addition to the growth of crystalline lamella, there is a secondary ordering process when polyethylene is cooled from the melt, during which the familiar spherulites form. Spherulites are not completely crystalline, but contain amorphous material as well.

In the crystalline phase, molecules have practically no translational or rotational motion, but vibrate about fixed centres of equilibrium.

(b) Amorphous Phase

The amorphous phase is characterized by a short range order where molecular architecture extends over a distance of 30-100 Å. The molecules are entangled and can assume arbitrary configurations.

Amorphous phase represents a non-equilibrium state where various structural forms with different properties may be present in its limits. Three distinct amorphous regions in polyethylene can be identified from light scattering and electron microscopy, intralamellar, interlameller and interspherulitic amorphous phase.

Amorphous material is like a viscous liquid. The molecules possess translational, rotational and vibrational motions, and their centres of equilibrium are continually changing their positions, imparting mobility to various chain segments of a macromolecule.

3.2.1.1.2 Factors affecting permeability of oxygen through polyethylene

Solubility of gases, and their permeation through crystallites of polyethylene is negligible (Michaels and Parker, 1959; Michaels and Bixler, 1961).

Loose packing of polymer macromolecules and increased chain flexibility increase gas permeability.

3.2.1.2 Polyethylene Selected for Experimental Work

Branched low density polyethylene (density 0.916) manufactured by Union Carbide (India) Ltd., available in granule form under the trade name of DFDI 0114 was utilized for developing films. The films (60, 70, 100 gauge) were prepared at ambient temperature (30°C) by the blowing process.

Branches in polyethylene macromolecules hinder dense macromolecular packing but chain mobility decreases due to the

crosslinking effect of the branches. Crystallinity is less in low density polyethylene.

3.2.1.3 Increasing (a), Interfacial Area (A) to Volume (V) of the sample Ratio

3.2.1.3.1 By increasing interfacial area (A)

By imparting a stirring action to the wastewater sample, the effective interfacial area (A) increases, thereby increasing the area to volume ratio (a).

3.2.1.3.2 By decreasing volume (V) of the sample contained

If water is added in a plastic film bag, the bag will inflate till it contains maximum volume of water and has minimum area to volume ratio. However, if the sides of the bag are held to each other by means of a stiffening material, the bag will inflate partly and the volume of water contained therein shall be less, thereby increasing interfacial area (A) to volume (V) ratio.

Low density polyethylene films (LDPF) were utilized for the fabrication of

(i) respirometer in which effective interfacial area (A) is increased by imparting a stirring action to the wastewater sample.
(ii) disposable respirometric BOD bottle in which area to volume ratio (a) is increased by decreasing the volume of sample contained.

3.3 BOD EXERTION

Bacterial growth and substrate oxidation should be stoichiometric processes for a given set of environmental conditions. During substrate oxidation by bacterial cultures, rate of growth and

hence the rate of BOD exerted may vary, because (i) physiological changes within the cells occur in going from the lag phase, through the log phase and in the stationary phase of growth, (ii) energy transfer efficiencies of different cultures, for the same substrate, vary widely, (iii) food elements may be removed as a whole or preferentially by mixed cultures of bacteria, (iv) intermediate and terminal end products interfere with growth. (v) species rivalry and predators destroy otherwise effective bacterial cultures (vi) nitrification may produce increase in rate of BOD exertion. Thus with time, the successive bacterial growth or BOD exerted is usually interfered with.

3.3.1 BOD As An Indirect Measure Of Growth

Cell growth can be measured by increase in weight or COD of suspended solids (Busch, 1958; Myrick and Busch, 1960; Busch and Myrick 1961). Measurements of deoxyribonucleic acid (DNA) and dehydrogenase activity are the indirect measures of cell growth (Giese, 1962; Lenhard et al. 1964). All these parameters lack simplicity.

For heterotrophic growth, a portion of the substrate is used for energy and a portion for biosynthesis. BOD exertion consists of two energy requirements i.e. energy for cell maintenance and for cell synthesis. During active cell replication (logarithmic growth and declining growth), maintenance energy requirement is small and for simplicity may be neglected. Thus if cell growth is taking place under constant environmental conditions, during active cell replication, growth is approximated by the BOD exerted.

Consideration of various types of growth curves and formulation of the diphasic carbonaceous BOD are presented.

3.3.2 Growth Curves

Let t denote time which influences the size y of the phenomenon observed. Then the differential co-efficient (dy/dt) denotes the rate of growth i.e. the increase in y per unit time. The growth process may be characterized by the differential equation (Hald, 1952),

$$(dy/dt) = f(y)\, g(t) \qquad \ldots.(3.3)$$

Where f(y) and g(t) are functions of y and t respectively.

or $dy/f(y) = g(t)\, dt$

By integration,

$$F(y) = G(t)$$

or y is determined as a function of t.

Introducing f(y) = 1, y, (L_1-y) and $y(L_1-y)$ in (3.3) following four differential equations result in;

$$(dy/dt) = \begin{cases} g(t) \\ y g(t) \\ (L_1-y)\, g(t) \quad (0<y<L_1) \\ y(L_1-y)\, g(t) \quad (0<y<L_1) \end{cases} \qquad \ldots.(3.4)$$

In the last two equations, the growth is limited, as L_1 denotes the maximum value of y. The four equations, respectively, indicate that at a given time the growth rate, (dy/dt),

(i) depends on the time, but is independent of the size reached,

(ii) is proportional to the size reached and to a function of the time,

(iii) is proportional to the remaining size, that is, the maximum size (L_1) minus the size reached, and a function of the time,

(iv) is proportional to both the size reached and the remaining size as well as a function of the time.

In the simplest case when $g(t) = \alpha_o$ the equations (3.4) upon integration become

$$y = b + \alpha_o t$$
$$y = b\, e^{\alpha_o t} \qquad \ldots.(3.5)$$
$$y = L_1(1-e^{\alpha_o t})$$
$$y = L_1/(1+m\, \bar{e}^{nt})$$

Where in the last equation $n = \alpha_o L_1$ and $m = (L_1-y_o)/y_o$ and $y=y_o$ when $t = 0$

Here the phenomenon of interest is the BOD exerted as presented below.

3.3.3 Formulation Of Diphasic Carbonaceous BOD

The diphasic carbonaceous BOD curve consists of two sequential autocatalytic growth curves. First growth of bacteria called the bacterial growth phase (BGP) and the second, cyclic degradation of cell mass (CDCM) through lysis-synthesis-lysis and/or the growth of protozoa feeding on bacteria. The two curves are separated by a stationary phase (plateau).

The entire diphasic carbonaceous BOD curve, comprising of exogenous carbon source during one phase and the endogenous carbon source during the other, and entirely different ecosystems and

widely varying maintenance energy requirements, cannot, justifiably, be approximated by any one of the growth curves represented by equations (3.5).

Thus, for valid interpretation and translation of the "analytical" information to actual systems, the two sequential autocatalytic growth curves are represented separately by appropriate growth curves from equations (3.5).

3.3.3.1 Formulation of the Bacterial Growth Phase (BGP)

BOD exertion during BGP proceeds through lag pahse (if any), logarithmic growth, declining growth and the stationary phase. Neglecting lag, the shape of the BOD curve is sigmoidal or autocatalytic.

Rate of BOD exerted during "autocatalytic" growth is proportional to both the cell mass or BOD exerted and remaining cell growth or BOD remaining to be exerted and is represented by the last growth curve of equatons (3.5). This curve is called the logistic growth curve.

3.3.3.2 Formulation of the Endogenous Phase

During endogenous phase, cells mainly require energy for maintenance which is supplied by respiration of the intracellular components of the cell. Endogenous respiration also proceeds through cyclic, lysis-synthesis-lysis, metabolism of the cell mass. Increased maintenance energy requirements result in little energy for synthesis. Rate of biomass decrease is proportional to the remaining biomass. Protozoan growth essentially proceeds at an ever declining growth rate. Rate of BOD exerted during endogenous

phase is proportional to the remaining BOD to be exerted and is given by the first order decreasing rate law and is represented by the 3rd growth curve of equations (3.5).

3.3.4 Estimation Of The Parametrs Of The Logistic Curve

3.3.4.1 Estimation of K_s and L_1

Logistic curve in the differential form as represented by last equation of (3.4) is

$$(dy/dt) = K_s\, y(L_1-y) \qquad \ldots.(3.6)$$

When $g(t) = K_s$

in which $g(t) = K_s$ = a constant equal to the rate constant of the logistic curve or sigmoidal curve.

L_1 is the limiting growth BOD and is defined as the BOD exerted till equilibrium (maximum) population of the bacterial cells is attained and exogenous substrate is exhausted.

Dividing both sides of equation (3.6) by y

$$(1/y)\ (dy/dt) = K_s\ (L_1-y)$$

or $$d(\ln y)/dt = K_s\ (L_1-y)$$

or $$\frac{\ln (y_2/y_1)}{\Delta t} = K_s\ (L_1-y)$$

Putting

$$\frac{\ln (y_2/y_1)}{\Delta t} = Z$$

$$Z = K_s\ (L_1-y) \qquad \ldots.(3.7)$$

Z is termed as the relative rate of growth. Equation (3.7) was utilized to examine whether BOD exerted during bacterial growth phase is represented by the logistic curve or not.

Plot of Z Vs $\bar{y}$ results in a straight line if the data is represented by a logistic curve.

Slope of the line of equation (3.7) = K_s and y-Intercept = K_sL_1

Thus K_s and L_1 can be determined.

3.3.4.2 Estimation of m and n

Last equation of (3.5) after transformation is written as

$$(L_1-y)/y = m \exp(-nt)$$

A plot of $(L_1-y)/y$ Vs t on a semilogarithmic paper results in a straight line.

Slope of the line = $-n$

exp (y-Intercept) = m

and also from (3.5) $n = K_sL_1$

Check

y - intercept of Z Vs $\bar{y}$ = slope of $(L_1-y)/y$ Vs t on a semilog paper.

CHAPTER IV

RESPIROMETRIC AND EXPERIMENTAL TECHNIQUES

4.1 INTRODUCTION

The idea that membranes, having permeability to oxygen, can be utilized to overcome a serious limitation of the BOD dilution technique was presented in chapter I. It was further elaborated in chapter III sec. 3.2 that the rate of diffusion of oxygen in the polyethylene chamber would be increased if either the sample in the chamber is imparted a stirring action or the volume of sample contained in the chamber is decreased. Respirometric systems were developed on these considerations which are explained in the following section. Experimental techniques are also presented in this chapter.

4.2 THE RESPIROMETRIC SYSTEMS

4.2.1 Respirometer

4.2.1.1 Construction

The respirometer is a polyethylene chamber to contain wastewater samples for estimating the BOD values (Fig. 4.1). It consists of a base plate and a cover plate held together, at a clear distance of 88mm, by three equidistant supports. This provided the framework of the chamber fabricated from perspex. The cover plate was provided with a central hole for mounting dissolved oxygen (D.O.) probe and another hole for a thermometer. Cover plate and base plates are provided with a groove for accomodating O-rings. An annular polyethylene membrane is held to base plate and cover plate by means of collars made of fibre plastic. The collar and

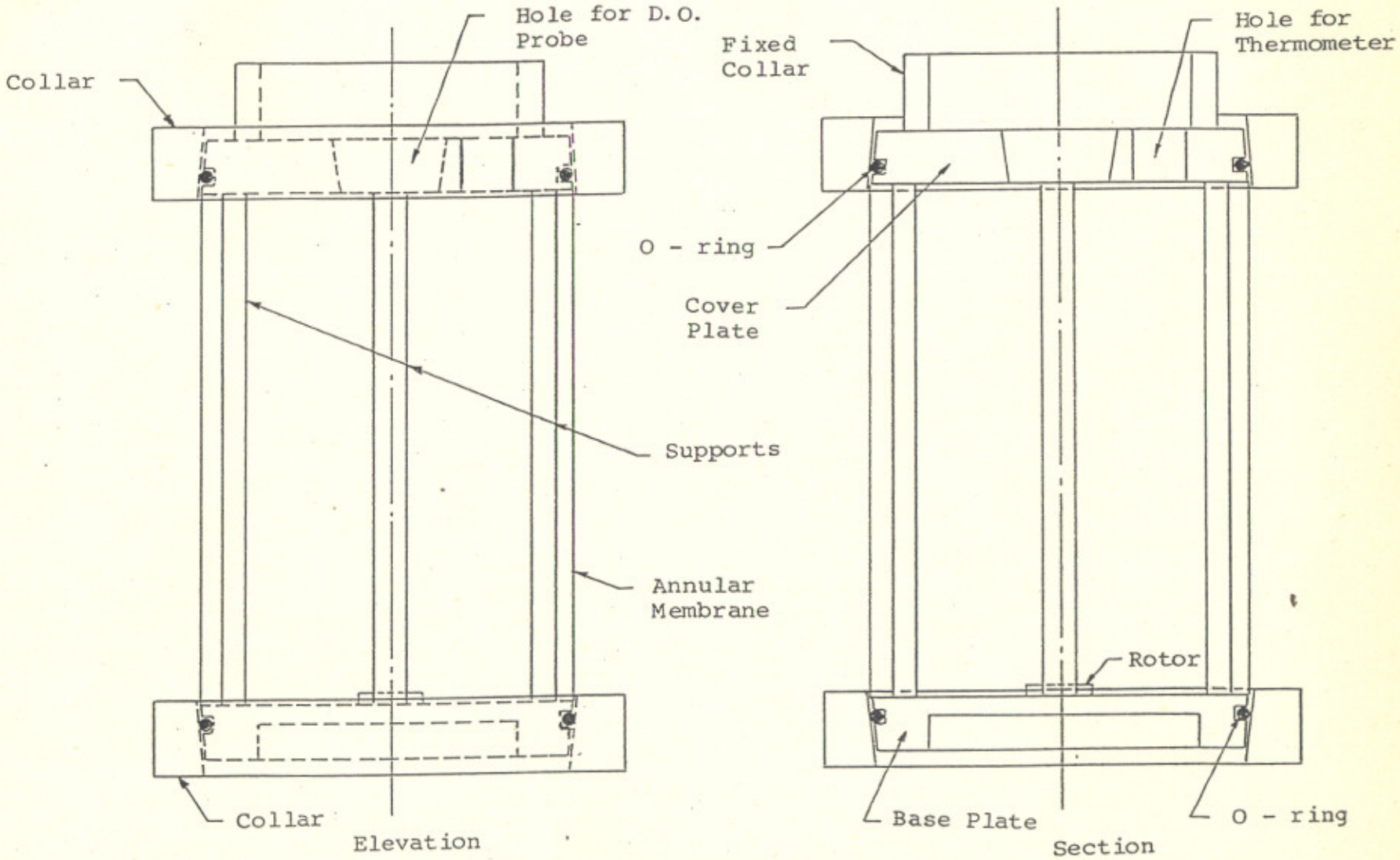


Fig. 4.1 Respirometer

O-rings hold the membrane tightly and make them leakproof. The annular membrane forms the cylindrical wall surface of the chamber. A collar is fixed on the cover plate for storing the liquid under test and preventing any leakage of vapours.

Stirring of the liquid was caused by a magnetic bit (rotor, 20mm x 4mm dia. enclosed in teflon) placed on base plate and rotated at 100 rpm with a magnetic stirrer.

4.2.1.2 Specifications

Diameter of top and base plates	=	65 m.m.
Height of the cylindrical membrane surface	=	88 m.m.
Total membrane area for diffusion	=	179.7 cm^2
Volume of liquid contained (D.O. probe and thermometer inserted)	=	300 ml.
Membrane area to volume ratio (a)	=	0.6 cm^{-1}

4.2.1.3 Operation

Annular membrane was slipped over base plate and cover plate to form the wall surface. The membrane was held tightly on the base plate with the collar and any extra membrane was cut and removed. Liquid sample was introduced through the holes in the cover plate so as to completely fill the respirometer chamber. Entrapped air was removed from the sides of the cover plate by slightly pulling the membrane and creating a space inbetween the sides of the cover plate and the membrane.

The membrane was then held tightly on the cover plate with the help of the collar and the rotor, D.O. probe and thermometer mounted.

The respirometer was placed on a magnetic stirrer and incubated at 20°C.

The probe was connected to a potentiometric chart recorder for measuring D.O.

4.2.2 Disposable Respirometric BOD Bottle

4.2.2.1 Fabrication

Respirometric BOD bottles were fabricated by heat sealing two layers of polyethylene membranes inbetween a pair of stiffening frames.

4.2.2.1.1 Stiffening frame

Figs. 4.2, 4.3 show a circular and a rectangular frame respectively. These consist of the frame, with a collar extending upward from the frame. A pin is provided on one side of the collar and a hole on the other to fit in so as to form a pair of frames. Further reinforcing ribs are provided with the collar. The membranes were heat sealed inbetween a pair of stiffening frames forming the respirometric BOD bottle. Stiffening frames were die casted from low density polyethylene.

4.2.2.1.2 Heat sealing operation

One of the frame was placed, on a base plate, with engraved impressions of the frame, so that the pin of the frame was on the upper side.

Two layers of the membrane were placed on the frame, extending upto the pin and the hole line and projecting outwardly in all the other three directions. A piece of cellophane paper was

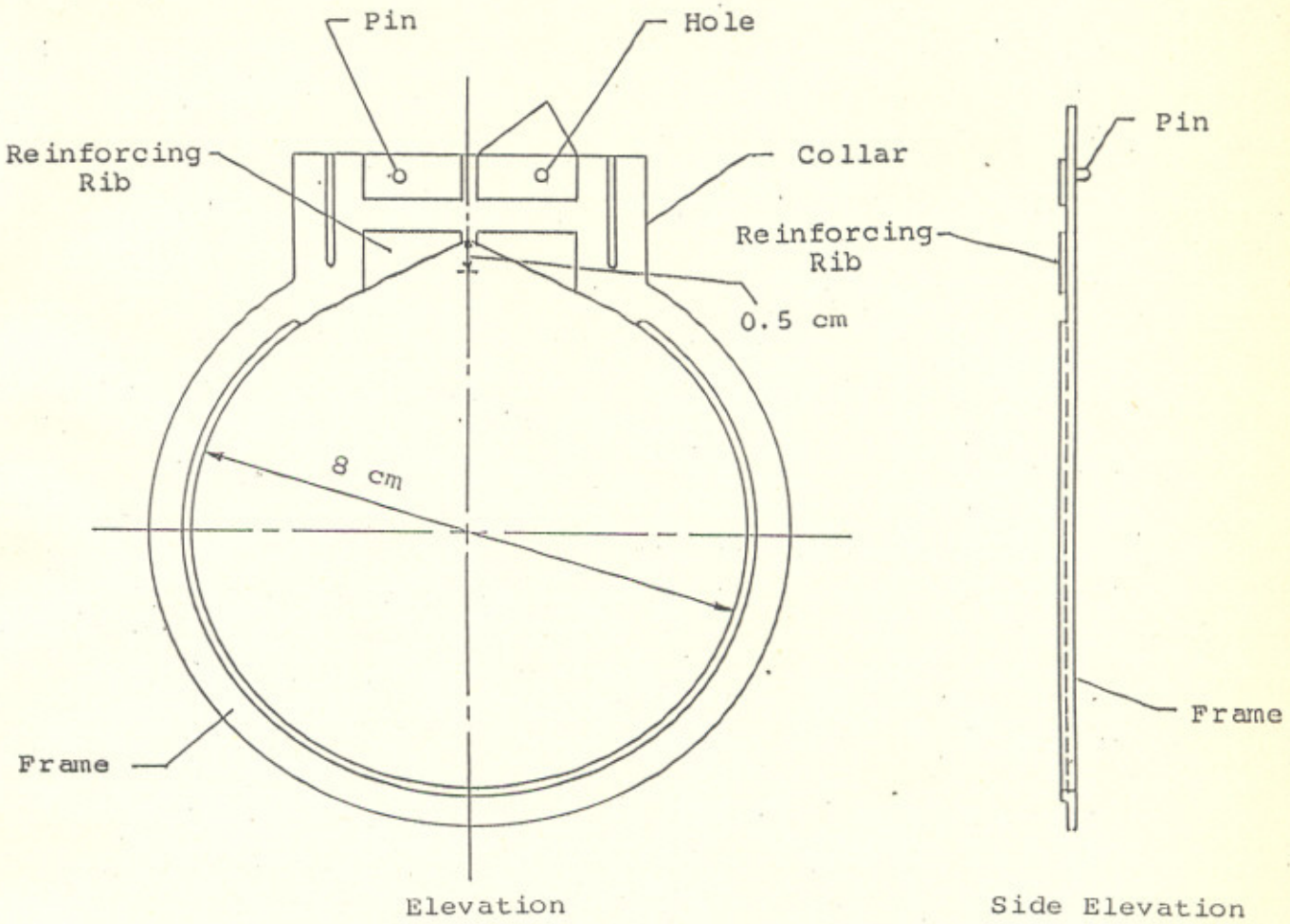


Fig. 4.2 Circular Stiffening Frame

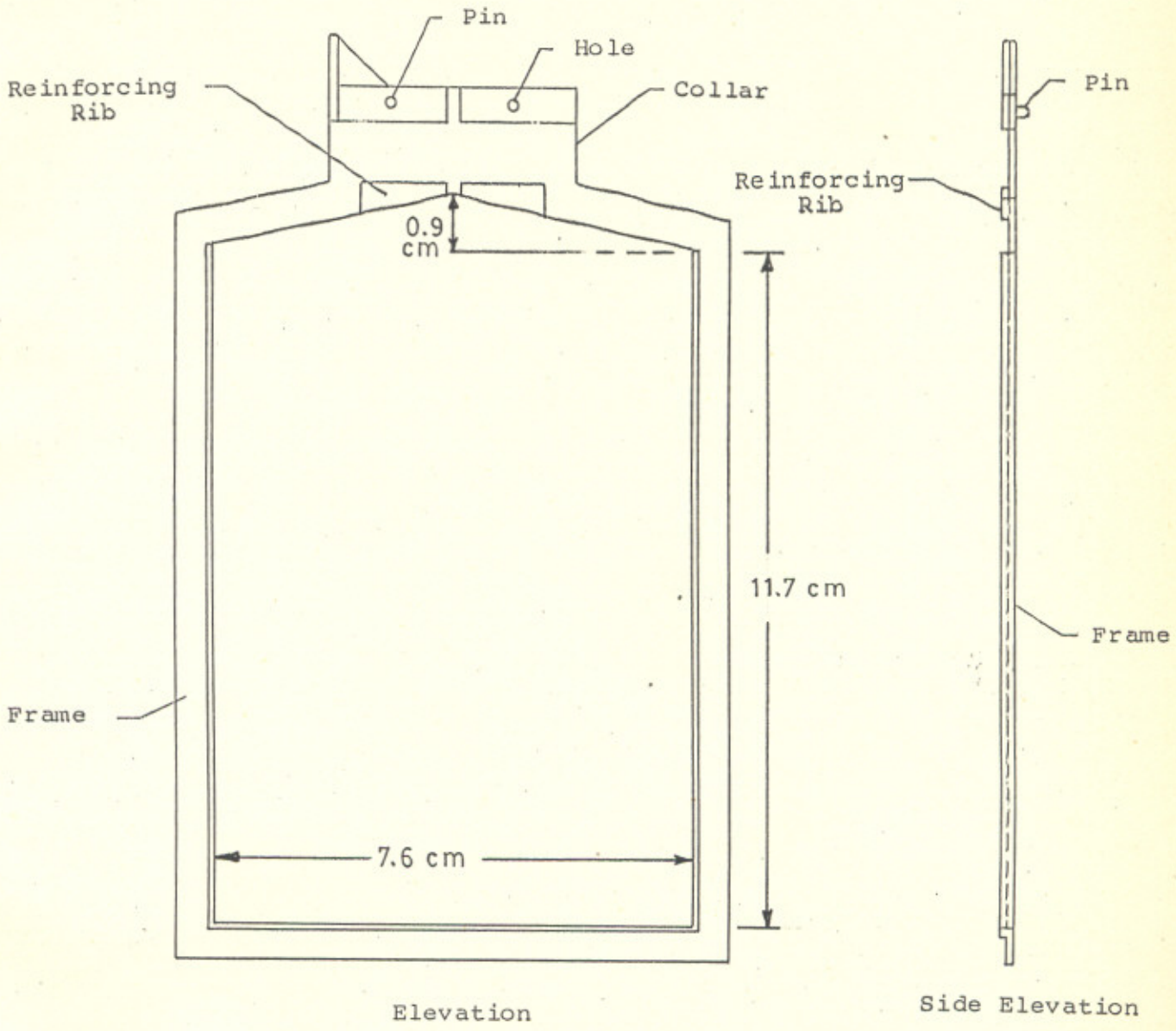


Fig. 4.3 Rectangular Stiffening Frame

placed inbetween the membranes. Another frame was placed over on the base plate so that hole of one and the pin of other fitted in to form a pair of frames.

The pair of frames was kept in position by fixing a piece of cellophane tape with the base plate and collar of the upper frame.

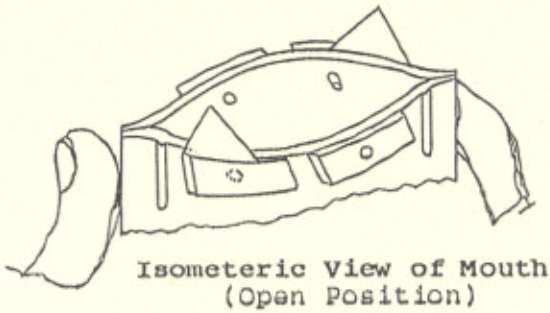
Hot soldering iron (125W) fitted with a roller was moved along the shaded area as shown in Figs. 4.4 to 4.7 till the membranes were sealed with the stiffening frames. The frames were subsequently removed from the base plate.

Extra membrane extending beyond the frames was cut and removed.

The two membranes make a leakproof joint. In the area between the reinforcing ribs and the pin and hole line, where a piece of cellophane paper was placed, the two membranes were not sealed with each other. However, the upper membrane was sealed with the upper collar and the lower membrane with the lower collar of the stiffening frames, forming a collapsible mouth of the bottle as illustrated in the three dimensional view of the mouth in Fig.4.4 and 4.5. The collapsible mouth and sealing of the membranes with the collars is also illustrated in Fig. 4.6 where the central section is shown in perspective with the mouth open.

4.2.2.2 Construction

The respirometric BOD bottle is in the form of a bag, consisting of a pair of membranes. The edges of the membranes were heat sealed to a pair of stiffening frames as described earlier. The



Isometeric View of Mouth
(Open Position)

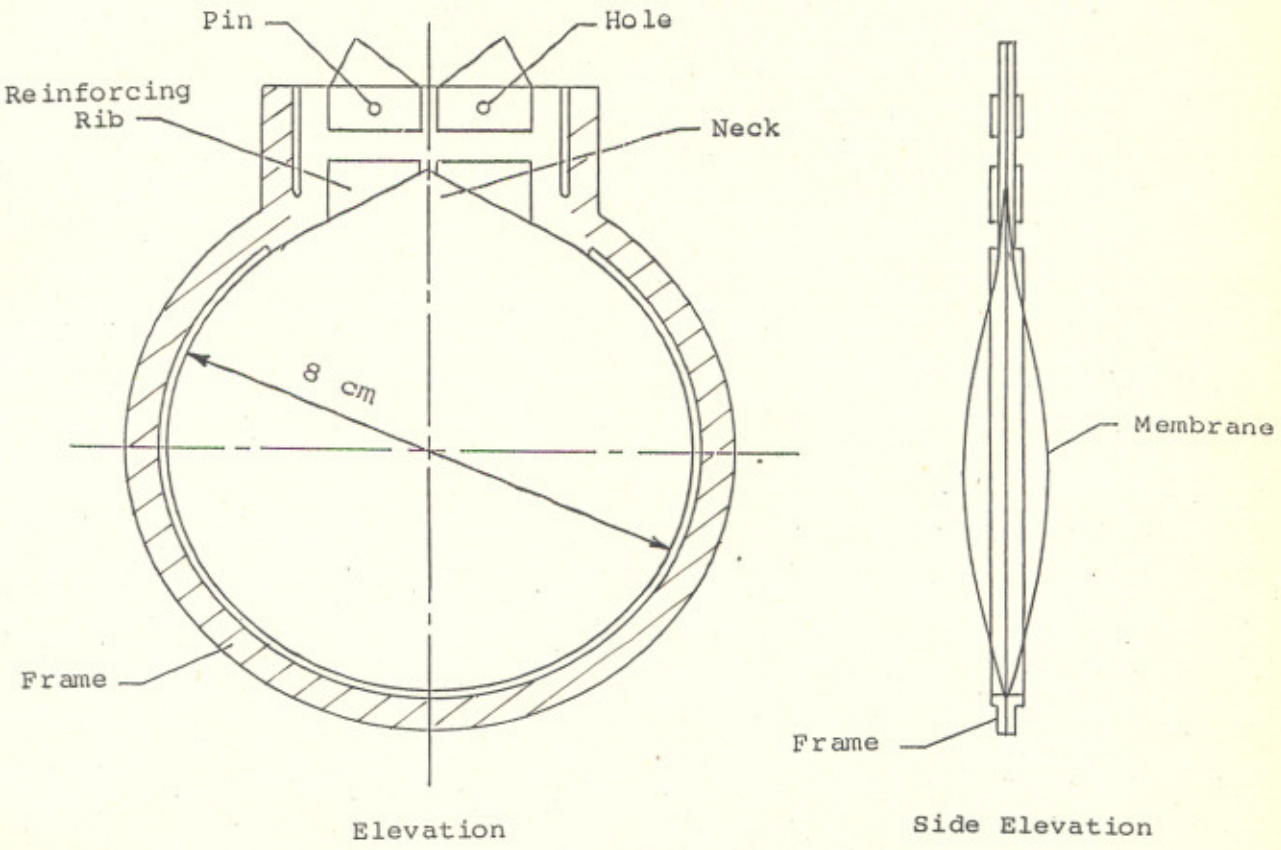


Fig. 4.4 Respirometric BOD Bottle

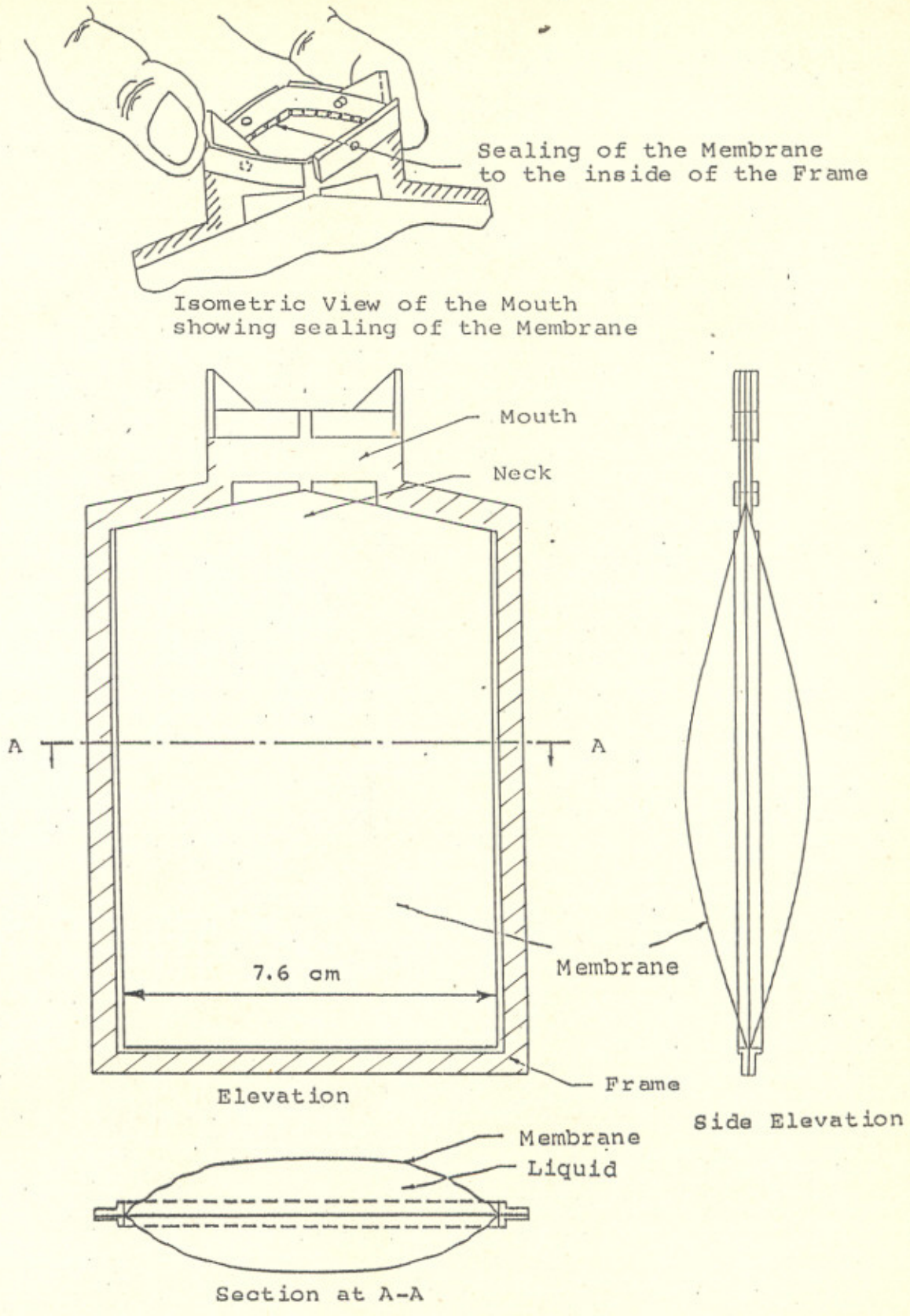


Fig. 4.5 Respirometric BOD Bottle, Rectangular, Single Compartment

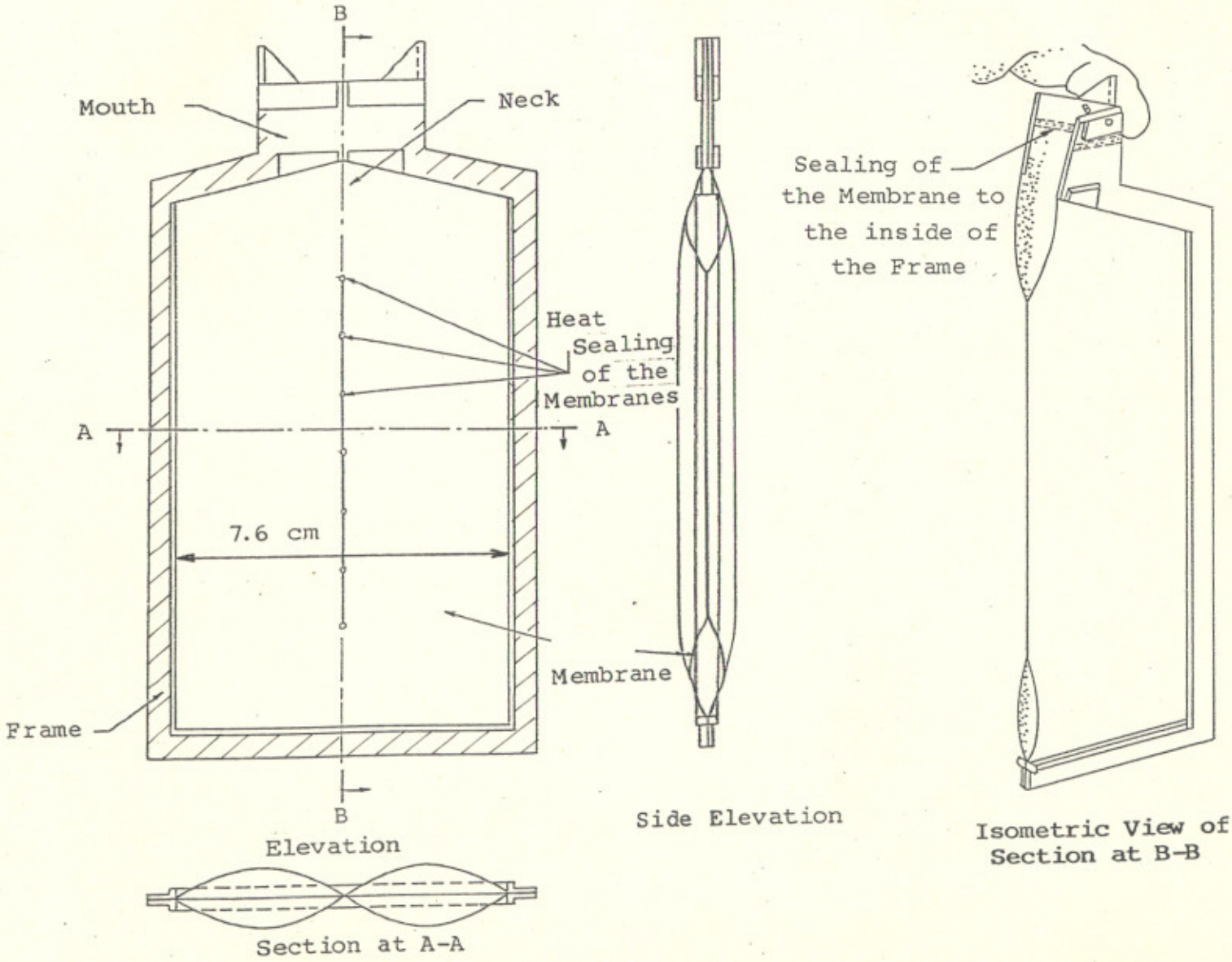


Fig. 4.6 Respirometric BOD Bottle, Rectangular, Two Compartments

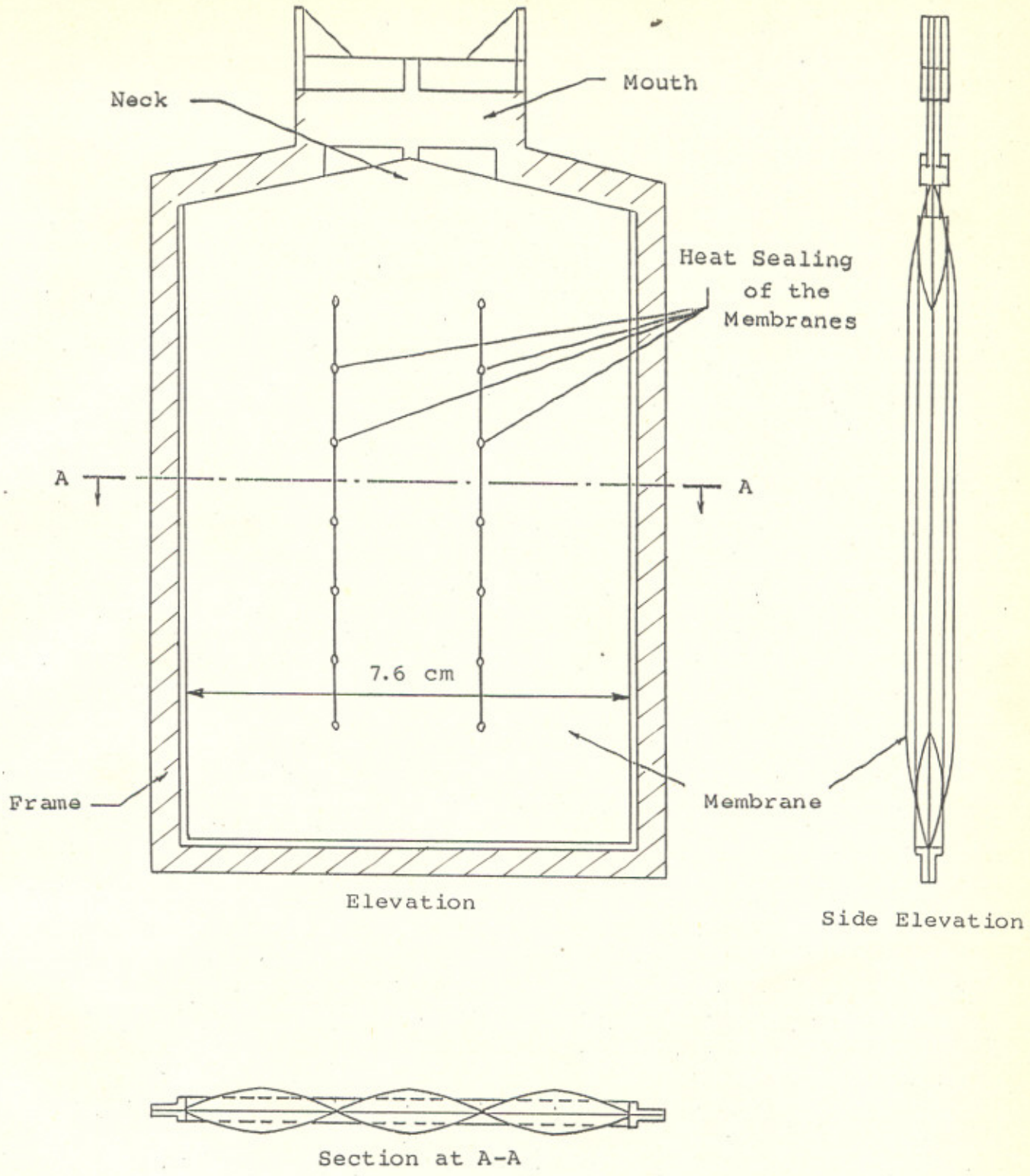


Fig. 4.7 Respirometric BOD Bottle, Rectangular, Three Compartments

bag has a collapsible mouth. A pin provided on one side of the collar cooperates with a hole provided on the opposite side of the collar for an aligned closure of the mouth.

After unlocking the pins, the collar is pressed with the thumb and first finger of the hand (Figs.4.4 and 4.5), thereby moving the two collars apart and creating an opening for the introduction or discharge of the wastewater sample. To close the mouth, the two collars are pressed against each other locking the pins with the holes. The reinforcing ribs help in keeping the mouth in properly closed position. The stiffening frames ensure the constant volume. Further, the stiffening frames restrain the inward movement of the edges of the bottle and hence allow the membranes to inflate only partly, whereby, the volume of liquid contained therein is minimum. The interfacial area for diffusion is constant and area to volume ratio (a) of bottles of any one particular construction is constant.

In another modification of the respirometric BOD bottle, the bag has two, three or more interconnected compartments. These compartments were created by heat sealing the two membranes together at one, two or more dashed lines as shown in Fig. 4.6 and 4.7. When the liquid was added in the respirometric BOD bottle and the bulging, and hence the liquid capacity, of the bag was considerably reduced. By way of example, if a given bag with a single compartment had a volume capacity of X cm^3, the same bag having two interconnected compartments would have a volume capacity of less than x/2 cm^3 for each compartment and, whereby

the total volume contained would be much less than X cm^3. However the interfacial surface area of the bag remained constant whether it was a single or double compartment bag. Thus in a bag having two or three interconnected compartments, ratio of area to volume increased in comparison to a bag having only a single compartment.

4.2.2.3 Specifications of Various types of Respirometric BOD Bottles

4.2.2.3.1 Circular

The dimensions are as shown in Fig. 4.4.

In Fig. 4.4 is shown the elevation of the respirometric BOD bottle with mouth closed. Bulging of the membranes when filled with liquid is shown in side elevation of Fig. 4.4. On top of the elevation is an isometric view of the mouth when it is opened by pressing its sides by the thumb and forefinger of the hand.

Stiffening frame inner diameter	=	80 m.m.
Membrane thickness	=	60 gauge
Total interfacial area for diffusion	=	101.5 cm^2
Volume of liquid contained	=	32 ml.
Area to volume ratio (a)	=	3.17 cm^{-1}

4.2.2.3.2 Rectangular single compartment

The dimensions are as shown in Fig. 4.5.

In Fig. 4.5, the respirometric BOD bottle is shown in elevation. Extent of bulging of the membranes (when full) is shown in the side elevation and also in section at A-A. At the top of the figure is an isometric view of the mouth in open position.

Heat sealing of the membrane inside the collar of the mouth is also shown in the isometric view.

Membrane thickness	=	100 gauge
Total interfacial area for diffusion	=	184 cm^2
Volume of liquid contained	=	125 ml.
Area to volume ratio (a)	=	1.47 cm^{-1}

4.2.2.3.3 Rectangular two interconnected compartments

The dimensions are as shown in Fig. 4.6.

In Fig. 4.6, the respirometric BOD bottle is shown in elevation. Extent and manner of bulging of the membranes is shown in the side elevation, also in section at A-A and in the isometric view of section at B-B. Open position of mouth and heat seals of membranes to inside of the collars are distinctly illustrated in the sectional isometric view.

Membrane thickness	=	100 gauge
Total interfacial area for diffusion	=	184 cm^2
Volume of liquid contained	=	68 ml.
Area to volume ratio (a)	=	2.7cm^{-1}

4.2.2.3.4 Rectangular, three interconnected compartments

The dimensions are as shown in Fig. 4.7.

In Fig. 4.7, the respirometric BOD bottle is shown in elevation. Extent and manner of bulging and formation of interconnected compartments is shown in the side elevation and also in section at A-A.

Membrane thickness	=	100 gauge
Total interfacial area for diffusion	=	184 cm^2
Volume of liquid contained	=	38 ml.
Area to volume ratio (a)	=	4.84 cm^{-1}

4.2.2.4 Operation

After unlocking the pins, mouth of the R.BOD bottle was opened and held inbetween the thumb and first finger of the left hand (Fig.4.4 and 4.5).

Measured quantity of the liquid (water or wastewater) was added in the bottle.

The mouth of the R.BOD bottle was closed by pressing the collars whereby the pins were also locked in the holes. A little liquid (0.5 ml or less) usually spilled out of the bottle during this operation. The water/wastewater remained filled upto the neck and completely expelled air from the respirometric BOD bottle.

The mouth of the respirometric BOD bottle was gripped tightly with a paper clip and the bottle was hung in the incubator. Temperature of the incubator was maintained at 20°C $\pm$ 0.5°C.

After a certain interval of time, the bottle was taken out from the incubator, the liquid sample was siphoned out in the sample tube, and dissolved oxygen, in the sample, was determined.

4.3 DETERMINATION OF DISSOLVED OXYGEN

D.O. was determined by Alsterberg azide modification of the Winkler method. Reagents required for the test were prepared and standardized as described in Standard Methods.

4.3.1 Siphoning Of The Sample

4.3.1.1 Apparatus

4.3.1.1.1 Sample tube

Sample tube is in te form of test tube with B19-cup joint, so that when whole liquid is siphoned out from the respirometric BOD bottle, it filled the tube approximately and upto level A-A Fig.4.8. Sample tubes of different capacities were prepared for different capacity R.BOD bottles.

Sample was enclosed in the sample tube, without entrapping air, with a conical tip B19 stopper Fig. 4.8.

4.3.1.1.2 Siphon Pipe

It is a plastic pipe, Fig. 4.8, 150 to 200 m.m. long and 5 m.m. diameter and a chisel point at one end of the pipe. Alternatively 10 to 15 m.m. long glass or silica tube, with a chisel point, was fitted in one of the ends of the plastic pipe.

4.3.1.2 Procedure

Sample tube was placed in the test tube stand.

Siphon pipe was placed in the sample tube, with the chisel point projecting out of the tube.

Respirometric BOD bottle was held in the left hand and the chisel point (with siphon pipe in the sample tube) was held inbetween the thumb and the forefinger of the right hand.

A hole was made in the membrane of the R.BOD bottle with the chisel point and pushed 2 to 5 m.m. inside the bottle. The liquid

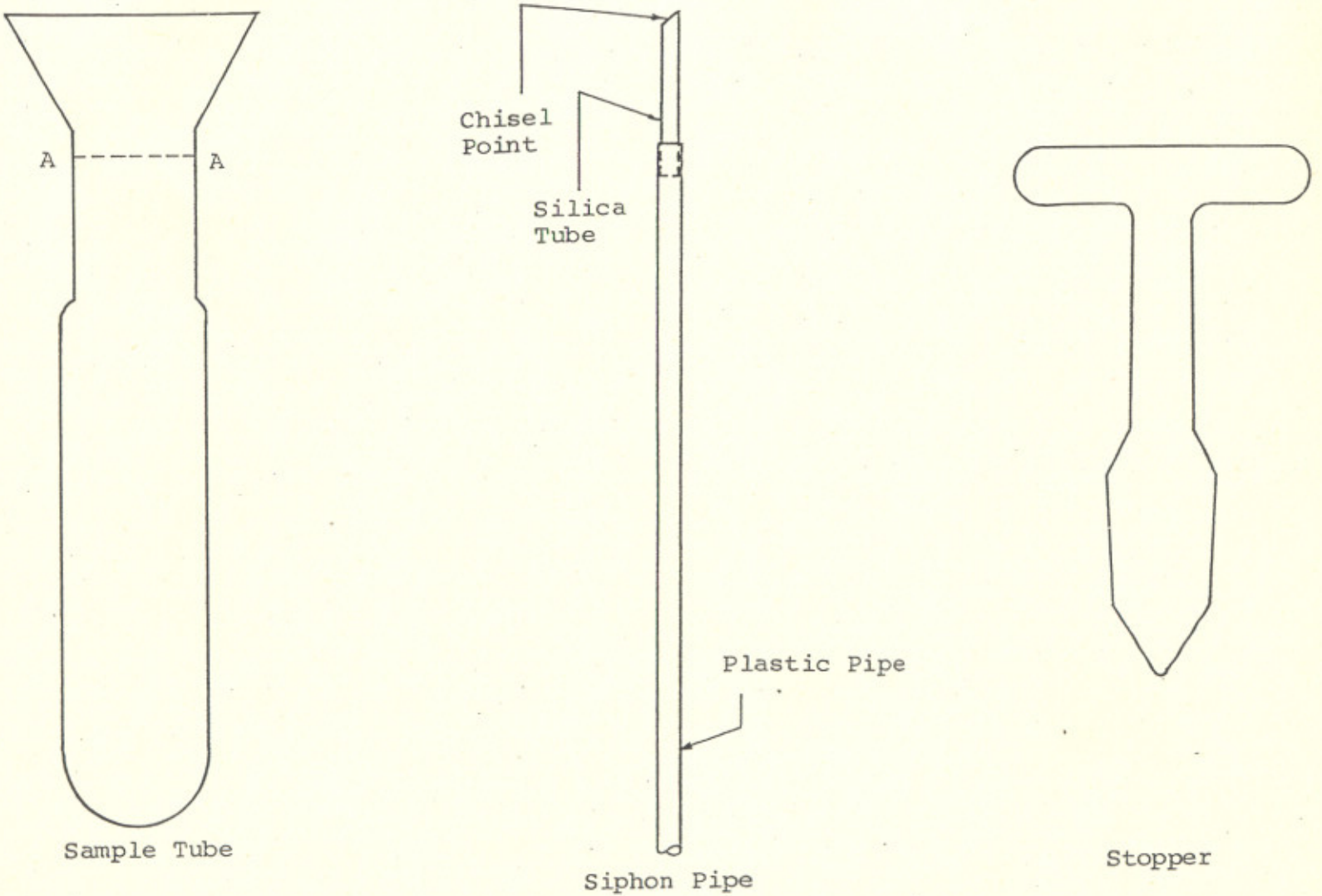


Fig. 4.8 Apparatus for Siphoning of the Sample

was siphoned out in the sample tube and the membranes collapsed from up to downwards.

4.3.2 Fixation Of Dissolved Oxygen And Release Of Iodine

Immediately after siphoning out the sample glass beads (2 to 5 No) were added in the sample tube.

With separate pipettes, 0.2 ml of manganous sulphate solution* followed by 0.2 ml of alkaline potassium iodide azide solution* were released in the middle of the sample tube, and the tube stoppered with the conical tip stopper.

The sample tube was turned up and down, keeping the stopper in position. Glass beads, added earlier, also moved up and down in the sample tube and helped in bringing the precipitate in intimate contact with oxygen dissolved in the liquid sample.

The precipitate was allowed to settle down at the bottom of the sample tube.

After removing the stopper, 0.2 ml of concentrated sulphuric acid* was released at the surface of the liquid in the sample tube and the tube again stoppered.

The tube was tilted up and down to dissolve the precipitate. Movement of the glass beads helped in breaking the settled precipitate and its dissolution.

The released iodine was determined by microtitrimetric analysis.

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* 0.5 ml of the reagent solution when volume of sample was more than 50 ml.

4.3.3 Titration Of The Released Iodine

4.3.3.1 Apparatus

4.3.3.1.1 Titration flask

It consists of an ordinary conical titration flask with a very wide mouth and humped bottom. The hump in the bottom of the flask was so designed, that after adding measured quantity of the solution to be titrated, top surface of the hump remained clear of the liquid Fig. 4.9.

4.3.3.1.2 Micro-burette

It consists of a vertical burette and a pressure controlling device.

Vertical burette was fabricated from a 1 ml pipette subdivided into 200 parts. The pipette was made from a uniform bore capillary and was accurately graduated with zero mark at the top. The tip of the pipette has a fine elongated bore.

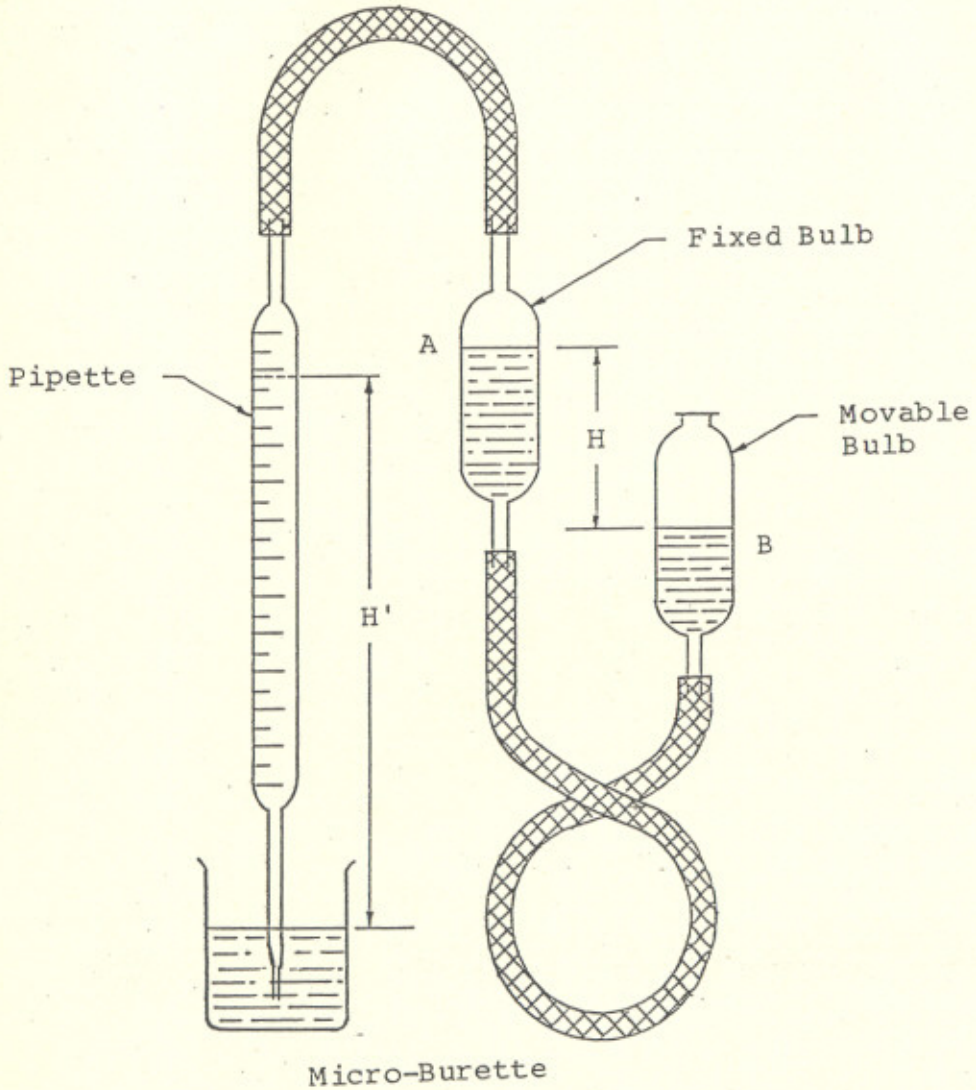
Pressure controlling device consists of one fixed bulb (A) and the other (B) mounted on a stand with rack and pinion arrangement, having motion in the vertical direction.

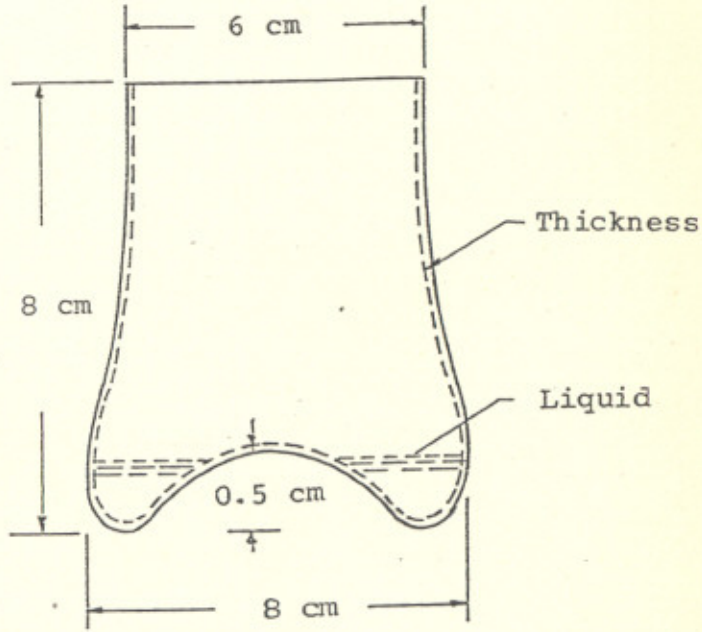
Connections between various ends of the fixed and movable bulbs and the pipette were made with PVC tubing Fig. 4.9.

Movable and fixed bulbs were partly filled with mercury.

4.3.3.2 Titration Procedure

Movable bulb B was raised to its upper position with the rack and pinion arrangement. Level of mercury in the bulbs A and B was the same.





Humped Bottom Titration Flask

Fig. 4.9 Apparatus for Titration of the Sample

Tip of the burette was dipped in the standard sodium thiosulphate solution taken in a 100 ml beaker. The burette was filled by lowering bulb B. At equilibrium, pressure H equals pressure H'.

Tip of the burette was taken out from the standard solution and the 100 ml beaker placed on one side. Zero level of the solution in the burette was adjusted by slightly raising bulb B and touching the tip of the burette with a wet filter paper or wet finger. Minute quantities of the solution were released on the wet surface. The process is repeated until the solution in the burette is at zero level.

20.3 ml of the solution, to be titrated, were transferred from the sample tube into the titration flask.

During the titration process, tip of the burette was kept above the liquid level in the titration flask and bulb B is slowly raised. Standard thiosulphate solution was released in drops into the titration flask which was being swirled continuously.

As the color of the solution fades to pale yellow, starch indicator solution (2 to 4 drops) was added and the color became blue.

Bulb was no longer moved and left in the last position. Tip of the burette was touched with the top surface of the hump that was clear of the liquid. It was repeated again and again at short intervals simultaneously swirling the flask until the end point was reached.

If no further change occured in the burette reading, bulb B was raised slightly and the process repeated till the solution turned colorless.

Due to surface forces at the capillary tip of the burette, liquid was not released from the burette if bulb B was slightly raised and then left in position. However when the tip of the burette was touched with a wet surface, minute quantities of the solution (around 0.005 ml) were released on the wet surface. As the end point was approached, further addition of the standard solution was in minute quantities by touching burette tip with the wet surface.

In the standard procedure 203 ml of the sample are titrated and the volume of standard solution used is read with an accuracy of $\pm$0.05 ml. Whereas in the microtitration procedure as explained above, 20.3 ml of the sample were titrated and the volume of standard solution used was read with an accuracy of $\pm$0.005 ml.

4.4 OVERALL MASS TRANSFER CO-EFFICIENT ($K_L^\circ a$) DETERMINATION

4.4.1 Procedure For Respirometer

Distilled water was filled in two aspirator bottles (3 litre capacity) and kept overnight in the incubator at constant temperature $\pm$0.5°C. The temperature of water was checked in both the aspirator bottles next day and if found to be the test temperature, the experiment was carried out further.

Compressed nitrogen gas was bubbled through water in one of the aspirator bottles for 30 min to reduce D.O. below 1 mg/l.

In the beginning of the experiment, D.O. probe was calibrated.

Using the membrane for which the mass transfer co-efficient was to be determined, the respirometer was filled with distilled water with reduced D.O. The D.O. probe was connected to the potentiometric chart recorder and the experiment was terminated when D.O. concentration of water in the respirometer remained constant for about 12 hours.

4.4.2 Procedure For Respirometric BOD Bottle

Distilled water was filled in a 3 litre aspirator bottle and kept in the incubator overnight. Following the various steps outlined in para 4.4.1, D.O. concentration of the water was reduced below 1 mg/l. The procedure outlined in para 4.2.2.4, was followed and measured quantity of the water, from the aspirator bottle, was added in the R.BOD bottle. The volume of water to be added depended on the capacity of the R.BOD bottle.

The bottle was hung in the incubator with a numbered paper clip. The time of filling was recorded.

After suitable interval of time (depending on the type of respirometric BOD bottle) three R.BOD bottles were taken out and the water siphoned out in the three separate numbered sample tubes as outlined in para 4.3.1.

D.O. in water in various sample tubes was determined as outlined in sec. 4.3.

In about 1.5 to 8 hours, (depending on the R.BOD bottle) water was saturated with D.O. and the experiment was terminated.

4.5 COMPOSITION OF SYNTHETIC WASTE

In addition to a source of available carbon, the synthetic waste should contain all the accessory nutrient elements and have a favourable pH and osmotic conditions. Potassium, sodium, calcium and magnesium salts were added to give buffering capacity and proper osmotic conditions. These also served to provide the micro-organisms with any of these elements that were needed in growth and metabolism. Ferric chloride, magnesium sulphate and ammonium chloride supplied the requirements for iron, sulphur and nitrogen. The phosphate buffer, in addition to proper pH conditions furnished any phosphorus that was needed for metabolism.

Composition of synthetic waste used for respirometric studies is shown in Table 4.1. Concentrations of various constituents were taken from Bhatla and Gaudy (1964) and 'Standard Methods.'

TABLE 4.1

Composition of Synthetic Waste

Constituents	Concentrations
Substrate : Glucose, Glutamic Acid or Glucose only	50-200 mg/l
Phosphate Buffer Solution	10 ml/l
$MgSO_4 \cdot 7H_2O$	100 mg/l
$FeCl_3 \cdot 6H_2O$	0.5mg/l
$MnSO_4 \cdot H_2O$	10 mg/l
$CaCl_2$	7.5mg/l
Ammonium Phosphate Solution	10 ml/l
TapWater	100 ml/l

Made upto one litre with distilled water.

Phosphate Buffer Solution

34.0 gms of KH_2PO_4, 87.0 gms of K_2HPO_4 and 44.5 gms of $Na_2HPO_4 2H_2O$ in one litre of distilled water.

Ammonium Phosphate Solution

33.0 gms of $(NH_4)_2 HPO_4$ dissolved in one litre of distilled water.

Ammonium Phosphate solution was ommited when glutamic acid was used as one of the substrates.

4.6 SEED

Settled domestic sewage was used as seeding material. Acclimation of the seed was carried out in the respirometer (Fig. 4.1). Synthetic medium, containing 100 mg/l of glucose and glutamic acid and the inorganic salt concentrations shown in Table 4.1, was prepared and 25 ml of settled domestic sewage in one litre of synthetic medium were added. The respirometer (with synthetic waste and seed) was maintained at 20°C. After 24 hours 10 ml seed was transferred into one litre fresh medium and kept in the respirometer at 20°C. The contents of the respirometer were continuously stirred. Three serial dilutions were made on successive days. During the course of experimentation, the respirometer contents were replaced daily with acclimated inoculum and fresh medium. Near the end of the log growth phase (within 15 to 20 hours), 5 ml/l of the medium from the respirometer was used for experimentation. Glucose was the sole source of carbon during acclimation for experiments on BOD exertion on glucose. Synthetic medium was prepared as in Table 4.1 and ammonia was added as source of nitrogen.

4.7 COD DETERMINATION

COD was determined using the standard COD technique as explained in 'Standard Methods' with the exception that digestion was carried out at elevated temperature in a pressure cooker at a pressure of $1 kg/cm^2$ for half an hour. Filterate COD was determined on the membrane filterate.

4.8 CELLULAR PROTEIN

Cellular protein was determined by Folin Phenol reagent (Herbert et al. 1971).

CHAPTER V

MASS TRANSFER MODELS

5.1 INTRODUCTION

Oxygen transfer in the respirometer and respirometric BOD bottle is interphase mass transfer and the rate of oxygen transfer is also affected by the membrane surrounding the liquid.

A brief review of the flow of inert gases through polymers followed by mechanism and mathematical model of diffusion of gases through polyethylene are presented in this chapter.

Mass transfer models for the respirometer and respirometric BOD bottle are presented in the later part of the chapter.

5.2 FLOW OF INERT GASES TRHOUGH POLYETHYLENE

The main, qualitative features concerning the movement of gases through polymer membranes, were described by Graham (1866), as ref. by Crank and Park (1968) . The process was described as the condensation and solution of gas at one surface followed by diffusion through, in the form of a liquid, and evaporation to the gaseous state at the other surface. Those gases, which are most easily condensable and most soluble in water, penetrate most readily. The non-porous nature of rubber and its similarity to a liquid rather than to a solid were emphasized. The effect of increasing temperature was discussed as causing an increase in the liquid nature of the rubber leading to a greater rate of penetration, even though the solubility of the gas was reduced by increasing temperature due to reduced ease of condensation.

5.3 MEASUREMENT OF THE DIFFUSION CONSTANTS OF GASES

Von Wroblewski (1879), as ref. by Crank and Park (1968), described the solubility of gases as proportional to the partial pressure above the rubber membrane. It was also speculated that Fick's linear law applies for free gases and liquids. Daynes (1920), as ref. by Crank and Park (1968), determined solubilities and diffusibilities from measurements of permeability and time lag to acquire the steady state of permeation. Barrer (1939), as ref. by Crank and Park (1968), fully developed the time lag method for determination of diffusion constants and the solubility coefficients for gases in high polymers. Barrer (1939) and Van Amerongen (1946), as ref. by Crank and Park (1968), also opined that the flow of gases through an amorphous polymer is a diffusion controlled process to which Fick's Laws of diffusion apply.

Full experimental details for studying diffusion across membranes have been given by Crank and Park (1968).

5.4 MECHANISM OF DIFFUSION

As the heat motion of gas molecules are rapid as compared with those of the polymer chains. The rate of permeation is entirely controlled by the latter. If the minimum void volume required to disperse the penetrant molecule in the polymer is smaller than the average free volume, the dominating mechanism for diffusion occurs by the initial dispersal of the penetrant molecule into pre-existing, unfilled local cavities large enough to accomodate the penetrant molecules at or near the polymer surface. Subsequent diffusion occurs by localized activated jumps, into neighbouring pre-existing cavities (Frisch, 1965; Kumins and Kwei 1968).

Diffusion of gases in polyethylene is an activated process. Energy of activation for diffusion is that required to cause a volume fluctuation which results in decrease in the local density and formation of a hole. Analysis of polymer segmental mobility by Bueche (1953), as ref. by Crank and Park (1968), after studying the diffusion of hydrogen, helium and other larger gases through polymers, revealed that each element of the vibrating segment sweeps out the same volume. In the diffusion of larger gases, longer chain lengths are involved in the "hole forming" process. An individual hole may not be large enough to accomodate a diffusing molecule; the co-operative motion of several neighbouring molecules may allow two or more holes to merge into one hole large enough for a diffusional jump to occur. The idea that molecular transport in polymers occurs through the co-operation of several degrees of freedom was advanced by Barrer (1941).

5.5 MATHEMATICAL MODEL FOR DIFFUSION

The process of diffusion in polymers is exceedingly complicated; it is presently not possible to predict permeabilities from known properties of the polymer and of the diffusing substance. Model for diffusion, in a microcrystalline polymer, such as polyethylene, is based on the "Two phase" concept. Partly crystalline polymer polyethylene consists of a large number of tiny crystalline regions dispersed in an amorphous matrix.

The crystalline component of the polymer functions as an impermeable, dispersed phase, thereby constraining the gas

molecules to move through irregular, constricted amorphous polymer conduits in the intercrystalline regions. The impedance offered to diffusional transport depends on the geometry of the impermeable phase and its volume concentration.

The crystallites may reduce polymer chain segment mobility in the interstitial amorphous phase, thereby raising the energy barrier for diffusional transport of gas molecules. Degree of segment immobilization will become greater as the volume fraction of crystalline phase increases and/or average crystallite size decreases (Michaels and Parker, 1959). This effect will manifest itself in an increased activation energy for diffusion and will be more pronounced for larger gas molecules.

Michaels and Parker (1959) considered the polymer polyethylene as a "Porous medium", the particles of which are the crystallites and "Pores" of which comprise the amorphous phase.

Completely analogous expression, for gas permeation in a partly crystalline polymer such as polyethylene, can be taken from the classical physics of flow through porous solids.

Steady state gas permeation is given by Fick's first law of diffusion (Fair et al. 1981)

$$(1/A)\ (dW/dt) = D_p\ (dc/dx) \qquad \ldots.(5.1)$$

Where $(1/A)\ (dW/dt)$ = Amount of gas diffusing per unit area.

D_p = Diffusivity of the polymer.

(dc/dx) = Concentration gradient of the diffusing gas along the direction of flow x.

Where $P = D_pS = (\alpha D_p'S'/\tau x \beta)$ .....(5.2)

P, D_p and S are the permeability, diffusivity and solubility of the gas in the semicrystalline polymer.

S' is the solubility of the gas in a hypothetical completely amorphous polyethylene.

D_p' is the diffusion constant in the homogenous amorphous phase in the absence of any restrictive influence imparted by the crystallites.

$(1/\tau)$ represents the fractional reduction in diffusivity due to the neccessity of diffusing molecules to bypass crystallites and move through irregularly shaped, elongated channels of non-uniform cross sectional area of the amorphous material.

$(1/\beta)$ represents the fractional reduction in diffusivity attributable to reduction in amorphous chain segment mobility due to the proximity of crystallites. Cross linking action of crystallites partly restricts the co-operative movement of chain segments thereby decreasing diffusivity.

τ and β increase with decreasing α,[Fractional Crystallinity].

5.6 MASS TRANSFER MODELS FOR RESPIROMETER AND RESPIROMETRIC BOD BOTTLE

5.6.1 Steps In The Diffusion Process

Diffusion of oxygen from the atmospheric air to the liquid in the respirometer and the respirometric BOD bottle involves a number of elementry steps as given below:

Diffusion of oxygen from the bulk gas phase to the gas membrane interface.

Sorption of oxygen at the surface of the membrane from the interface.

Permeation of oxygen in the membrane.

Desorption of oxygen at the other end of the membrane and its sorption at the membrane liquid interface.

Diffusion of oxygen from the interface to the bulk liquid phase by molecular diffusion and/or by mixing.

5.6.2 Dissolved Oxygen Saturation Concentration

Limiting concentration of D.O. in the liquid phase (distilled water) in the respirometer and respirometric BOD bottle was 7.75 mg/l at 20°C and 745 m.m. of Hg pressure at Patiala. Limiting concentration of D.O. was the same (7.75 mg/l) when 60 and 100 gauge thick polyethylene membranes were used in both the respirometer and respirometric BOD bottle.

5.6.3 Mass Transfer Model For Respirometer

Oxygen transfer in the respirometer has been modelled on the "Stagnant-film" model. Due to mixing in the bulk liquid phase, resistance to mass transfer was confined to the membrane and a thin stagnant liquid film adjacent to the membrane. Following assumptions were made:

Equilibrium at the gas membrane interface was established instantaneously and the equilibrium D.O. concentration (in terms of D.O. concentration in water) was the limiting D.O. concentration (C_s=7.75 mg/l) at 20°C and 745 m.m. of Hg. Fig. 5.1.

No hold up of D.O. in the thin stagnant liquid film and hence diffusion proceeded under steady state conditions.

Laminar flow near the membrane liquid interface and hence the thickness of liquid film remained constant.

Rate of mass (oxygen) transfer in the respirometer, according to stagnant film model is given by equation (3.1)

$$(dc/dt) = K_L^{\circ} a (C_s - c) \quad \ldots.(3.1)$$

Where

C_s = Limiting D.O. conentration in the respirometer at test temperature and pressure.

Putting $C_s - c = D$ and $K_L^{\circ} a = r$

Equation (3.1) is transformed to

$$(dD/dt) = - r D \quad \ldots.(5.3)$$

Where

D is referred to as the saturation deficit or oxygen deficit, and

r is termed as the respirometer constant and is the overall mass transfer co-efficient.

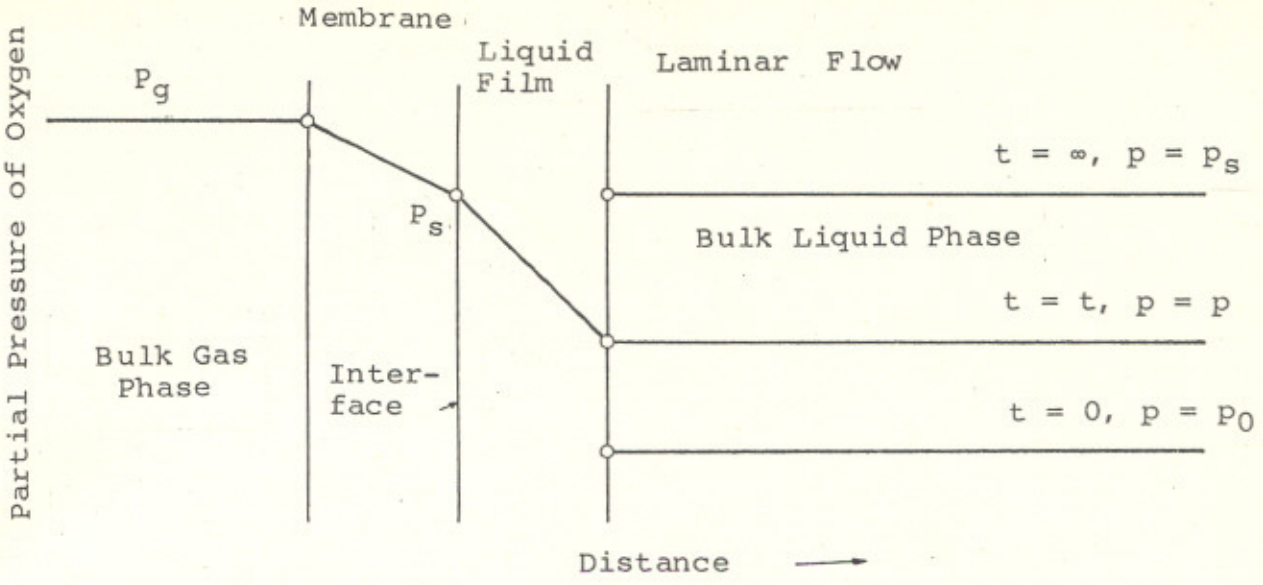
The respirometer constant, r, specifics the rate of oxygen transfer in the respirometer under specified conditions of temperature, pressure, polyethylene membrane thickness and rate of stirring.

Integrating (5.3) between the limits,

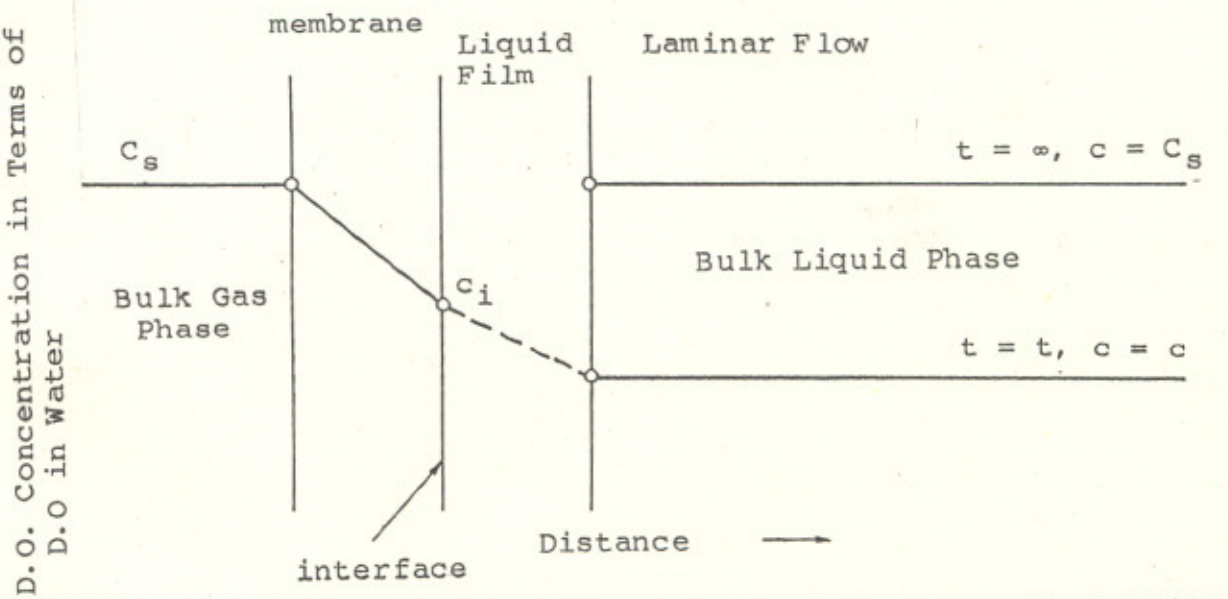
$t = 0$, $D = D_o$ and $t = t$, $D = D$

$$D = D_o e^{-rt} \quad \ldots.(5.4)$$

Pressure and concentration gradients are shown in Fig. 5.1.



Pressure Gradient in Membrane and Liquid Film



Concentration Gradient in Membrane and Liquid Film

Fig. 5.1 Concentration and Pressure Gradients in the Respirometer

5.6.4 Mass Transfer Model For Respirometric BOD Bottle

The liquid in the respirometric BOD bottle (denoted as R.BOD bottle) is not stirred. Concentration gradients exist in the bulk liquid phase. Hence membrane surrounding the liquid as well as liquid phase offer resitance to mass transfer. Diffusion of oxygen in the R.BOD bottle is a transient process and the membrane liquid interface (herein after called interface) saturation deficit changes with time.

Mass transfer from the membrane liquid interface to the bulk liquid phase has been modelled on Higbie Penetration Model. Higbie (1935), as ref. by Sherwood et al. (1975), proposed that the principal mechanism of mass tranfer across an interface involved the motion of turbulent eddies from the core of the fluid to the interface, followed by a short interval of unsteady state molecular diffusion from the interface into the fluid before it was displaced by subsequent eddies.

5.6.4.1 Rate of Mass Transfer

5.6.4.1.1 Assumptions

R.BOD bottles have finite dimensions and as such cannot be compared with infinite or semi-infinite solids. Since the "depth of penetration" is too less in comparison to the dimensions of the R.BOD bottles, these have been assumed to be semi-infinite.

Membrane liquid interface has been assumed to be plane for the surface elements of the liquid. This assumption is true in any practical case since curvature radius of the surface is much larger than the "depth of penetration" that is, much larger than 10^{-3} cms (Astarita, 1967).

At the gas membrane interface, equilibrium is established instantaneously and the limiting D.O. concentration (in terms of D.O. concentration in water) is C_s (7.75 mg/l at 20°C and 745 m.m. of Hg.)

Pressure gradients in membrane and liquid phase are shown in Fig. 5.2. Dissolved oxygen concentrations in the membrane are not known and difficult to be determined. Concentration gradients in terms of D.O. concentration in water are shown in Fig. 5.2.

Mass transfer during transient diffusion, from a region of constant composition into a stagnant fluid of infinite depth, is given by (Sherwood, Pigford and Wilke, 1975) the equation,

$$\frac{(C_s - c)}{(C_s - c_o)} = \text{erf}\frac{x}{2\sqrt{(D_L t)}} + \exp[(K_m . x/D_L) + (K_m{}^2 . t/D_L)]\,\text{erfc}[x/2\sqrt{(D_L t)} + K_m \sqrt{(t/D_L)}] \quad \ldots.(5.5)$$

with the initial and boundary conditions

$$\begin{aligned} c &= c_o && \text{when } t=0 \text{ and } x \geq 0 \\ c &= C_s && \text{at the gas membrane interface} \\ c &= c && \text{when } t=t \text{ and } x \geq 0 \end{aligned} \quad \ldots.(5.6)$$

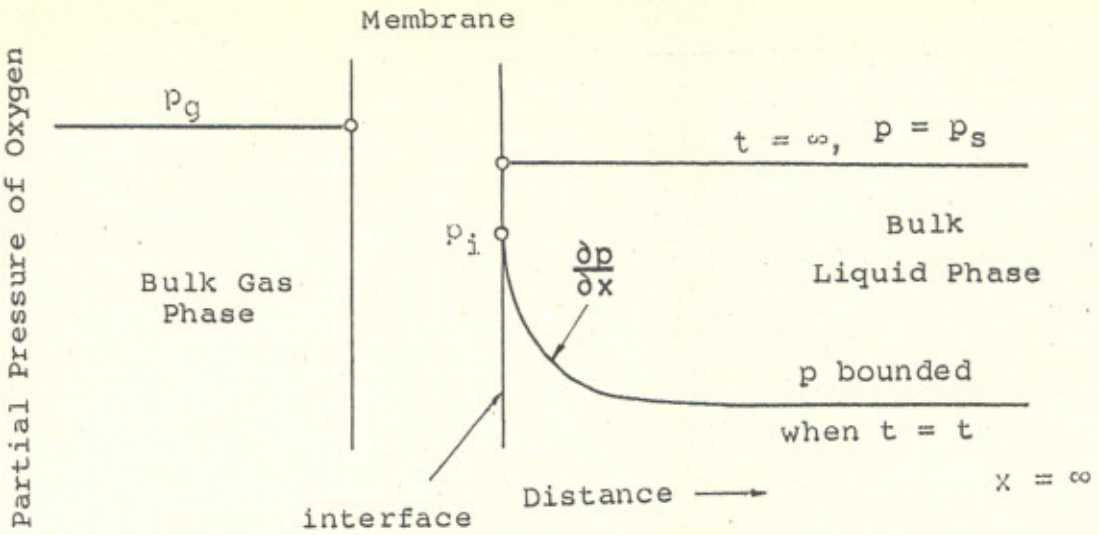
c is bounded when t=t and $x \to \infty$, and

K_m is the oxygen transfer rate of membrane.

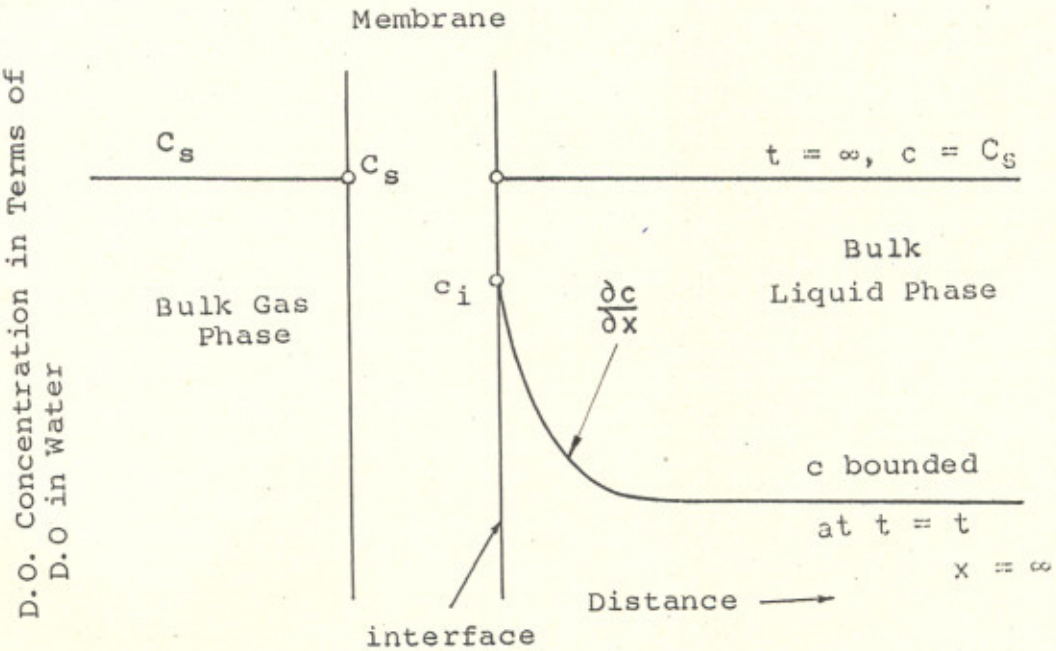
x = "depth of penetration" in the stagnant liquid medium.

D_L = Diffusivity of oxygen in the liquid phase.

For large value of K_m (Table 6.7) and time of exposure, effect of second term in equation (5.5) is negligible,



Pressure Gradient in Membrane and Liquid Phase



Concentration Gradient in Membrane and Liquid Phase

Fig. 5.2 Concentration and Pressure Gradients in Respirometric BOD Bottle

Equation (5.5) may be written as

$$\frac{(C_s - c)}{(C_s - c_o)} = \text{erf}\ \frac{x}{2\sqrt{(D_L t)}} = \text{erf}\ (X) \qquad \ldots.(5.7)$$

Where erf(X) is the error function of X and

$$X = \frac{x}{2\sqrt{(D_L t)}}$$

Mass (oxygen) transfer in the respirometric BOD bottle is given by equation (5.5)

or $(D/D_o) = \text{erf}(X)$ ....(5.8)

Where $D = C_s - c$ and $D_o = C_s - c_o$

Differentiating (5.8) w.r.t. t, rate of mass transfer at any value of x is given by

$$(dD/dt) = -[D_o/\sqrt{(\pi t^2)}]X\ e^{-X^2} \qquad \ldots.(5.9)$$

Where $X = \frac{x}{2\sqrt{(D_L t)}}$

5.6.4.2 Analogy with Heat Conduction

Equation (5.8) is of little value, since interface saturation deficit D_i is not known and cannot be determined experimentally. Only the average saturation deficit is known at various time intervals.

In order to estimate the mass transfer rate(s) from the experimental data, an analogy was drawn between the conducton of heat through infinite bodies with surface and internal resistance and the mass transfer in the R.BOD bottle. Following analogous conclusions were drawn (Schneider, 1955).

5.6.4.2.0 Analogous conclusions

5.6.4.2.1 The average saturation deficit (D_{AV}) lags behind the interface saturation deficit (D_i) by the time interval (t_L) termed as transfer lag.

The transfer lag t_L is given by

$$t_L = (V/K'_m.A) \quad \ldots.(5.10)$$

Where

V is the volumetric capacity of the R.BOD bottle and A the membrane area.

K'_m is the oxygen transfer rate of the membrane per unit surface area.

5.6.4.2.2 Interface saturation deficit (D_i) and average saturation deficit (D_{AV}) act like the saturation deficit when the contents of the bottle are stirred i.e. analogous to Newtonian Heating.

5.6.4.3 Effect of the Finite Thickness of the Bulk Liquid Phase

Higbie Penetration model of mass transfer in the R.BOD bottle applies only as long as the medium is semi-infinite. The geometry of the respirometric BOD bottle is such that the thickness of the bulk liquid phase decreases from the centre towards the edges. When the concentration, at central plane, perpendicular to the direction of diffusion, becomes appreciable, this solution must be replaced by one in which the finiteness of the bulk liquid phase is recognised. Under such a condition, entire transfer resistance is considered to lie in a laminar surface layer of thickness x_L where D.O. concentration(c) is uniform for all x greater than x_L.

Thus according to the Film Penetration Theory, (Skelland, 1974) surface renewal occurs by eddies which penetrate the surface from the bulk of the liquid phase. Thus transfer through young elements of surface follows the penetration theory, transfer through old elements follows the film model and transfer through elements of intermediate age combines both mechanisms. The key difference from the penetration theory lies in the last boundary condition of (5.6).

5.6.4.4 Different Phases in the Mass Transfer Model for Respirometric BOD Bottle

In the R.BOD bottle, the membrane surrounding the liquid as well as the bulk liquid phase offer resistance to mass transfer and the interface saturation deficit changes with time. The mass transfer is divided into the following phases.

Phase I

When water, having uniform D.O. concentration is added in the R.BOD bottle, instability exists at the membrane liquid interface. Movement of turbulent eddies from the core of the fluid to the interface is rapid and eddy contact time is very small. Concentration gradients are being established and the rate of mass transfer is controlled by the resistance of the membrane.

Phase II

After a time interval equal to the transfer lag(t_L) of the R.BOD bottle, bulk liquid phase as well as the membrane control the rate of mass transfer. Eddy contact time increases. Average saturation

deficit (D_{AV}) lags behind the interface saturation deficit (D_i) by the time interval equal to transfer lag (t_L).

Phase III

When the D.O. concentration at the central plane perpendicular to the direction of diffusion becomes appreciable, transfer resistance in the bulk liquid phase lies in a laminar surface layer of certain thickness where beyond this thickness D.O. concentration is uniform in the bulk liquid phase. Rate of mass transfer is controlled by the Film Pentration Model. The average saturation deficit (D_{AV}) lags behind the interface saturation deficit (D_i) by the time interval equal to transfer lag (t_L) of the R.BOD bottle.

5.6.4.5 Calculation of Mass Transfer Co-efficients

If the average saturation deficit is D_{AV} at particular t=t, then the interface saturation deficit D_i at that particular value of t=t will be equal to the average saturation deficit when $t=t+t_L$, where t_L is the transfer lag (analogous conclusion 5.6.4.2.1).

Also from analogous conclusion 5.6.4.2.2,

$$D_{AV} = D_o \exp(-r_b' t) \qquad \ldots.(5.11)$$

When t=t and r_b' respirometer constant of the respirometric BOD bottle as if the contents of the bottle are stirred.

Also interface saturation deficit D_i when t=t is,

$D_i = D_{AV}$ when $t = t + t_L$

i.e. $$D_i = D_o \exp -r_b'(t+t_L) \qquad \ldots.(5.12)$$

Differentiating (5.12) w.r.t. t, we get

$$(dD_{AV}/dt) = -r_b' D_o \exp(-r_b' t) \quad \ldots.(5.13)$$

Dividing eq. (5.13) by eq. (5.11).

$$(dD_{AV}/dt)/D_{AV} = -r_b' \quad \ldots.(5.14)$$

Differentiating equation (5.12) w.r.t. t

$$(dD_i/dt) = -r_b' D_o \exp -r_b'(t+t_L) \quad \ldots.(5.15)$$

Dividing equation (5.15) by equation (5.11)

$$(dD_i/dt)/D_{AV} = -r_b' \exp(-r_b' t_L) = r_b \quad \ldots.(5.16)$$

Where r_b is the respirometer constant of respirometric BOD bottle when the contents of the bottle are not stirred.

5.7 Mass Transfer And Simultaneous BOD Exertion

Consumption of oxygen due to biochemical activity is assumed to be homogenous and independent of the concentration of D.O. Mass transfer co-efficients are not affected by the simultaneous exertion of BOD in the respirometer and R.BOD bottle.

CHAPTER VI

RESULTS AND DISCUSSION

6.1 INTRODUCTION

Considerable research in the past was directed to improve upon or to replace the first order decreasing rate model for BOD and the BOD test itself. These efforts did not prove fruitful except the understanding of the biological events taking place during the course of BOD exertion.

In the present investigations, a system of improvised respirometer and respirometric BOD bottles was visualized and fabricated as outlined in chapter IV. The respirometer and R.BOD bottle(s) were to be standardized to determine the rate of mass (oxygen) transfer before these could be utilized for the study of BOD exertion of samples.

Results of mass transfer co-efficients, (respirometer constants), BOD exertion in the respirometer and R.BOD bottle are presented in this chapter.

The results presented herein have provided explanation for some of the problems, but more areas for further research are thrown open in the field.

6.2 MASS TRANSFER CO-EFFICIENTS (RESPIROMETER CONSTANTS)

6.2.1 Respirometer

Following the procedure outlined in sec. 4.4.1, the respirometer was filled with oxygen free distilled water and a continuous record of D.O. was obtained through the D.O. probe connected to a

potentiometric chart recorder (Fig. 6.1). The experiment was terminated when D.O. concentration of water in the respirometer was maintained constant for 12 hours. Continuous record of D.O., for 60 and 100 gauge LDPF membranes, was obtained on the same chart (Fig. 6.1) at 20°C.

The record was also obtained for 100 gauge thick membrane at 15°C (Fig. 6.2), 25°C (Fig. 6.3) and 30°C (Fig. 6.4).

The experimental data was fitted to equation (5.4) as per film model (Sec. 5.6.3).

$$D/D_o = e^{-rt} \qquad \ldots.(5.4)$$

Where r = respirometer constant

D = Saturation Deficit (C_s-c)

and C_s = Saturation dissolved oxygen concentration in the respirometer at test temperature and pressure mg/l.

c = D.O. concentration in the respirometer at any time t mg/l

D = D_o when t = O

Figures 6.1 and 6.5 show the results of experiments accomplished under essentially identical conditions. The curves represent :

(a) continuous recorder plots of dissolved oxygen (reaeration curves) in the respirometer.

(b) regression lines (film model) for 60 and 100 gauge LDPF at 20°C (Fig. 6.1) and 100 gauge LDPF at 15°C, 20°C, 25°C and 30°C (Fig. 6.5).

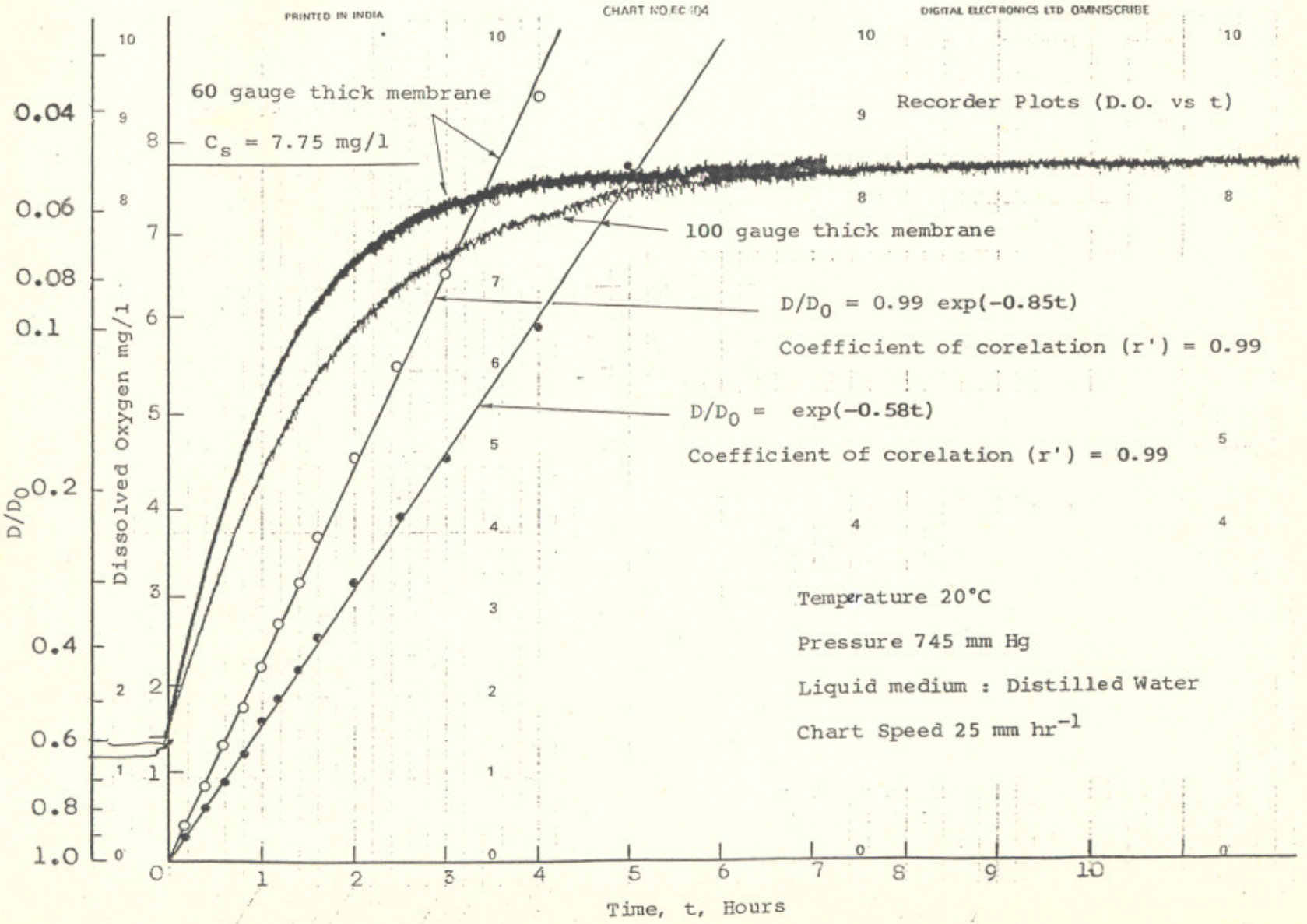


Fig. 6.1 Continuous Recorder Plots of Dissolved Oxygen and Regression Lines for Respirometer

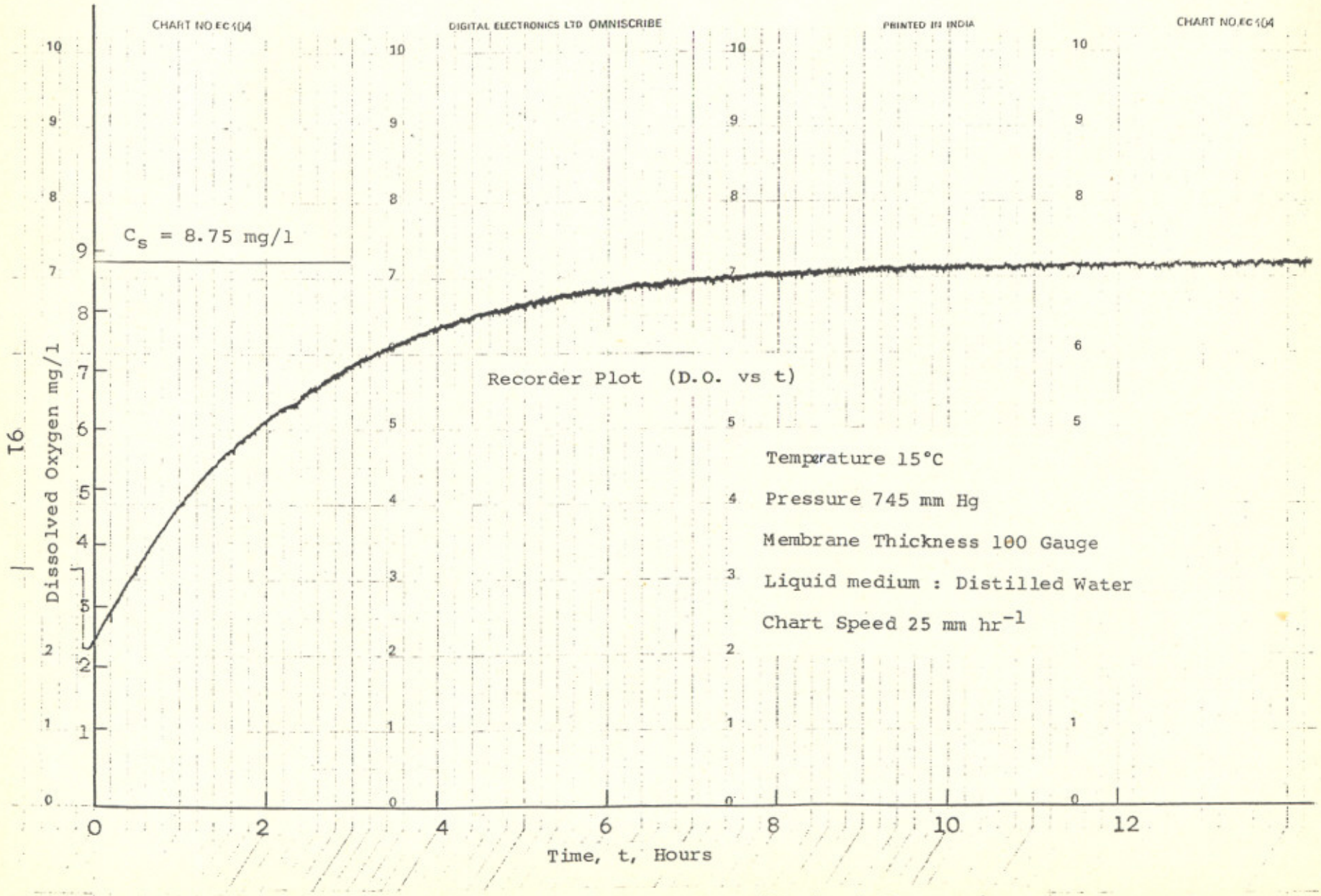


Fig. 6.2 Continuous Recorder Plot of Dissolved Oxygen in Respirometer

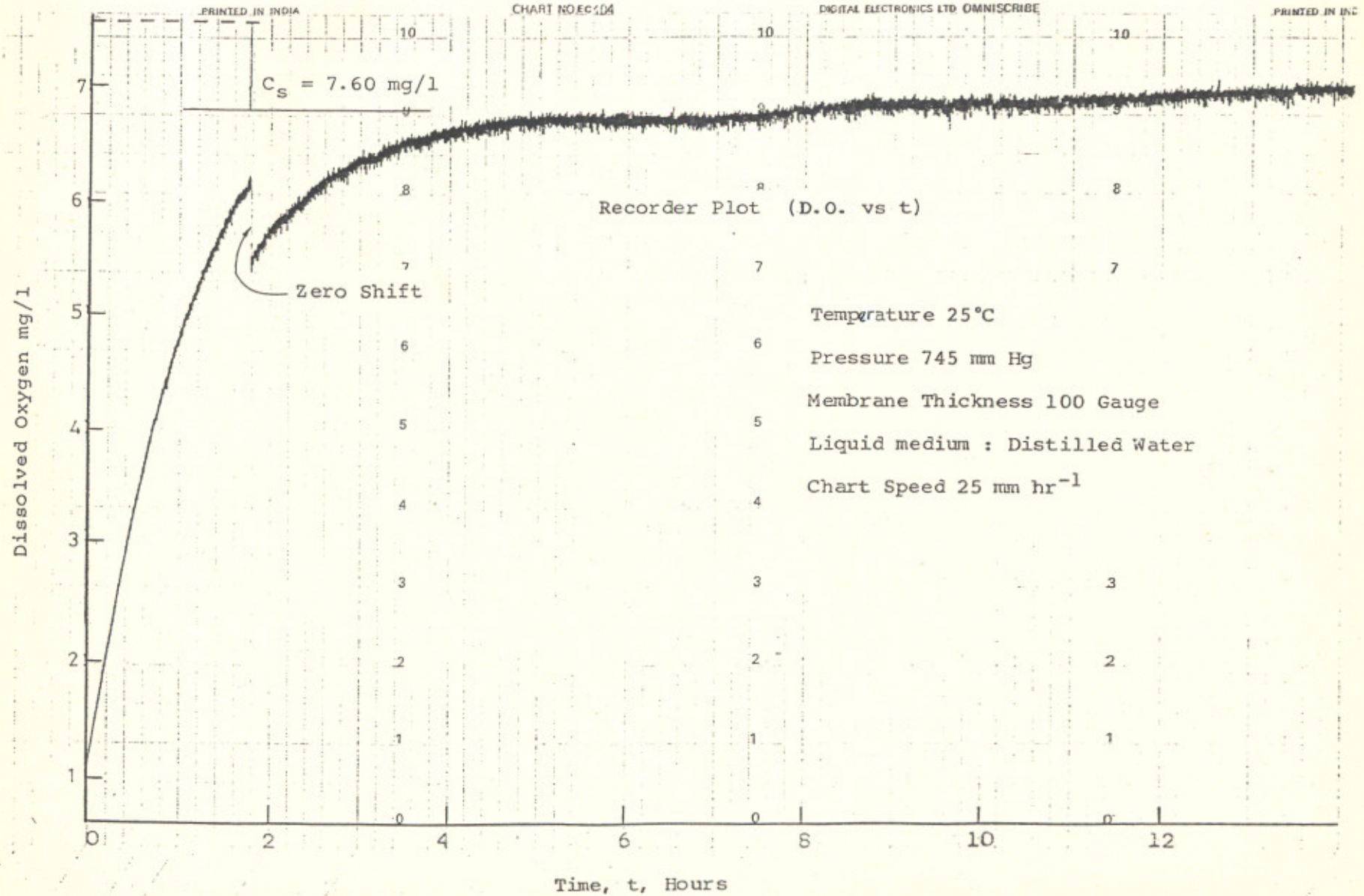


Fig. 6.3 Continuous Recorder Plot of Dissolved Oxygen in Respirometer

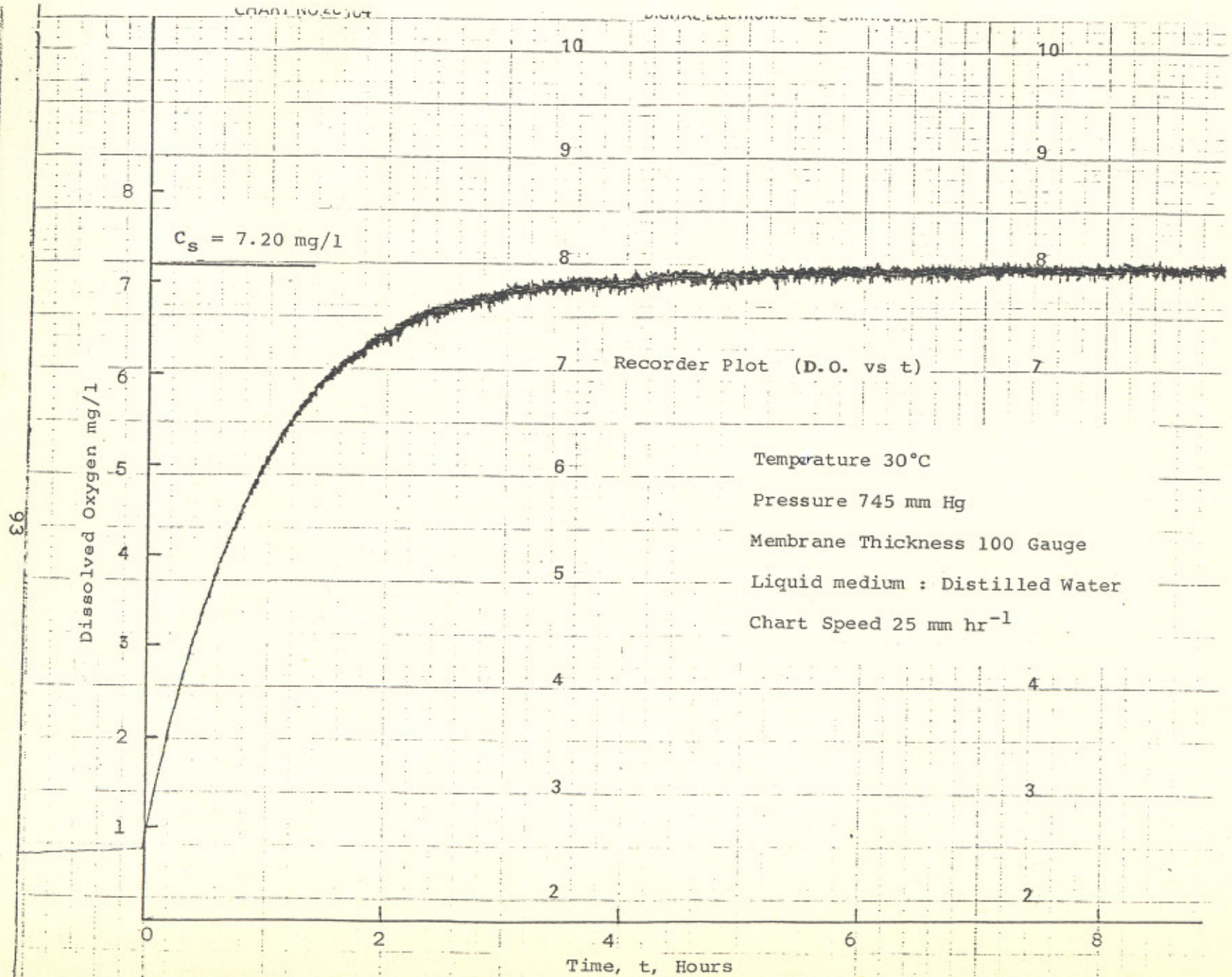


Fig. 6.4Continuous Recorder Plot of Dissolved Oxygen in Respirometer

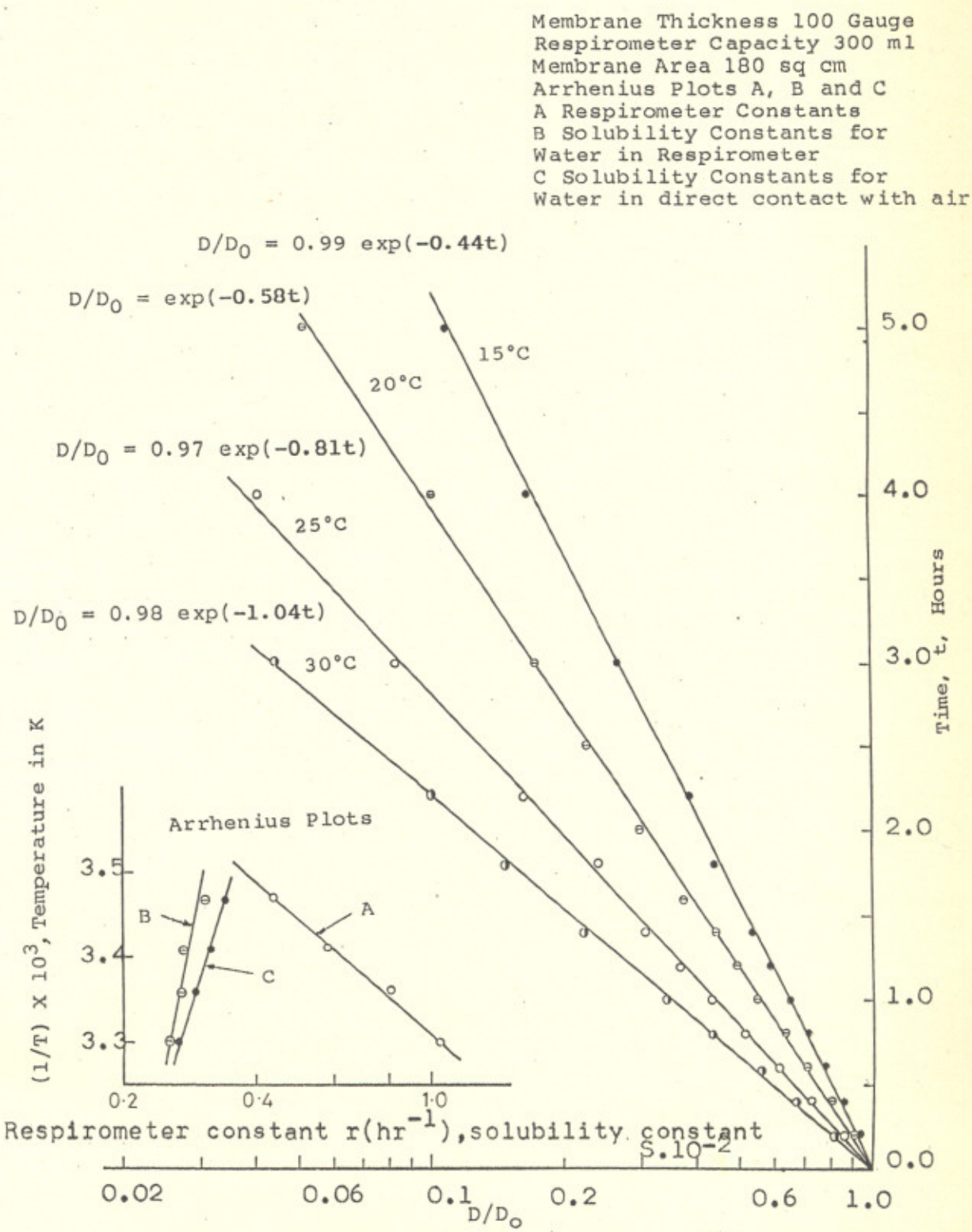


Fig. 6.5 Temperature Dependance of Respirometer Constants and Solubility Constants.

(c) arrhenius plot (A) for respirometer constant-permeation of oxygen from atmosphere to water; plot (B) solubility constant of D.O.-permeation through membrane and plot (C) solubility constant-permeation from atmosphere to water (Fig. 6.5). The results are summarised in Table 6.1.

TABLE 6.1

Results of Mass Transfer in Respirometer (Film Model)

Temperature	Membrane Thickness	Saturation D.O. Conc.	Respirometer Constant, r	Co-efficient of correlation (r')
°C		mg/l	hr^{-1}	
15	100 Gauge	8.75	0.44	0.99
20	60 Gauge	7.75	0.85	0.99
20	100 Gauge	7.75	0.58	0.99
25	100 Gauge	7.60	0.81	0.99
30	100 Gauge	7.20	1.04	0.99

The asymptotic nature of the D.O. recorder plots (reaeration curves) is clearly indicated in Figs 6.1 to 6.4 from which it can be concluded that the D.O. concentration in the respirometer reaches a saturation value. The D.O saturation values of water in the respirometer (Table 6.1) are less than the corresponding values of D.O saturation concentrations of water in direct contact with atmospheric air at the same temperature and pressure. Further the D.O. saturation values of water in the respirometer at 20°C and 745 mm of Hg pressure is of the same magnitude (as seen by the asymptotic nature of the two D.O. recorder plots merging at the same value Fig. 6.1) for both 60 gauge and 100 gauge LDPF.

The D.O. saturation concentration values of water in the respirometer decrease with rise in temperature (Figs. 6.1 to 6.4 and Table 6.1).

Rate of mass transfer in the respirometer is more with a 60 gauge LDPF than with a 100 gauge membrane as indicated by the slopes of the regression lines (Fig. 6.1).

Rate of mass transfer increaseswith rise in temperature as indicated by the slopes of regressions for 100 gauge LDPF at 15,20,25 and 30°C temperatures (Fig. 6.2, 6.1, 6.3 and 6.4).

6.2.1.1 Dissolved Oxygen Saturation Concentration

The dissolution of oxygen from the atmospheric air to the water inside the respirometer can best be described as the solution and condensation of oxygen from the atmospheric air at the outer surface of the LDPF, followed by diffusion through the membrane in the form of liquid, and the desorption and dissolution at the inner surface of LDPF and water in the respirometer.

The dissolution process takes place at the gas membrane interface and also at the membrane liquid interface. At the gas membrane interface, the frequency of molecular impacts (of the oxygen molecules in the gas phase) on the membrane face is high compared with the rate of diffusion of oxygen into LDPF. Thus equilibrium may be assumed to exist at every instant between the oxygen in the gas phase and the oxygen sorbed by the polymer at the gas membrane interface which has been assumed to be saturated with oxygen.

At the polymer liquid interface, the dissolution of oxygen may be imagined as taking place by (a) making a hole of molecular size in the polymer (b) making a hole of the molecular size in the liquid (c) transferring a molecule of oxygen from the hole in the polymer to a hole in the liquid. The success of the last step depends on the simultaneous opening of a hole, of the molecular size, in the polymer as well as in the liquid.

D.O. saturation values in the respirometer, at particular temperature and pressure thus depend on the frequency of simultaneous hole openings, of molecular size, in the polymer as well as in the liquid at the polymer liquid interface. Frequency of simultaneous hole openings at the polymer liquid interface is less than the frequency of molecular impacts, of the oxygen molecules at the gas liquid interface, when the liquid is in direct contact with atmospheric air. Thus the observed D.O. saturation values in the respirometer are less than the corresponding D.O. saturation values of water in direct contact with atmospheric air at the same temperature and pressure.

Since the D.O. saturation value of water in the respirometer depends on the thermodynamic properties of the polymer and water, the observed D.O. saturation values of water in the respirometer (Table 6.1) at 20°C and 745 mm of Hg pressure are of the same magnitude for 60 gauge and 100 gauge LDPF.

6.2.1.2 Temperature Dependence of Solubility Constants

The solubility of condensable gases decreases by increasing temperature due to reduced ease of condensation (Table 6.1).

Mears (1965) has described the solubility of a gas in a polymer as taking place in two stages :

(i) making a hole of molecular size in the polymer, an endothermic process.

(ii) transferring a molecule from the gas phase into the hole, an exothermic process.

The energy absorbed in the first stage depends upon the molecular volume of the gas and the cohesive energy density of the polymer. The energy evolved in the second stage depends on the Van der Waals bonds formed between the gas molecules and the surrounding polymer and for larger gas molecules heat of solution ΔH is negative.

Slope of Arrhenius plot B (Fig. 6.5) (Heat of solution ΔH=-2.0 k cal/g mol) is less than the slope of Arrhenius plot C (Heat of solution ΔH=-4.0 k cal/g mol). This sounds reasonable because when permeation of oxygen takes place from the atmospheric air through the membrane to water in the respirometer, the energy absorbed in making a hole of molecular size in the polymer is more than when permeation of oxygen takes place directly from the atmospheric air to water and the energy absorbed in making a hole of molecular size in water.

6.2.1.3 Temperature Dependence of Respirometer Constants

The respirometer constants increase with increase in temperature because this causes an increase in the liquid nature of the polymer leading to greater rate of mass transfer.

Apparent energy of activation for permeability of oxygen in the respirometer through polyethylene membrane (density 0.916) as worked out from Arrhenius plot for respirometer constants (curve A Fig. 6.5) is 10.3 k cal/g mol.

Michaels and Bixler (1961) have reported value of apparent energy of activation for oxygen permeability through Alathon 14 (a branched polyethylene density 0.9135) as 10.2 k cal/g mol. However the experimental conditions of Michaels and Bixler (1961) are different than those of the present investigations. Apparent energy of activation for oxygen permeability is in close agreement.

6.2.2 Respirometric BOD Bottle

Following the procedure outlined in sec. 4.4.2, 40 respirometric BOD bottles (denoted as R.BOD bottles) were filled with oxygen free distilled water. The D.O. was analysed after suitable time intervals (section 4.3) in replicate samples.

The procedure of analysis was continued for replicate samples till the water in the R.BOD bottles was saturated with D.O.

This experiment was carried out with all the four types of R.BOD bottles illustrated in Figs. 4.4 to 4.7 i.e.

(i) Rectangular, single compartment (Fig.4.5). Respirometer constant denoted as r_1.

(ii) Rectangular, two compartments (Fig. 4.6). Respirometer constant denoted as r_2.

(iii) Rectangular, three compartments (Fig. 4.7). Respirometer constant denoted as r_3.

(iv) Circular, single compartment (Fig. 4.4). Respirometer constant denoted as r_c.

6.2.2.1 Estimation of Mass Transfer Co-efficients of R.BOD Bottles

Different phases in the mass transfer model for R.BOD bottle were elaborated in Sec. 5.6.4.4. It was mentioned that during phase II and phase III the average saturation deficit (D_{AV}) lags behind the interface saturation deficit (D_i), in the R.BOD bottle, by the time interval (t_L) termed as transfer lag.

The transfer lag (t_L) for the four types of R.BOD bottles were determined graphically by the intersection of the D/D_o vs time curves (Fig.6.6) and recourse to equation (5.10). The values are presented in Table 6.2.

TABLE 6.2

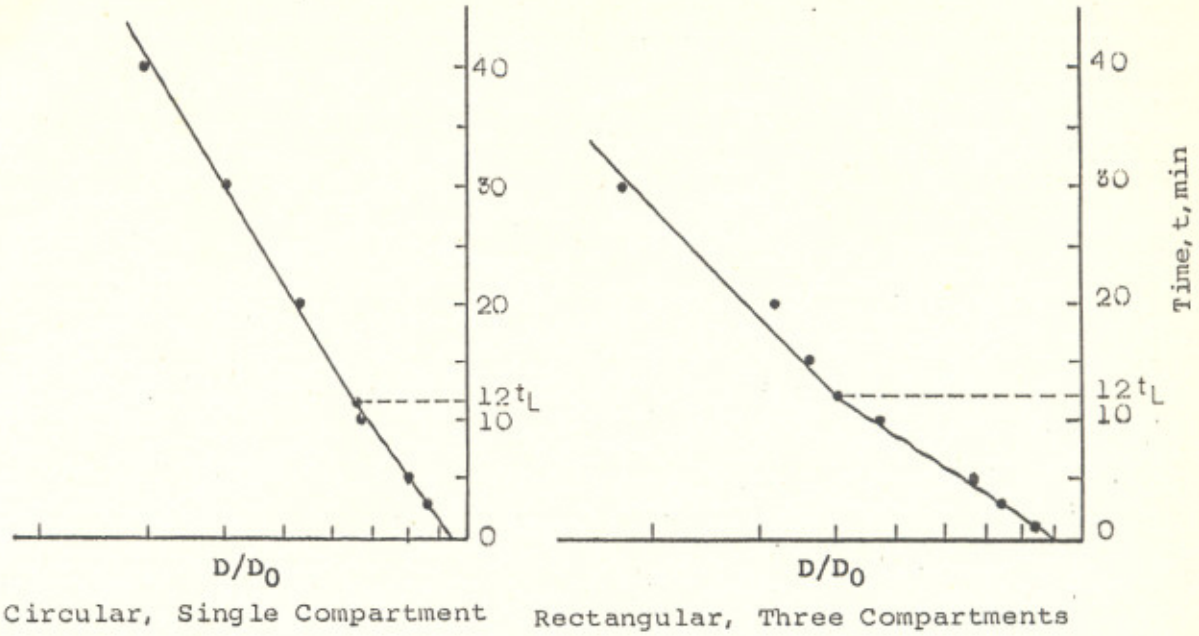
Transfer Lag (t_L)

Type of R.BOD Bottle	Graphically Estimated (Fig.6.6) t_L, min.	Theoretically Calculated Eq.(5.10) t_L, min.
1. Rectangular, Single Compartment	45.0	42.20
2. Rectangular, Two Compartments	24.0	22.95
3. Rectangular, Three Compartments	12.0	12.80
4. Circular, Single Compartment	12.0	13.49

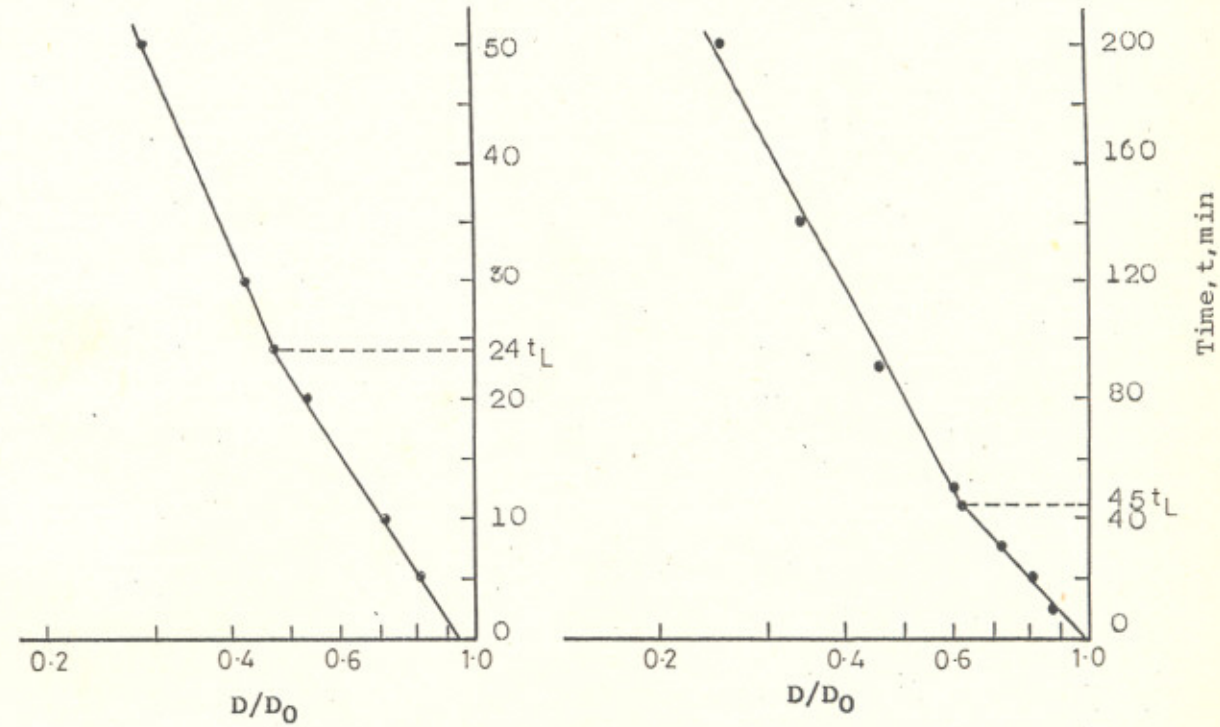
Theoretically calculated transfer lag is in agreement with graphically estimated t_L.

6.2.2.2 Calculations for Mass Transfer Co-efficients (Tables 6.3 to 6.7)

Results of the experiments (Sec. 4.4.2) are presented in Tables 6.3 to 6.7 for the four types of R.BOD bottles. Mass transfer



Circular, Single Compartment Rectangular, Three Compartments



Rectangular, Two Compartments Rectangular, Single Compartment

Fig 6.6 Transfer Lag for Respirometric BOD Bottles

TABLE 6.3

Estimation of Mass Transfer Co-efficients (r_1) for Rectangular Respirometric BOD Bottle, Single Compartment, Capacity 125 ml.

	(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	
S.No.	Time t (min)	D.O. Conc. mg/l B_1	B_2	B_3	Mean D.O. C	Saturation Deficit D=7.75-C	D/D_o = erf(X)	X	dD_{AV}/dt	$[dD_{AV}/dt]'$	r_1'	$e^{-r_1' t_L}$	r_1	Remarks
1.	0	0.72	0.80	0.88	0.80	6.95	1.000	-	-	3.46	0.50	0.69	0.34	Phase I
2.	10	1.77	1.63	1.63	1.68	6.07	0.873	1.08	7.930	3.03	0.50	0.69	0.34	Transfer Lag
3.	20	2.18	2.02	2.13	2.11	5.64	0.812	0.93	4.610	2.65	0.47	0.70	0.33	(t_L) 45 min.
4.	30	2.62	2.57	3.03	2.74	5.01	0.721	0.77	3.340	2.310	0.46	0.71	0.33	
5.	50	3.47	3.68	3.57	3.57	4.18	0.601	0.60	1.970	1.770	0.42	0.73	0.31	Phase II
6.	90	4.55	4.65	4.55	4.58	3.17	0.456	0.43	0.940	1.040	0.33	0.78	0.26	Penetration
7.	140	5.25	5.37	5.50	5.37	2.38	0.342	0.32	0.486	0.530	0.22	0.85	0.19	Model
8.	200	5.93	6.12	5.82	5.96	1.79	0.258	0.23	0.256	0.236	0.13	0.91	0.12	
		Empirical Equation for S.No. 5,6,7 and 8, $dD_{AV}/dt = 3.46 \exp(-0.013t)$, t min.												
9.	260	6.30	6.35	6.48	6.38	1.37	0.197	-	-	-	-	-	0.12	Transition
10.	320	6.93	6.53	7.08	6.84	0.91	0.131	-	-	-	0.43	0.73	0.31	Phase III
11.	410	6.77	7.47	7.58	7.27	0.48	0.069	-	-	-	0.43	0.73	0.31	Film Penetration Model

Film Model for S.No. 10 and 11, $D/Do = 1.28 \exp(-0.43t)$, t hours

TABLE 6.4

Estimation of Mass Transfer Co-efficients (r_2) for Rectangular Respirometric BOD Bottle, Two Compartment, Capacity 68 ml.

(1)	(2)			(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	
S.No.	Time t (min)	D.O. Conc. mg/l B_1	B_2	B_3	Mean D.O. C	Saturation Deficit D=7.75-C	D/D_o = erf(X)	X	dD_{AV}/dt	$[dD_{AV}/dt]'$	r_2'	$e^{-r_2't_L}$	r_2	Remarks
1.	0	0.95	0.90	0.95	0.93	6.82	-	-	-	8.29	1.21	0.62	0.75	Phase I
2.	1	1.30	1.25	1.30	1.28	6.47	0.949	1.38	47.50	7.97	1.23	0.61	0.75	Transfer Lag
3.	3	1.80	1.75	1.75	1.77	5.98	0.877	1.08	25.90	7.38	1.23	0.61	0.75	t_L=24 min.
4.	5	2.20	2.15	2.25	2.20	5.55	0.814	0.94	18.00	6.83	1.23	0.61	0.75	
5.	10	2.80	3.00	2.90	2.90	4.85	0.711	0.75	9.80	5.63	1.16	0.63	0.73	
6.	20	4.00	4.25	4.05	4.10	3.65	0.535	0.52	4.60	3.82	1.05	0.66	0.69	
7.	30	5.00	4.65	4.85	4.83	2.92	0.428	0.40	2.60	2.60	0.89	0.70	0.62	Phase II
8.	50	5.60	5.75	5.95	5.75	2.00	0.291	0.27	1.20	1.20	0.60	0.79	0.47	Penetration Model.
					Empirical Equation for S.No. 7 and 8, $dD_{AV}/dt = 8.29 \exp(-0.039t)$, t min.									
9.	80	6.90	6.75	7.05	6.90	0.85	0.125	0.11	-	-	0.82	0.72	0.59	Phase III
10.	110	7.20	7.15	7.30	7.20	0.55	0.081	0.075	-	-	0.82	0.72	0.59	Film Penetration Model
11.	150	7.20	7.55	7.55	7.42	0.33	0.048	0.040	-	-	0.82	0.72	0.59	

Film Model for S.No. 9, 10 and 11, $D/Do = 0.37 \exp(-0.82t)$, t hours

TABLE 6.5

Estimation of Mass Transfer Co-efficients (r_3) for Rectangular Respirometric BOD Bottle, Three Compartment, Capacity 38 ml.

S.No.	(1) Time t (min)	(2) D.O. Conc. mg/l B_1	B_2	B_3	(3) Mean D.O. C	(4) Saturation Deficit D=7.75-C	(5) D/D_o = erf(X)	(6) X	(7) dD_{AV}/dt	(8) $[dD_{AV}/dt]'$	(9) r_3'	(10) $e^{-r_3't_L}$	(11) r_3	Remarks
1.	0	0.75	0.70	0.80	0.75	7.00	-	-	-	15.2	2.17	0.65	1.40	Phase I
2.	1	1.90	1.75	2.00	1.88	5.87	0.839	0.99	88.16	14.1	2.39	0.62	1.48	Transfer Lag
3.	3	2.50	2.60	2.60	2.57	5.18	0.740	0.80	33.16	12.0	2.32	0.63	1.46	(t_L) 12 min.
4.	5	2.65	3.15	3.35	3.05	4.70	0.671	0.69	20.34	10.3	2.18	0.65	1.41	
5.	10	4.20	4.40	4.80	4.45	3.32	0.474	0.45	8.72	6.9	2.08	0.66	1.37	
6.	15	5.0	5.60	5.10	5.23	2.52	0.360	0.33	4.68	4.68	1.86	0.69	1.28	Phase II
7.	20	5.35	5.65	5.60	5.53	2.22	0.317	0.29	3.16	3.16	1.42	0.75	1.07	Penetration Model
		Empirical Equation for S.No. 6 and 7, $dD_{AV}/dt = 15.2 \exp(-0.079t)$, t min.												
8.	30	6.45	6.30	6.55	6.43	1.32	0.189	-	-	-	-	-	1.07	Transition
9.	40	7.15	7.25	7.00	7.13	0.62	0.089	-	-	-	2.39	0.62	1.48	Phase III
10.	60	7.50	7.40	7.50	7.47	0.28	0.040	-	-	-	2.39	0.62	1.48	Film Penetration Model

Film Model for S.No. 9 and 10, $D/Do = 0.44 \exp(-2.39t)$, t in hours

TABLE 6.6

Estimation of Mass Transfer Co-efficients (r_c) for Circular Respirometric BOD Bottle, Single Compartment, Capacity 32 ml.

(1)	(2)			(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	
S.No.	Time t (min)	D.O. Conc. mg/l B_1	B_2	B_3	Mean D.O. C	Saturation Deficit D=7.75-C	D/D_o = erf(X)	X	dD_{AV}/dt	$[dD_{AV}/dt]'$	r_c'	$e^{-r_c' t_L}$	r_c	Remarks
1.	0	0.95	1.00	0.95	0.97	6.78	-	-	-	14.1	2.08	0.66	1.37	Phase I
2.	5	2.25	2.30	2.30	2.28	5.47	0.807	0.92	18.14	10.6	1.94	0.68	1.32	Transfer Lag
3.	10	3.05	3.20	3.30	3.18	4.57	0.674	0.70	9.85	7.97	1.75	0.70	1.23	(t_L) 12 min.
4.	20	4.10	4.30	4.10	4.16	3.59	0.529	0.51	4.52	4.52	1.25	0.78	0.97	Phase II
5.	30	4.75	5.20	5.05	5.00	2.75	0.406	0.38	2.52	2.54	0.92	0.83	0.77	Penetration Model.
6.	40	5.40	6.30	5.60	5.77	1.98	0.292	0.27	1.44	1.43	0.72	0.87	0.63	
		Empirical Equation for S.No. 4,5 and 6, $dD_{AV}/dt = 14.1 \exp(-0.057t)$, t min.												
7.	50	6.25	5.80	6.30	6.12	1.63	0.240	-	-	-	-	-	0.63	
8.	70	6.60	6.30	6.40	6.43	1.32	0.195	-	-	-	-	-	0.63	Transition
9.	100	7.50	7.20	7.35	7.35	0.40	0.060	-	-	-	-	-	1.32	Phase III, Film Penetration Model

Not sufficient data for Phase III, respirometer constant taken from phase I.

TABLE 6.7

Respirometer Constants at 20°C

(a) Respirometer, capacity 300 ml.

(i) with 100 gauge thick polyethylene membrane, $r=0.58\ hr^{-1}$

$r_o = K_L a = 0.58\ hr^{-1}$

$K_L = 0.97$ since $a = 0.6$ (sec. 4.2.1.2)

K_L is not accurately known for the conditions of the experiment, assuming $K_L = 2.0$ (Fair et al.,1981)

$K_m = 1.9$ cm/hr (equation 3.2)

(ii) with 60 gauge thick polyethylene membrane, $r=0.85\ hr^{-1}$

(b) Respirometric BOD Bottles :

Type of Respirometric BOD Bottle

	Rectangular Single Compartment	Rectangular Two Compartment	Rectangular Three Compartment	Circular
Area/Volume ratio (a)	1.47	2.7	4.84	3.17
Respirometer Constant	r_1	r_2	r_3	r_c
D.O. Deficit (D)				
6.0 and above	0.34	0.75	1.48	1.36
5.5	0.33	0.75	1.47	1.34
5.0	0.33	0.73	1.44	1.28
4.5	0.32	0.72	1.41	1.24
4.0	0.30	0.71	1.39	1.09
3.6	0.28	0.69	1.38	0.97
3.2	0.26	0.63	1.37	0.88
3.0	0.24	0.60	1.34	0.83
2.6	0.20	0.55	1.28	0.74
2.2	0.16	0.50	1.07	0.67
2.0	0.13	0.48	1.00	0.63
1.5	0.12	0.47	0.98	0.45
1.0 and less	0.31	0.60	1.48	1.36

process was classified into three different phases in sec. 5.6.4.4. Accordingly the experimental data was segregated.

The experimental data for time interval equal to/or less than the transfer lag (t_L) was classified into phase I.

Due to the finite thickness of the bulk liquid phase, when the D.O., at the central plane perpendicular to the direction of diffusion, becomes appreciable, the mode of mass transfer in the R.BOD bottle changes from penetration model to film penetration model. A plot of D/D_o vs time on a semilog scale (Fig. 6.7), for the data of mass transfer in R.BOD bottle, rectangular, single compartment, clearly shows changes in slope of the D/D_o vs t curve at two points marked A and B in Fig. 6.7. The change in slope of the curve at A (Fig. 6.7) is due to a shift in mode of mass transfer from phase I (transfer lag) to phase II (penetration model). Whereas the change in slope of the curve at B (Fig.6.7) is due to the shift in mode of mass transfer from phase II (penetration model) to phase III (film penetration model) but the change is not sharp. This is due to the fact that the resistance to mass transfer is now shifting from the bulk liquid phase to a laminar surface layer of thickness x_L.

The saturation deficit at which the shift from phase II to phase III takes place depends on the depth of bulk liquid phase in the R.BOD bottle and will be different for different R.BOD bottles. However perusal of Fig. 6.7 and Tables 6.3 to 6.7, reveals that phase III is confined to a very narrow range of saturation deficit.

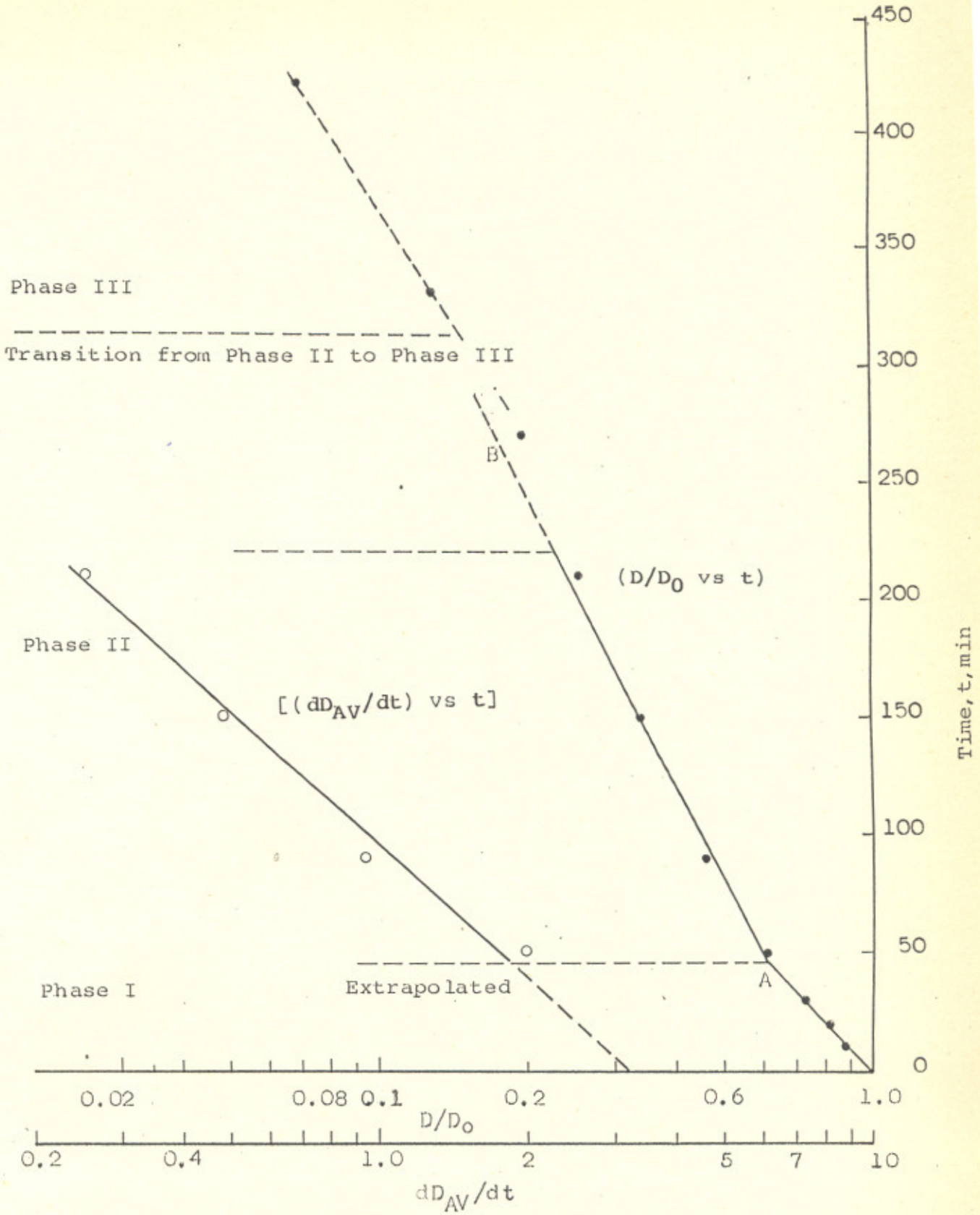


Fig. 6.7 Phases in Mass Transfer in Respirometric BOD Bottle, Rectangular, Single Compartment

From Fig. 6.7, it can be deduced that phase III starts at a saturation deficit of 1.0. In order to account for the gradual change in slope at B, a transition from phase II to phase III is assumed in the saturation deficit range of 1.1 to 1.75.

Since ranges of saturation deficits (1.0 to 0 for phase II to phase III and 1.75 to 1.1 for transition from phase II to phase III) are extremely narrow, the same ranges of saturation deficits for change from phase II to phase III (1.0 to 0) and for transition from phase II to phase III (1.75 to 1.1) have been assumed for all the four types of R.BOD bottles. Obviously slight shift in the above two ranges of saturation deficits will not cause any appreciable error in the calculated results.

The results of the experiments, for mass transfer for various types of R.BOD bottles and calculation for mass transfer coefficients are listed in Tables 6.3 to 6.6.

Col. (1) shows the time (from the start) against which three replicate samples of R.BOD bottles were drawn and analysed for dissolved oxygen.

Col. (2) shows the D.O. concentrations of three raplicate R.BOD bottles (B_1, B_2 and B_3).

Col. (3) shows the mean D.O. concentration of three replicate R.BOD bottles.

Col. (4) shows the saturation deficit D[7.75-Col.(2)]. 7.75 mg/l being the D.O. saturation value of water in the respirometer at 20°C and 745 mm Hg pressure.

Col. (5) shows the ratio of saturation deficit D at any time t to the initial saturation deficit D_o at t=0, and from equation (5.8), $D/D_o = erf(X)$

Col. (6) shows the value of X from error function table against the erf(X) in col.(5).

Col. (7) gives the calculated value of average rate of mass transfer dD_{AV}/dt as per equation (5.9) against value of X from col. 6.

Col. (8) gives the computed value of average rate of mass transfer $[dD_{AV}/dt]'$ when empirical exponential function was fitted (by regression) between dD_{AV}/dt (col 7) vs t (col 1) for the data of phase II. The results of average rate of mass transfer were extrapolated for phase I.

Col. (9) is the ratio of col.(8) and col.(4) and is the respirometer constant r_b' (r_1', r_2', r_3' and r_c' in Tables 6.3 to 6.6) according to equation (5.14).

Col. (10) is exp $(-r_b' t_L)$ and transfer lag (t_L) has been given in Table 6.2.

Col. (11) is the product of col.(9) and col.(10) and according to equation (5.16) is the respirometer constant of the R.BOD bottle when the contents of the bottle are not stirred.

By the above procedure, mass transfer co-efficients were estimated for phase II and (by extrapolation from the empirical equation between dD_{AV}/dt and t) for phase I.

Respirometer constant for the transition from phase II to phase III was taken from phase II as shown in Tables 6.3 to 6.6. Film model was fitted to the data of phase III, the repirometer constant was corrected for transfer lag (t_L) as shown in cols (9), (10) and (11) of Tables 6.3 to 6.6.

Respirometer constants for the respirometer and various types of respirometric BOD bottles are listed in Table 6.7.

Membrane, as well as , liquid phase offer resistance to mass transfer in the R.BOD bottle. Respirometer constants, for each type of R.BOD bottle are constant during phase I and decrease contiuously during phase II and again increase during phase III. When liquid, having uniform concentration, is added in the R.BOD bottle, instability exists at the membrane liquid interface and rate of mass transfer is controlled by the resistance of the membrane, till the concentration gradients are estblished. However during mass transfer and simultaneous BOD exertion, concentration gradients remain established even though D.O. concentration of water is in the range of phase I of the R.BOD bottle. Thus the respirometer constants, for phase I, were estimated by extrapolation from phase II. Eddy contact time and depth of penentration is so small that the mass transfer coeffiecients, during phase I, may be estimated by film model (Sherwood et al., 1975). Eddy contact time goes on increasing during phase II, thereby continuously decreasing the respirometer constants during this phase. In phase III, resistance to mass transfer is confined to a laminar layer of liquid of thickness x_L whereas, in phase II, resistance to mass transfer is offered by the bulk liquid phase.

6.3 BOD EXERTION IN THE RESPIROMETER AND RESPIROMETRIC BOD BOTTLE

6.3.1 BOD Exertion In Respirometer

Known quantities of substrates were dissolved in dilution water seeded with bacteria in accordance with procedures described in Secs. 4.5 and 4.6. The respirometer, using 60 or 100 gauge LDPF (Sec. 3.2.1.2) was filled. Continuous record of D.O. in the respirometer for various substrate concentrations were recorded (Figs. 6.8 - 6.21).

COD and cellular protein were determined on samples taken from a separate respirometer filled with the same wastewater sample and maintained under similar environmental conditions.

The synthetic waste of glucose and glutamic acid in 1:1 ratio was preferred as such a mixture has been reported to have a reasonably stable oxidation rate similar to that obtained in many municipal wastes (k_1=0.16 to 0.19) Standard Methods. Results of experiments are shown in Figs 6.8 to 6.21. The experiments were carried out under indentical conditions. The following curves are shown :

(a) the recorder plots, of D.O. sag in the respirometer, are shown in Figs 6.8 to 6.18, Figs 6.19(a), 6.20(a) and 6.21(a).

(b) the BOD exerted, for the synthetic wastewater, computed from the recorder plot data and the respirometer constant as determined in Sec. 6.2.1. The BOD calculations were made by integration of the basic differential equation of the sag curve as used by Issacs and Gaudy (1967) for their experiments and illustrated in Appendix I.

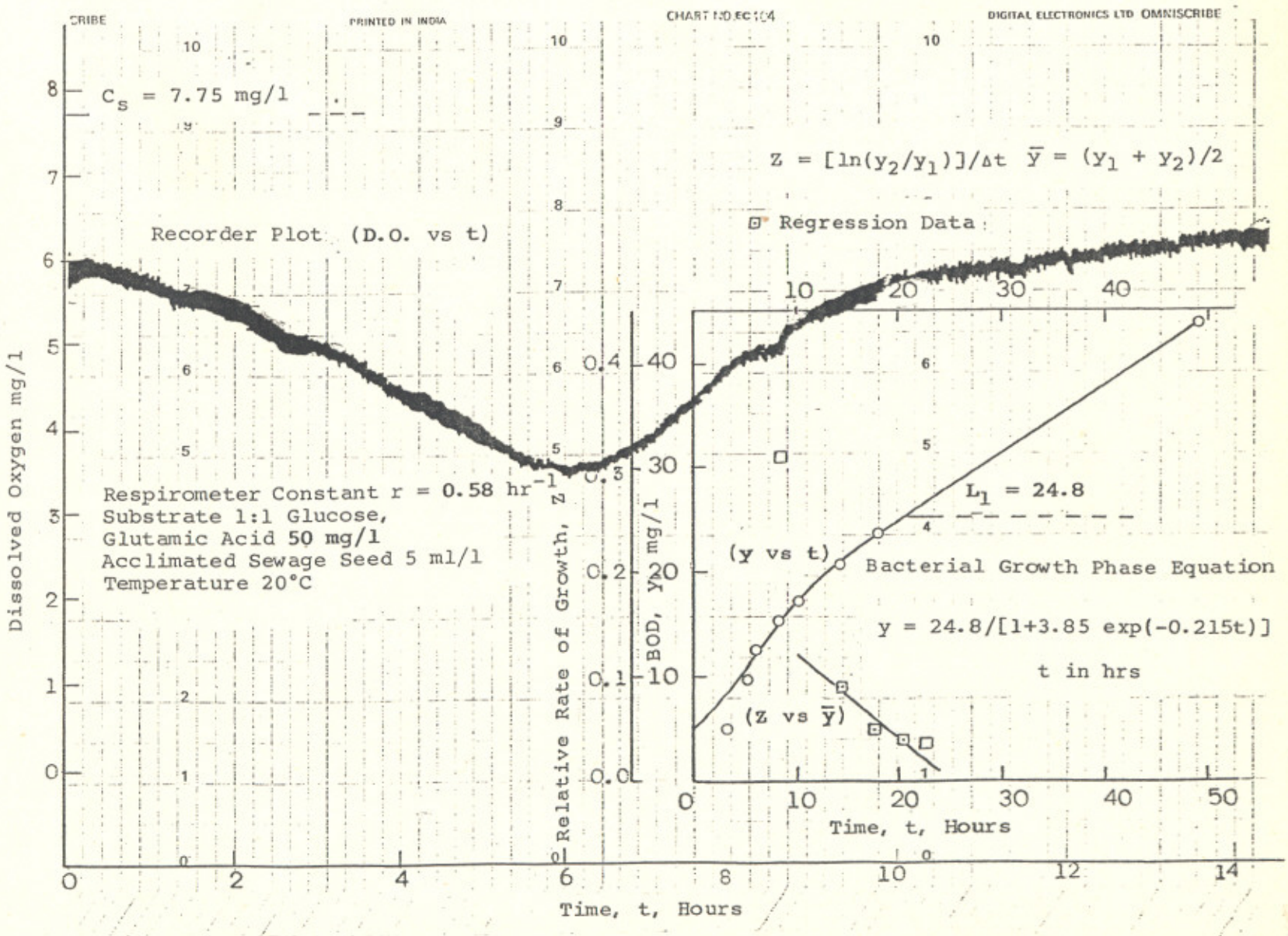


Fig. 6.8 Respirometric Study of Synthetic Waste

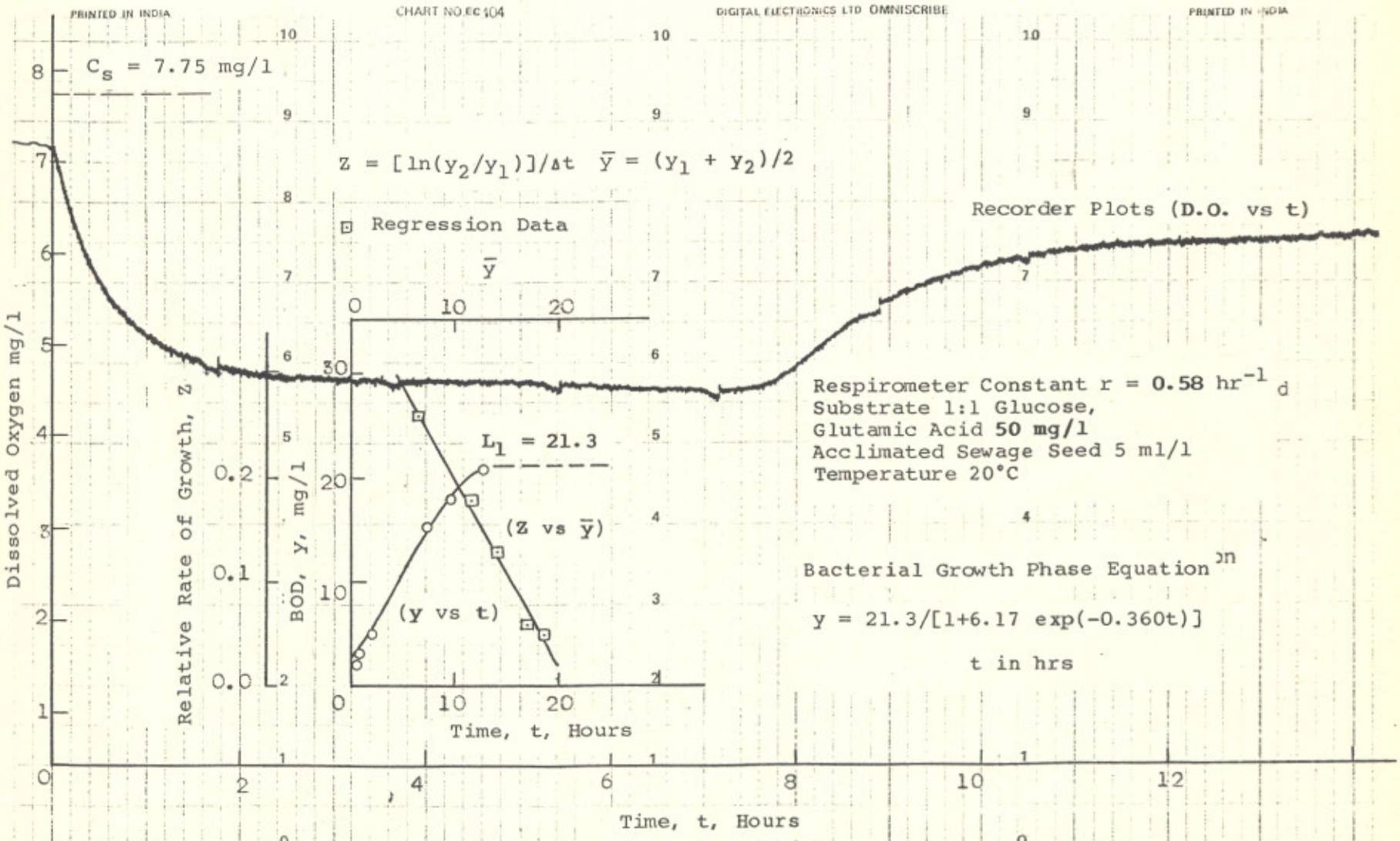


Fig. 6.9 Respirometric Study of Synthetic Waste

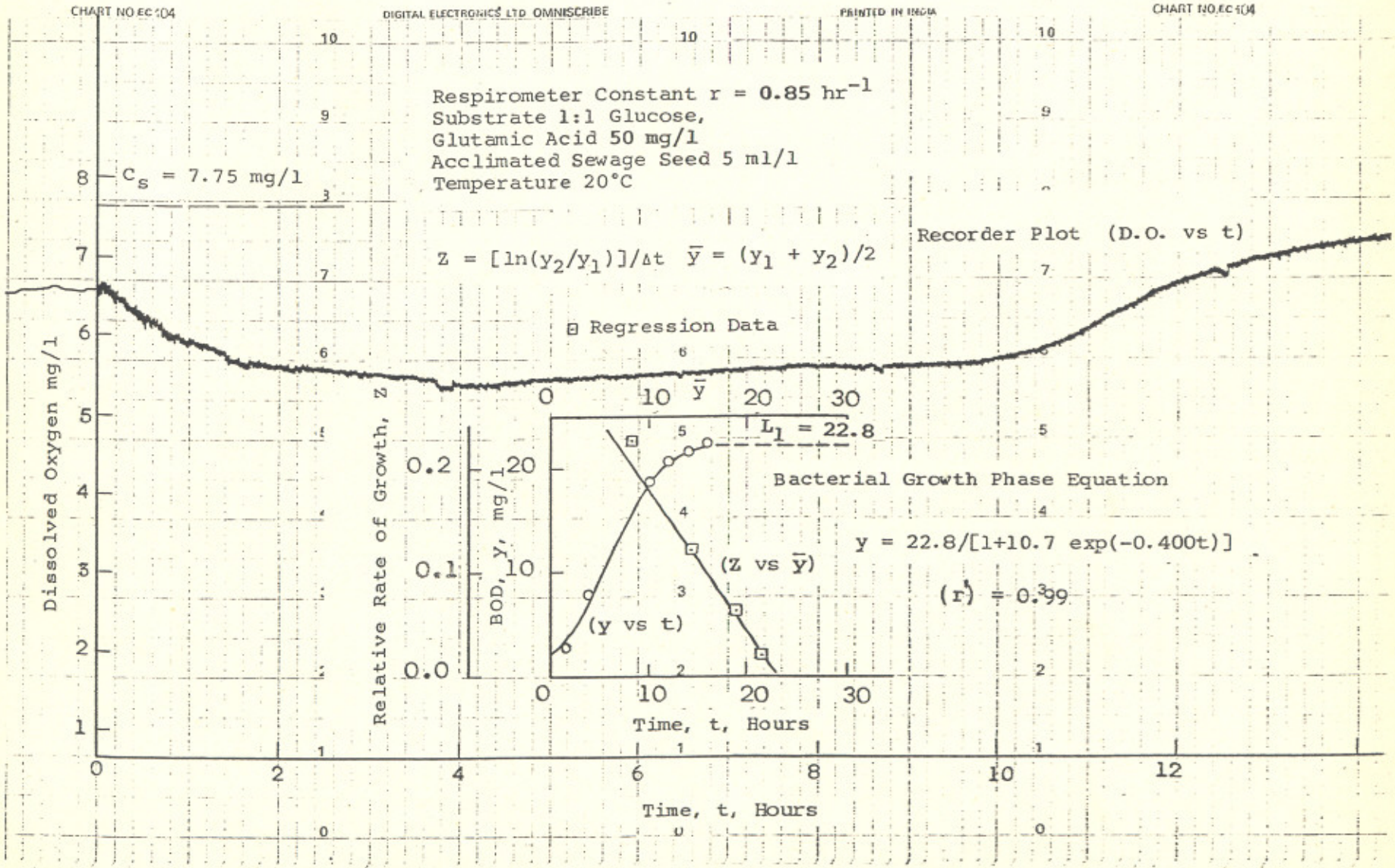


Fig. 6.10 Respirometric Study of Synthetic Waste

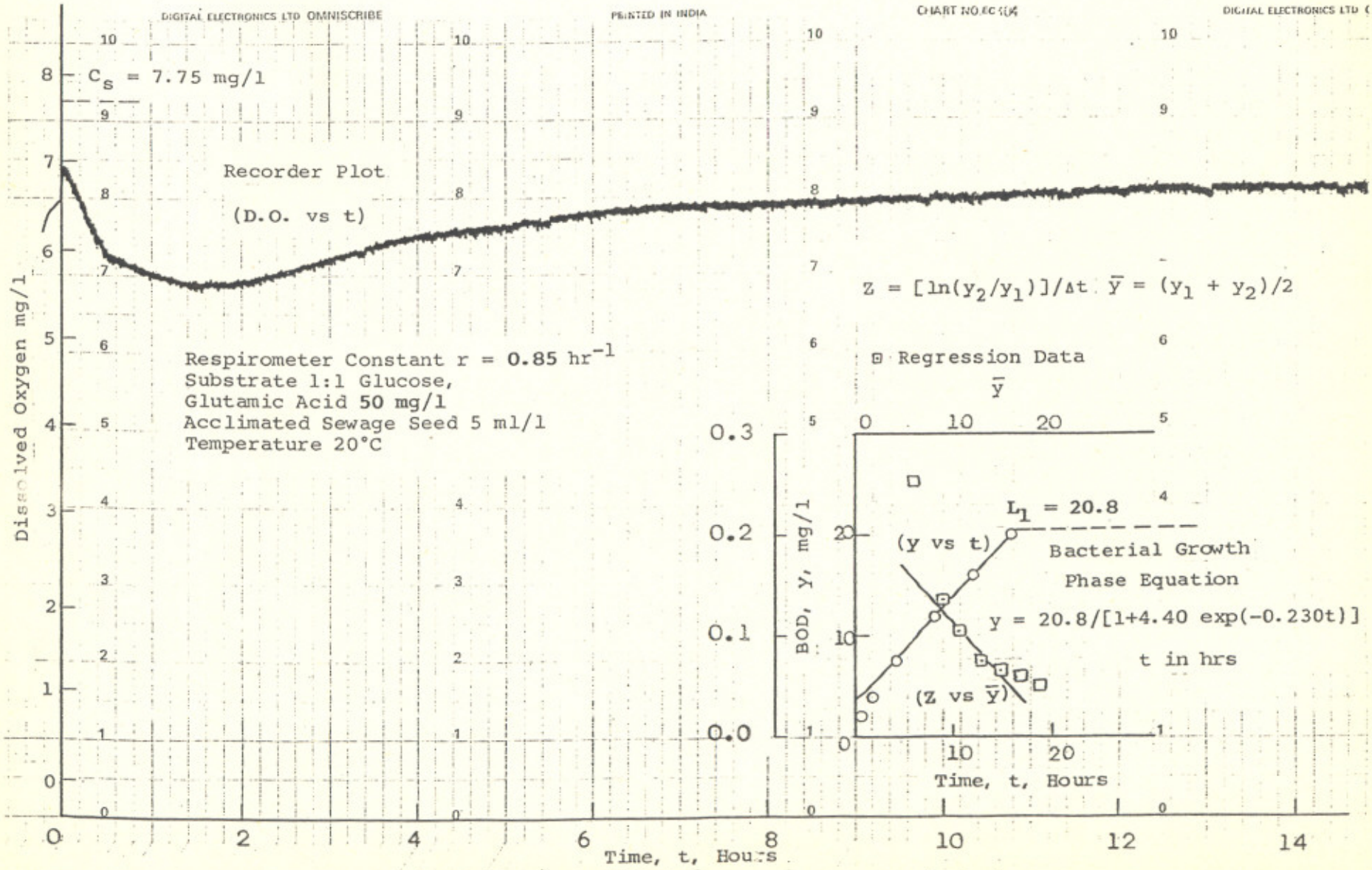


Fig. 6.11 Respirometric Study of Synthetic Waste

$z = [\ln(y_2/y_1)]/\Delta t$ $\quad \bar{y} = (y_1 + y_2)/2$

⊡ Regression Data

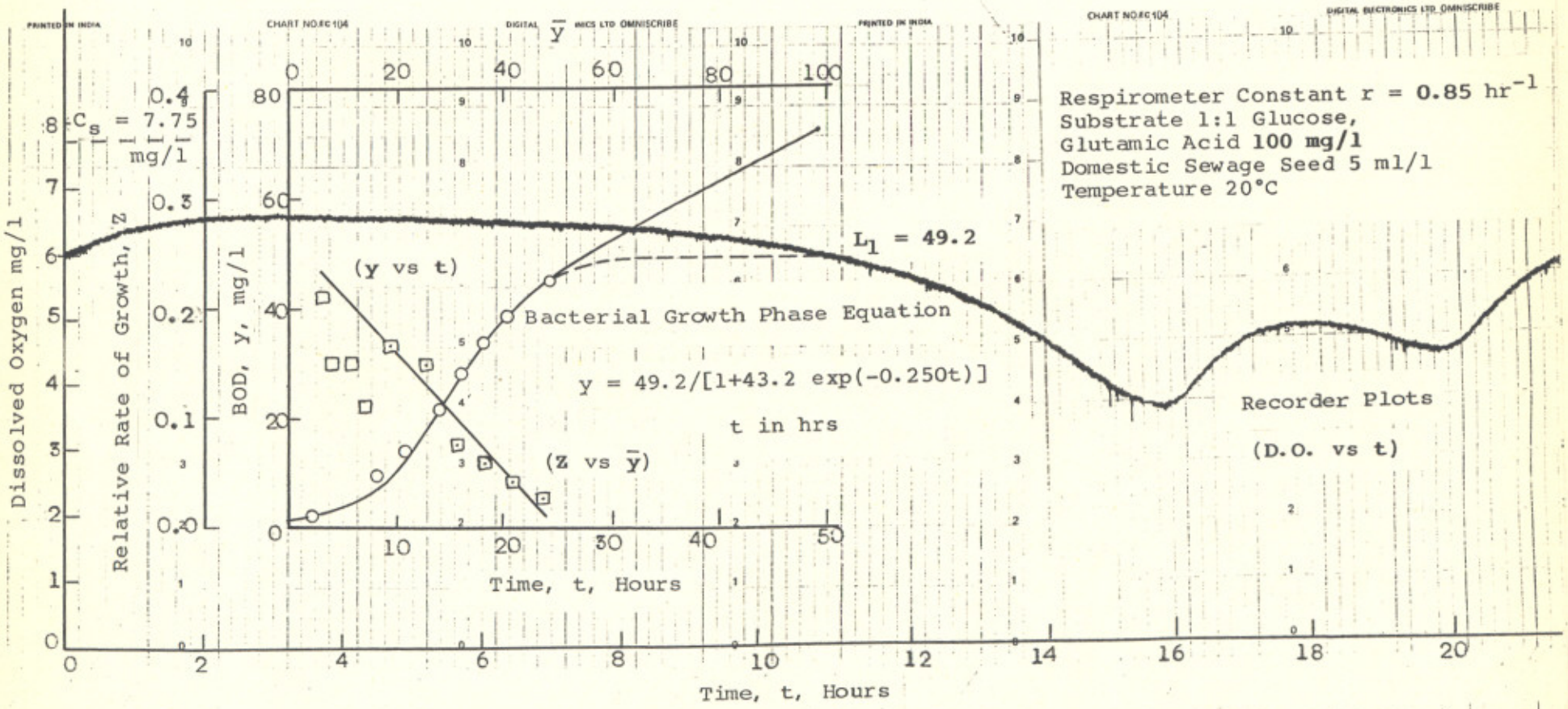


Fig. 6.12 Respirometric Study of Synthetic Sewage

$$Z = [\ln(y_2/y_1)]/\Delta t \quad \bar{y} = (y_1 + y_2)/2$$

⊡ Regression Data

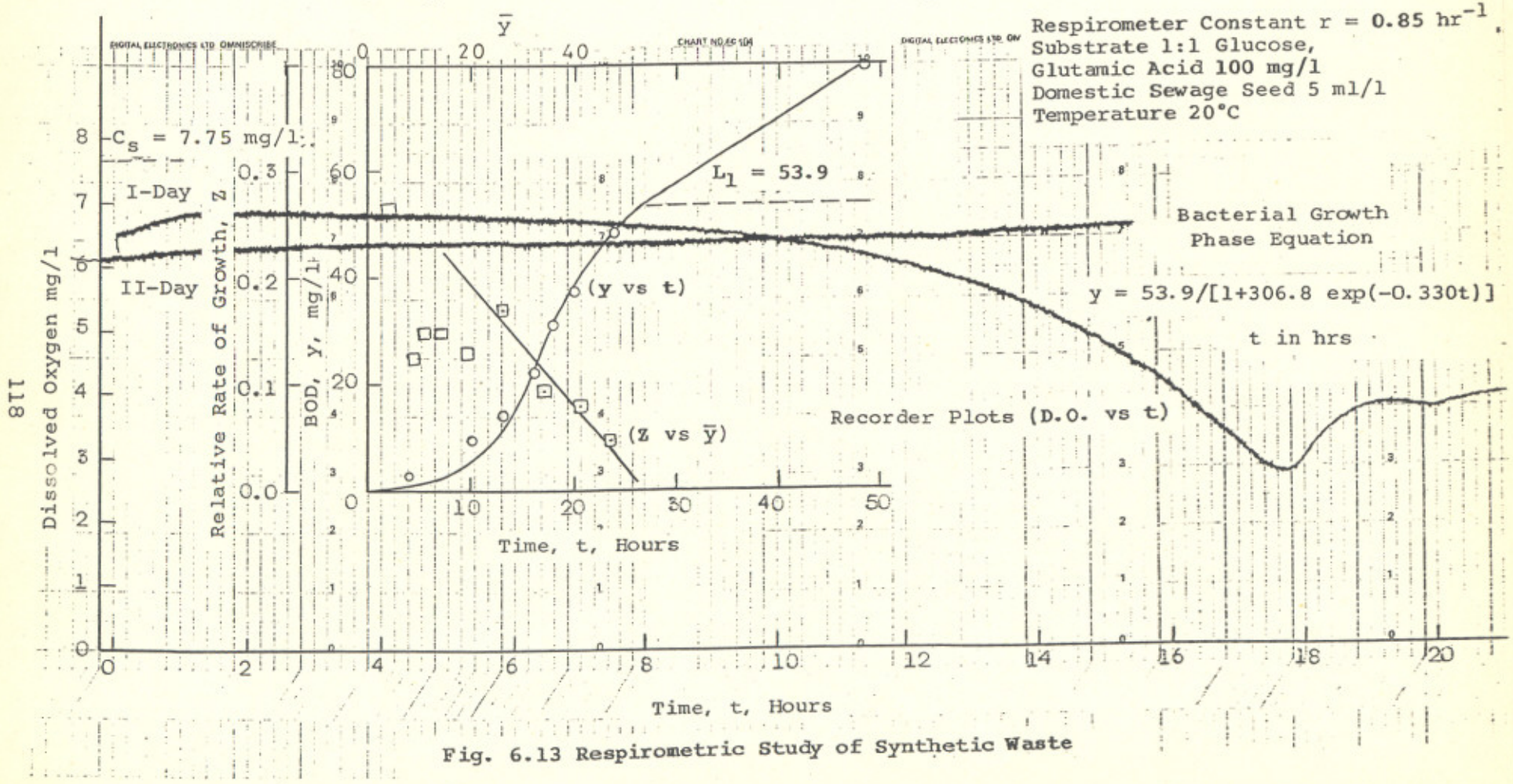


Fig. 6.13 Respirometric Study of Synthetic Waste

Respirometer Constant r = 0.85 hr^{-1}
Substrate 1:1 Glucose,
Glutamic Acid 100 mg/l
Acclimated Sewage Seed 0.5 ml/l
taken from Respirometer on 5th day
of incubation
Temperature 20°C

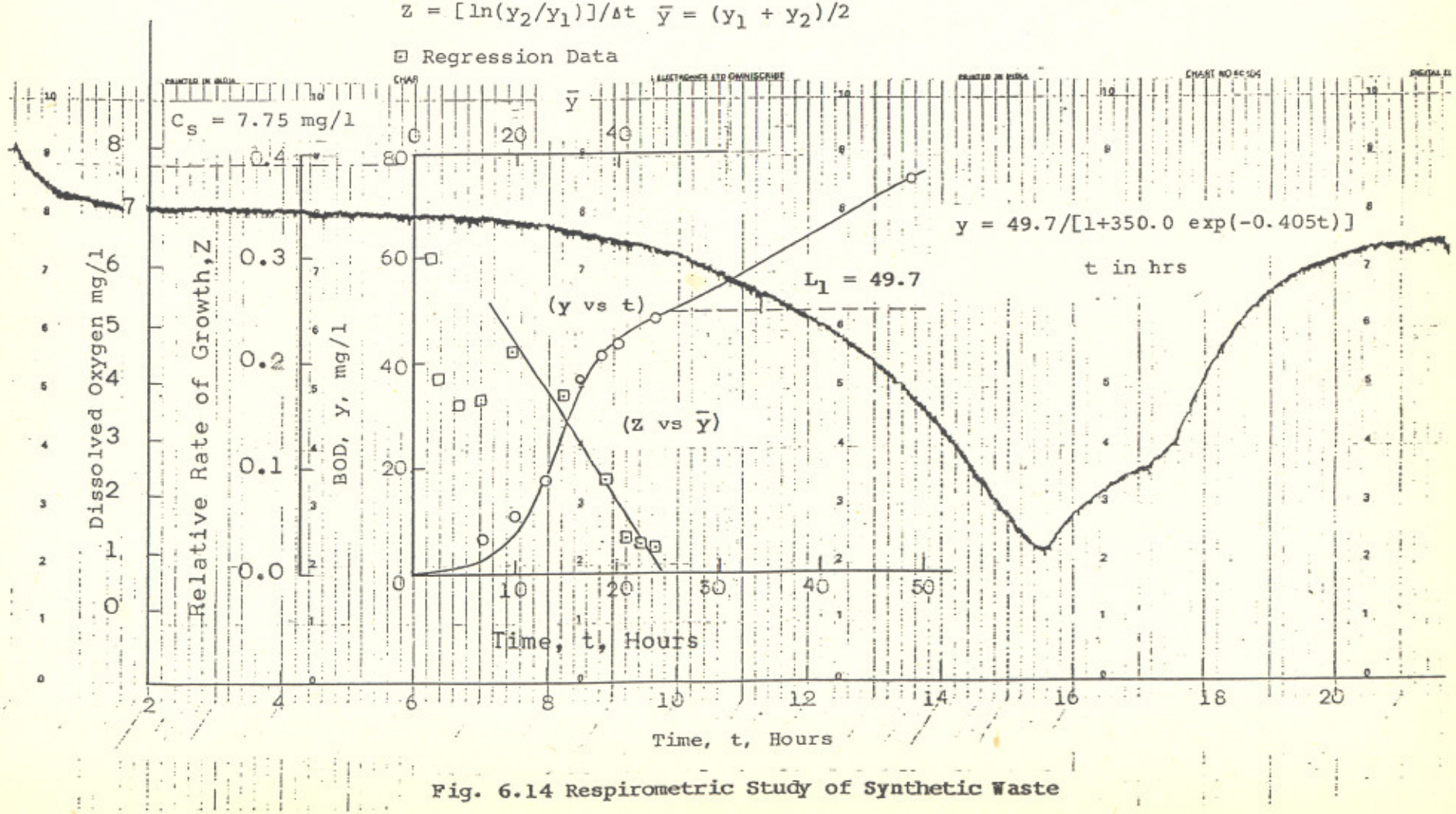


Fig. 6.14 Respirometric Study of Synthetic Waste

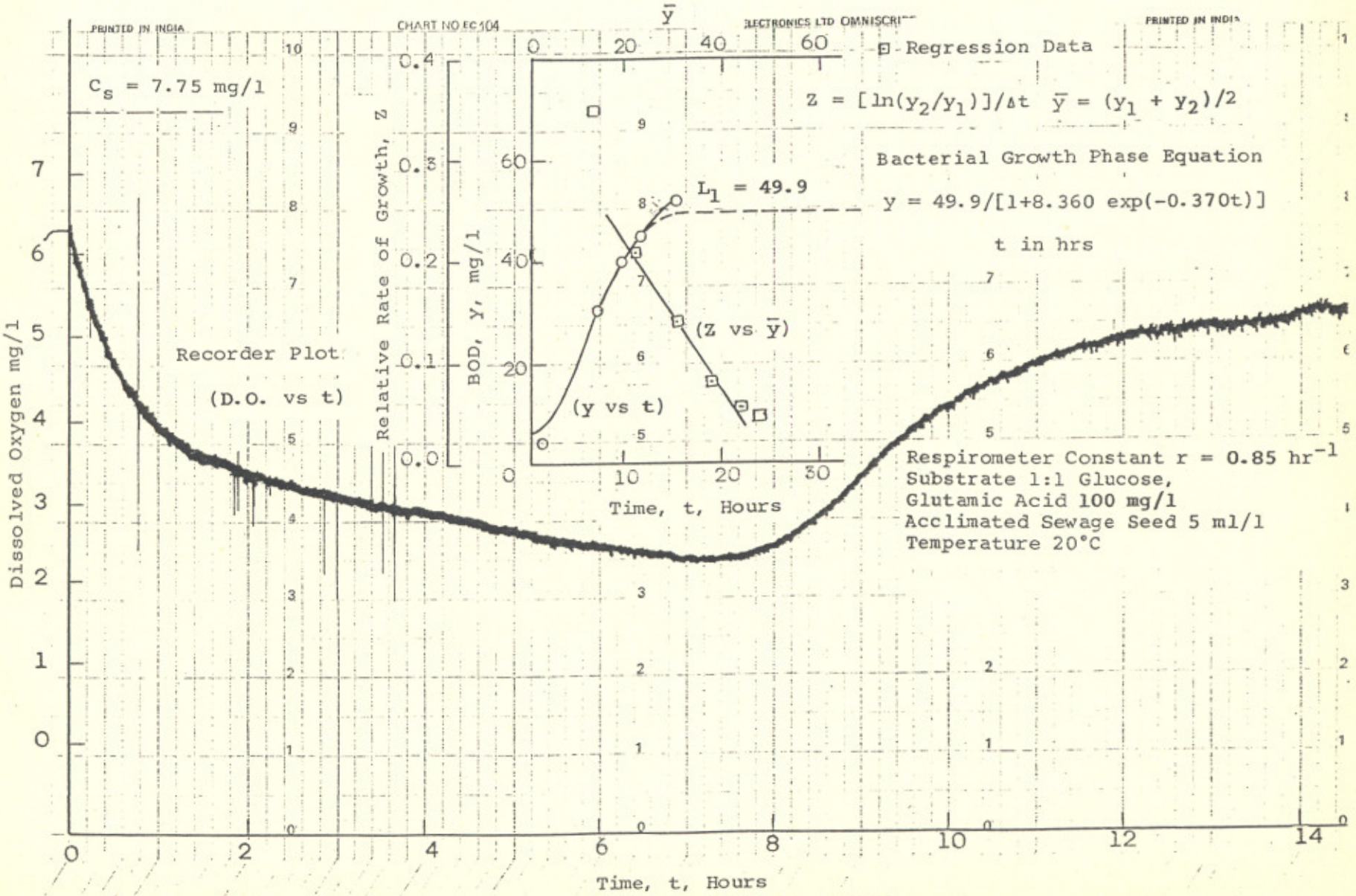


Fig. 6.15 Respirometric Study of Synthetic Waste

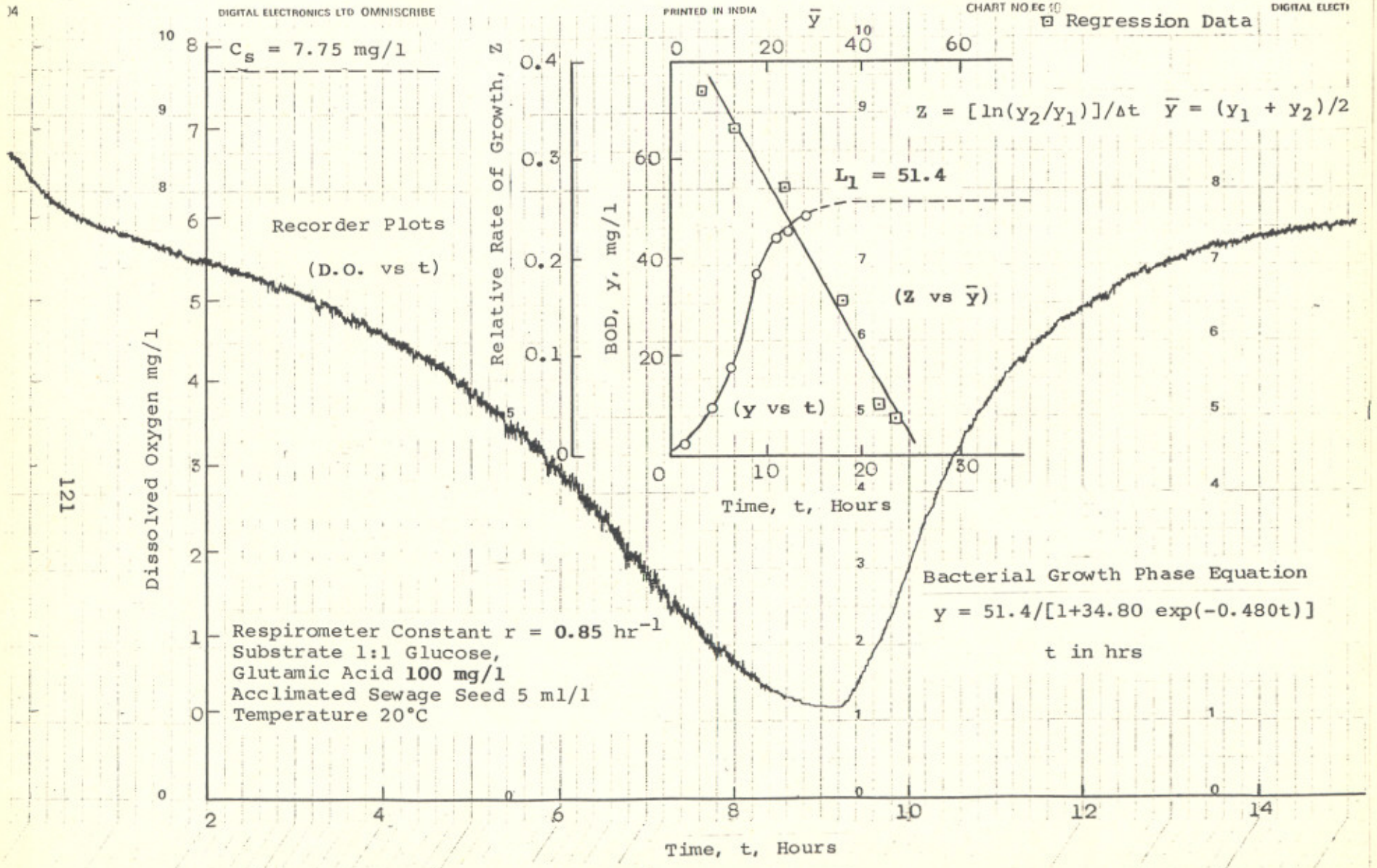


Fig. 6.16 Respirometric Study of Synthetic Waste

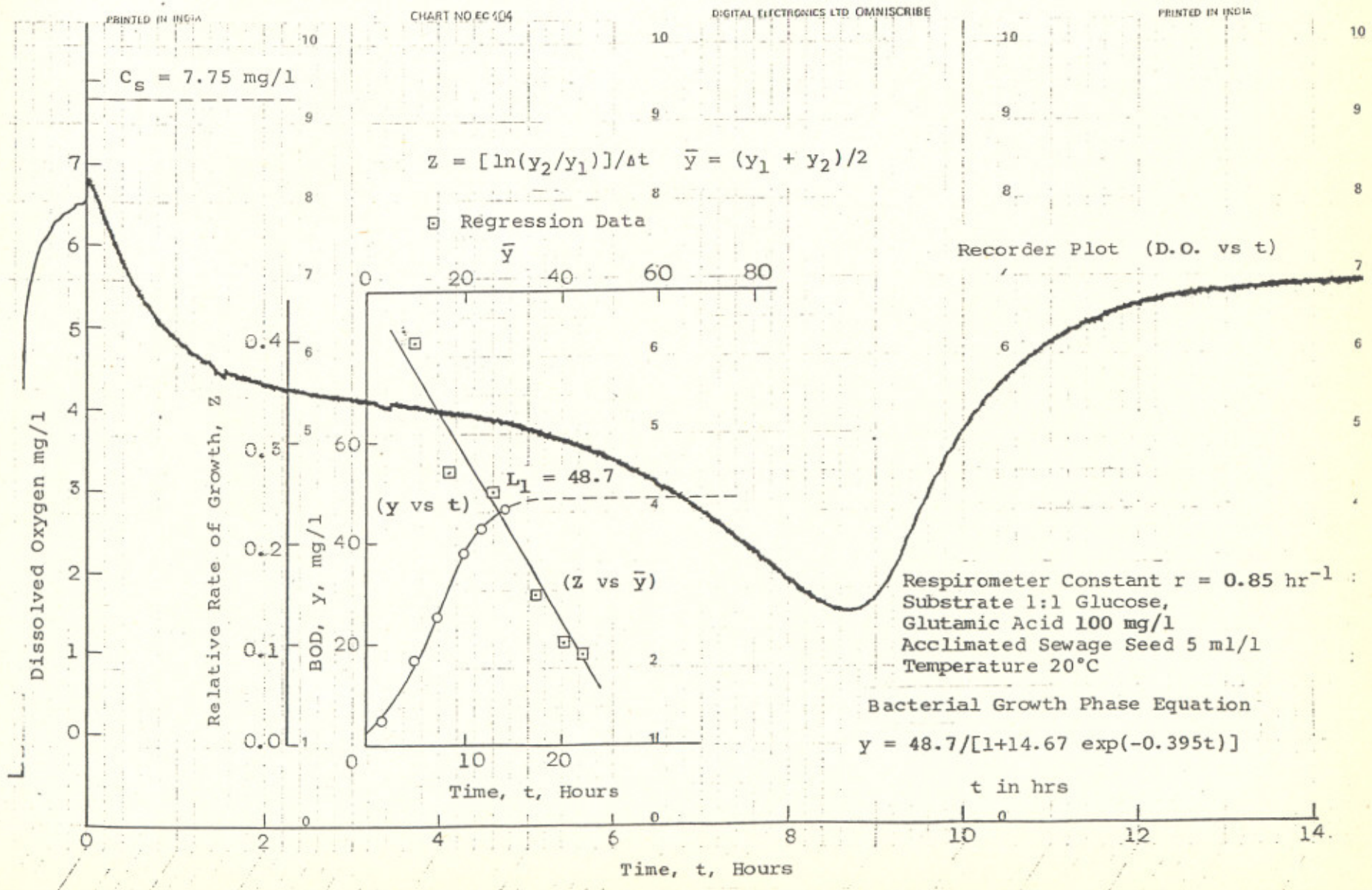


Fig. 6.17 Respirometric Study of Synthetic Waste

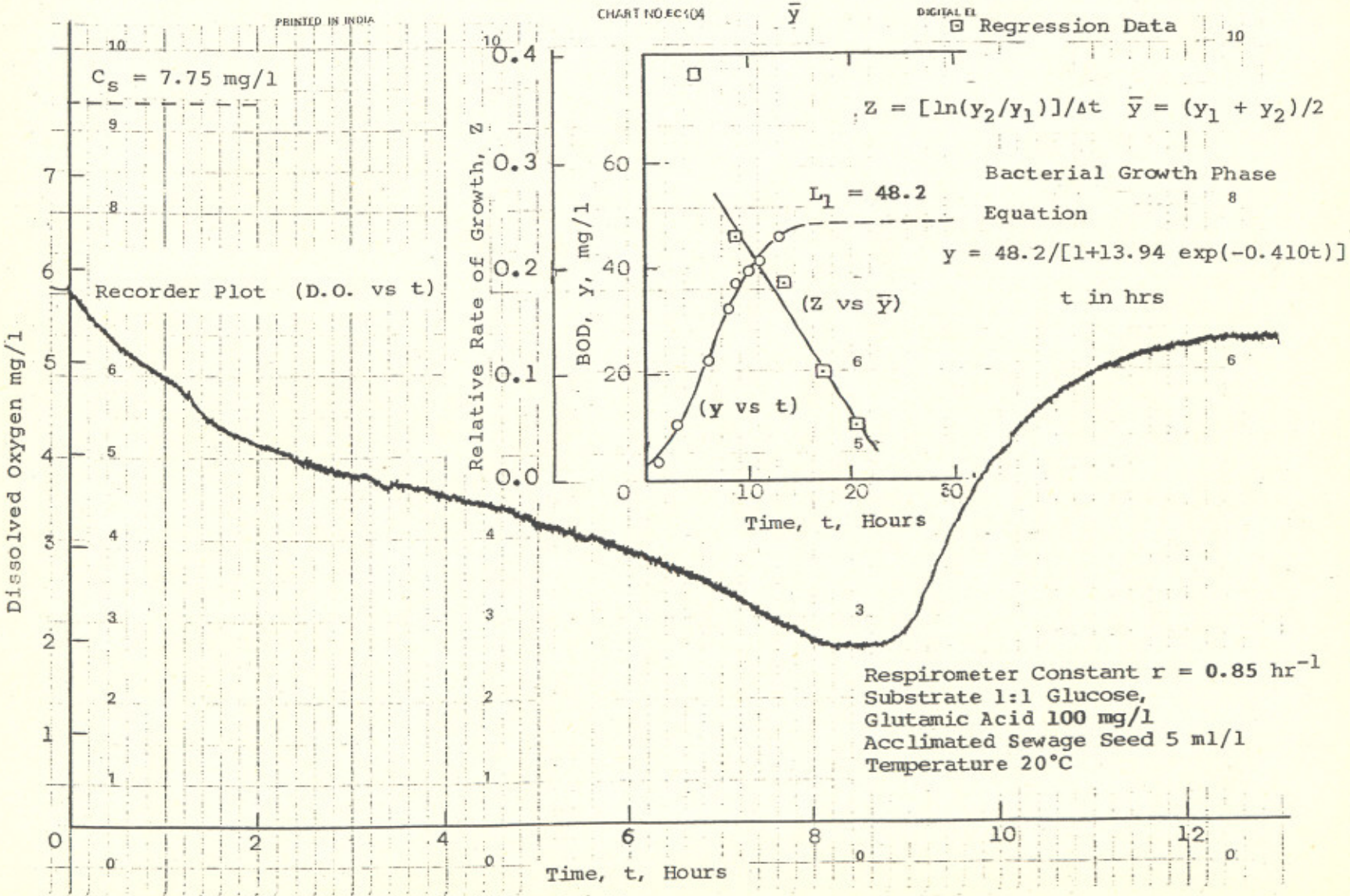


Fig. 6.18 Respirometric Study of Synthetic Waste

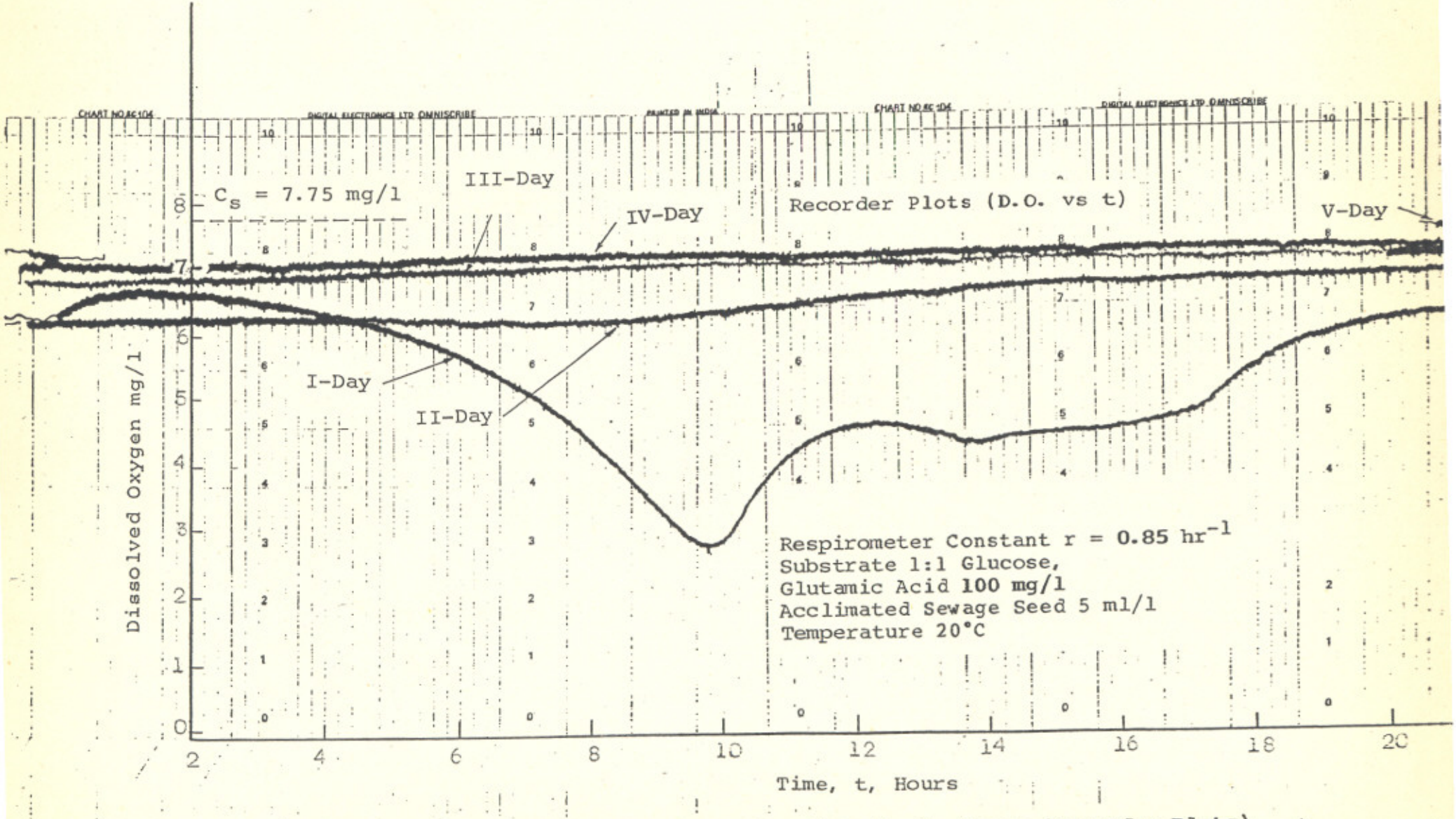


Fig. 6.19(a) Respirometric Study of Synthetic Waste (Recorder Plots)

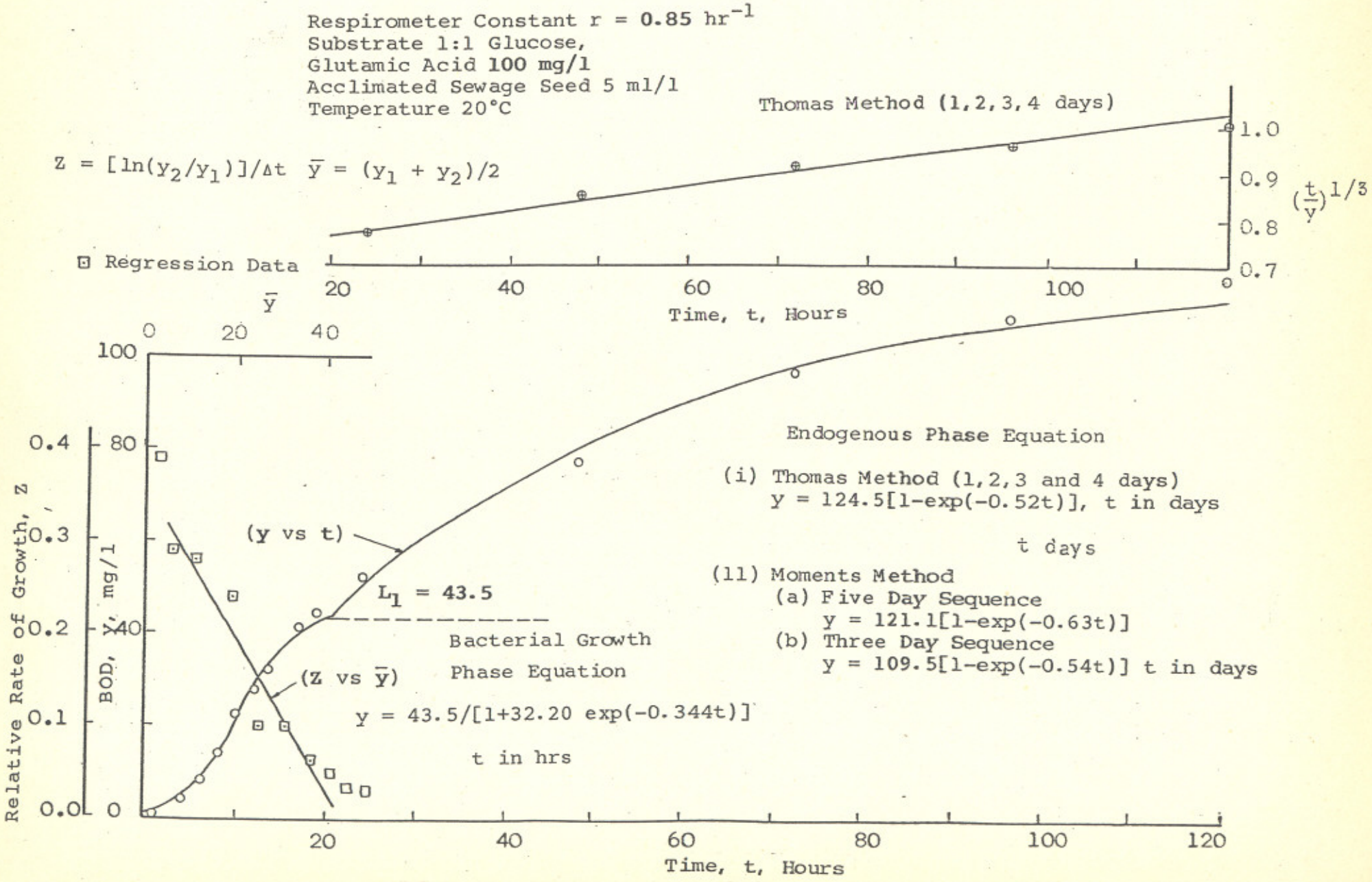


Fig. 6.19(b) Respirometric Study of Synthetic Waste

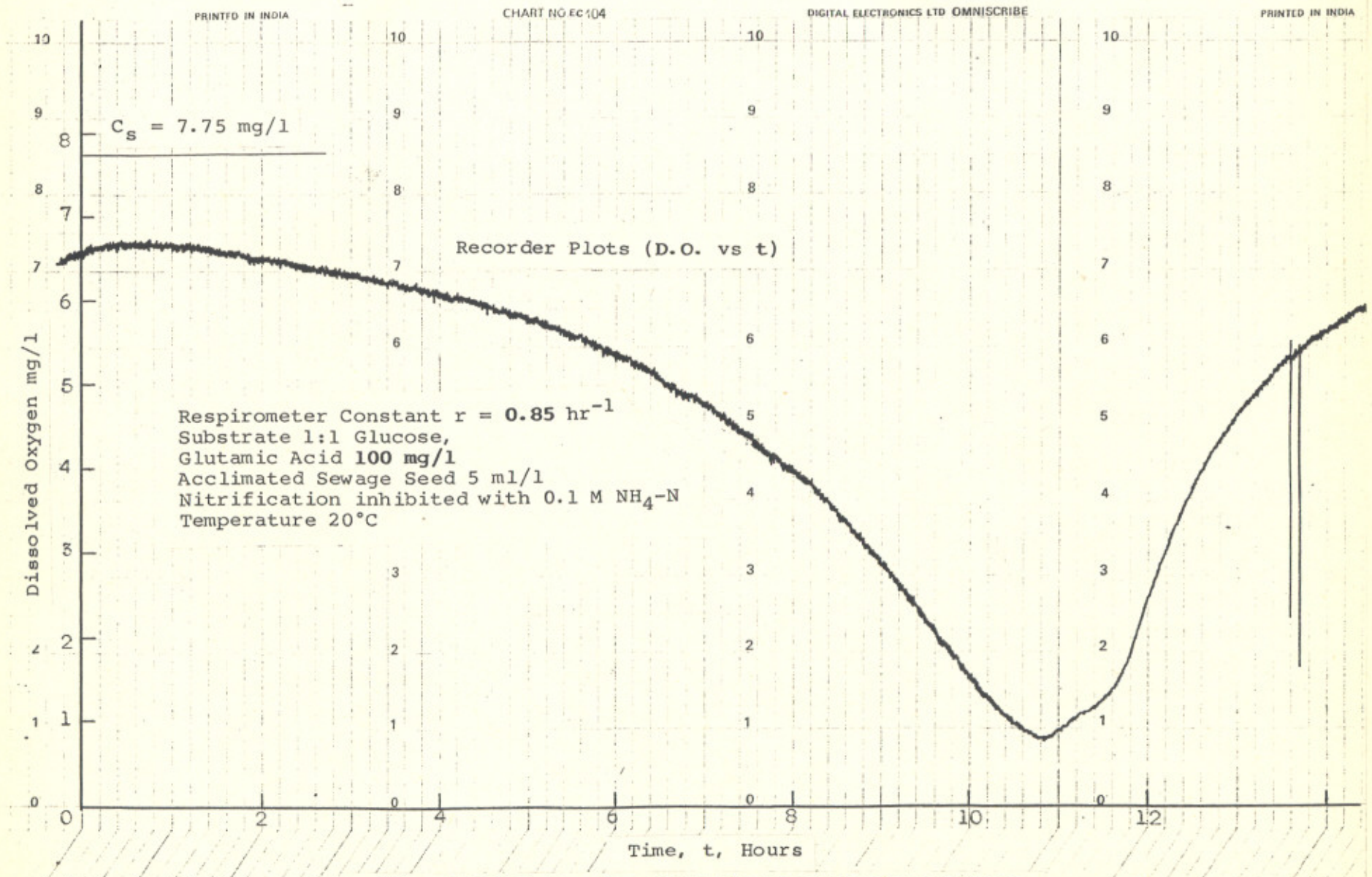


Fig. 6.20(a) Respirometric Study of Synthetic Waste

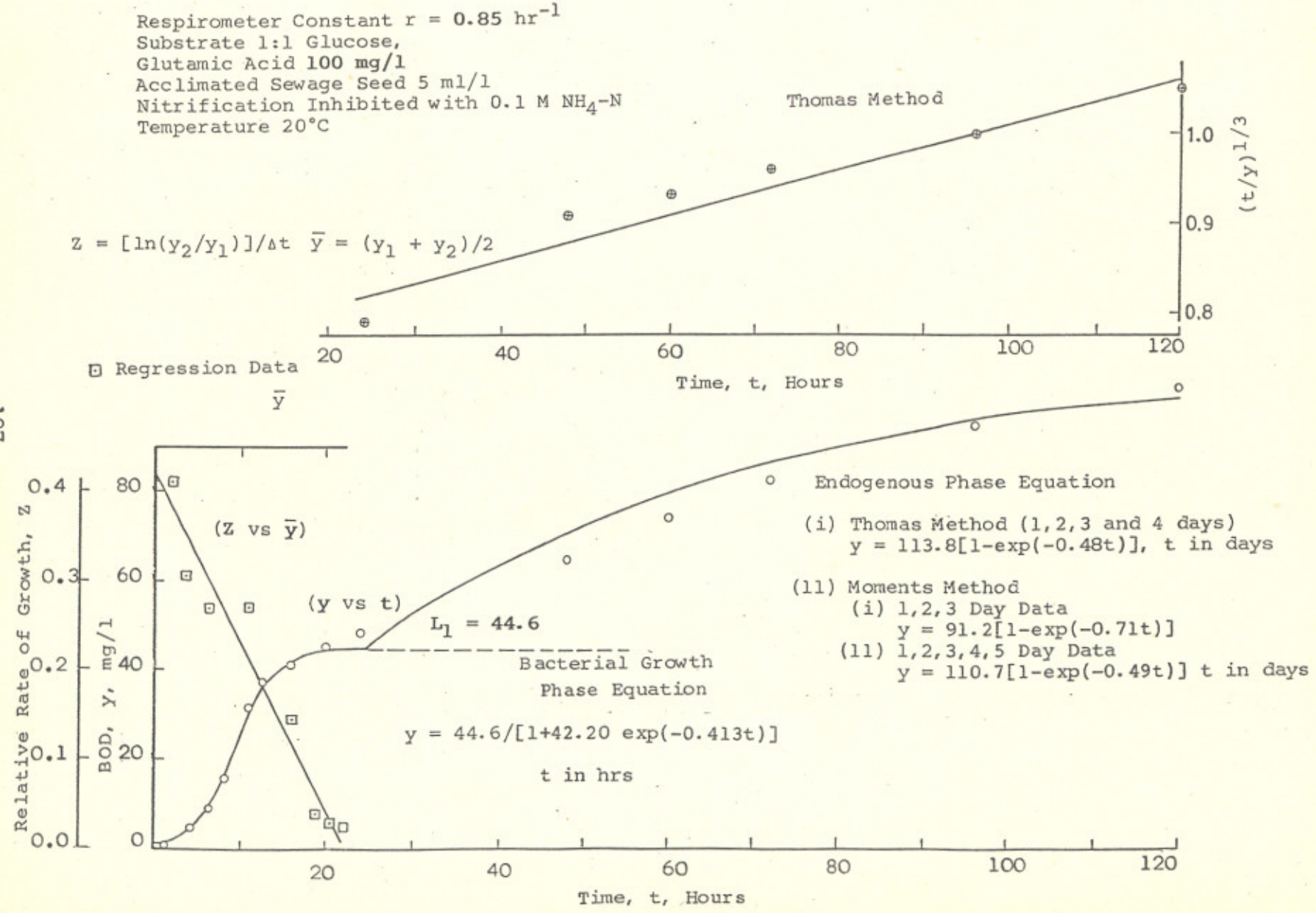


Fig. 6.20(b) Respirometric Study of Synthetic Waste

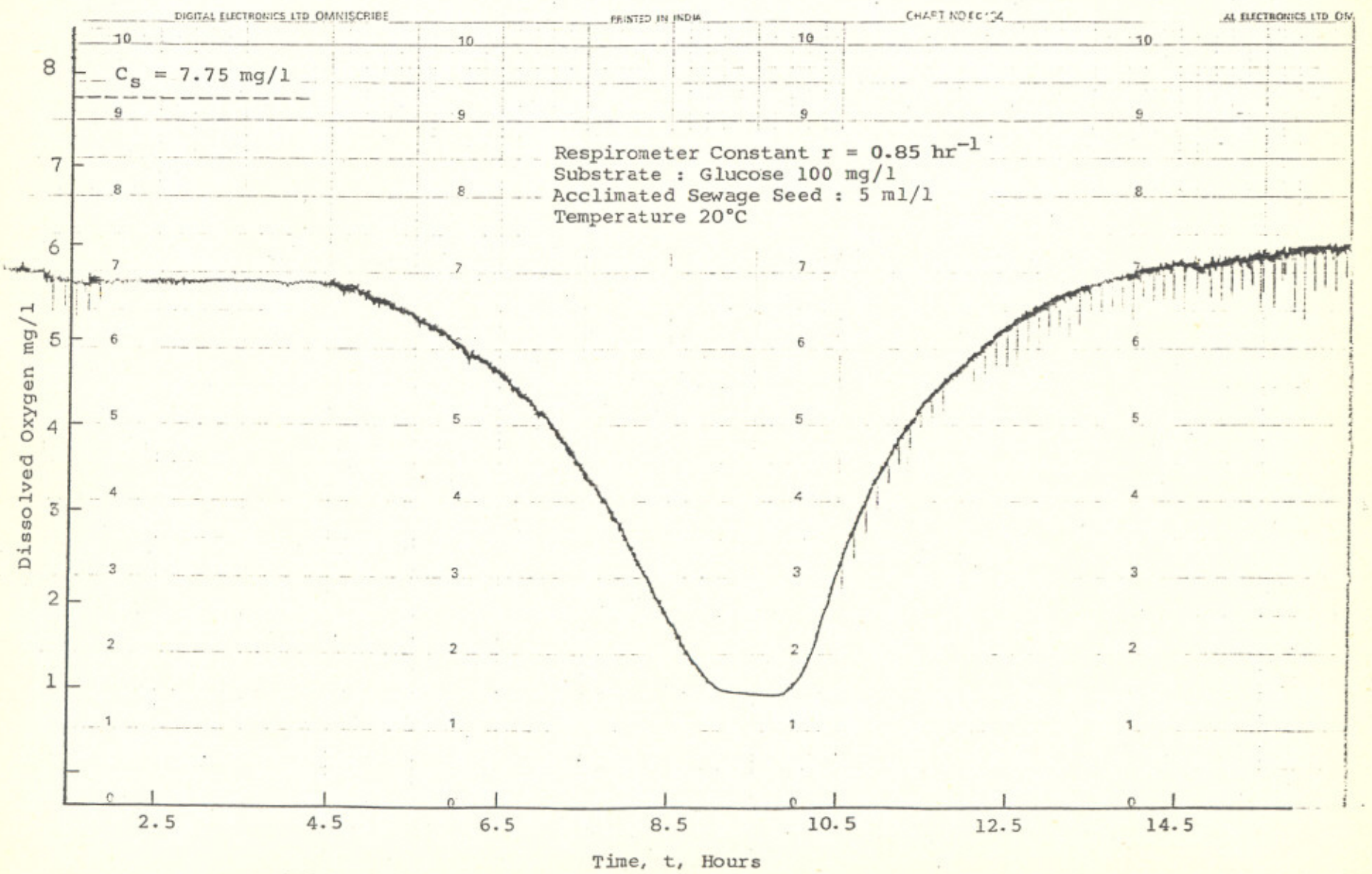


Fig. 6.21(a) Respirometric Study of Synthetic Waste (Recorder Plot)

Respirometer Constant r = 0.85 hr^{-1}
Substrate : Glucose 100 mg/l
Acclimated Sewage Seed : 5 ml/l
Temperature 20°C

$$Z = [\ln(y_2/y_1)]/\Delta t \qquad \bar{y} = (y_1 + y_2)/2$$

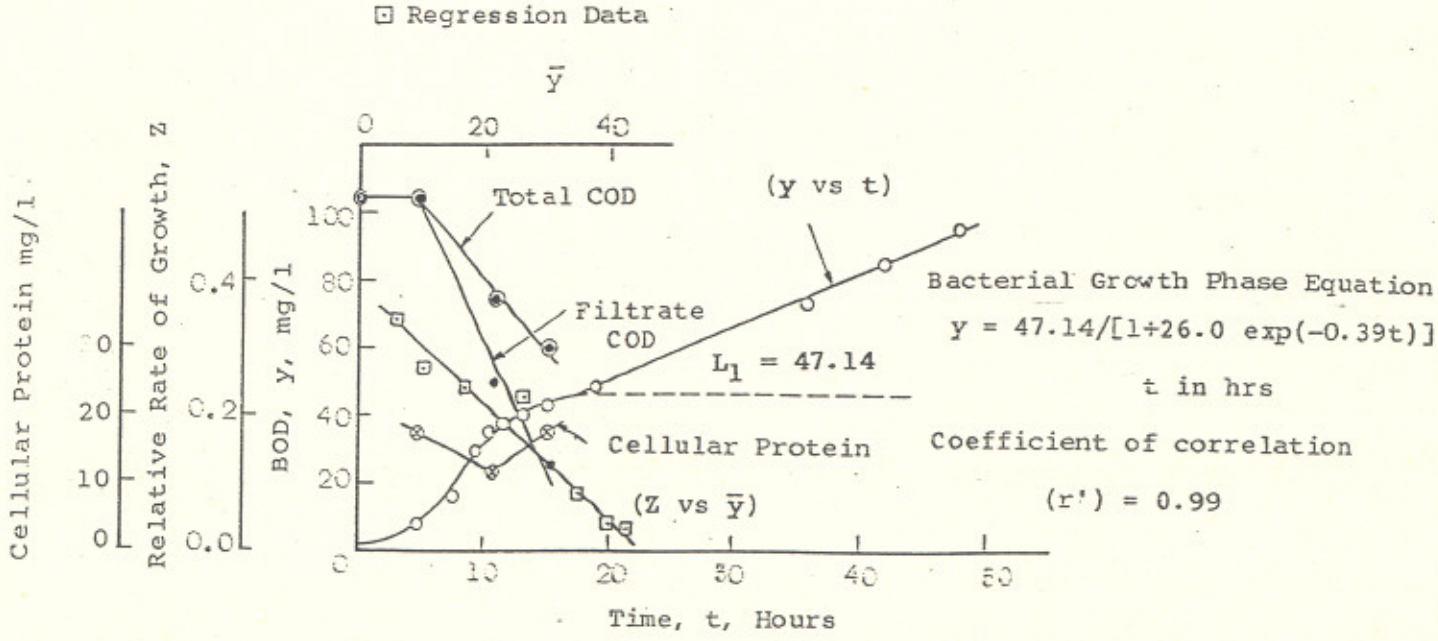


Fig. 6.21(b) Respirometric Study of Synthetic Waste

(c) the curves of relative rate of growth $Z[=\ln(y_2/y_1)/\Delta t]$ as a function of $\bar{y}$ $[=(y_1+y_2)/2]$. Relative rate of growth was estimated by calculating values of BOD exerted (y) at equal intervals of time as illustrated in Appendix I.

(d) the results of total COD, filterate COD and cellular protein as determined on samples from a separate respirometer kept in the incubator under essentially indentical conditions, (Fig. 6.21b).

During the investigations, the contents of the respirometer were continuously stirred to replenish oxygen through the polyethylene membrane surrounding the wastewater sample. This also maintained carbon dioxide in equilibrium with the atmosphere. Thus the respirometer represented a turbulent system that can be used for the study of BOD exertion of wastewater samples.

6.3.1.1 Recorder Plots for Respirometer

The general trend, of the recorder plots for respirometer (Fig.6.8 to 6.21), was similar i.e. a rapidly decreasing downward leg till a minimum value of D.O. was reached. This was followed by a rapidly increasing upward leg of the D.O. profile. After the rapid upward trend, slight change(s) in the slopes of the recorder plots were observed. A similar trend in D.O. profiles was observed by Issacs and Gaudy (1967) in their simulated stream experiments.

In the recorder plots (Fig.6.8 to 6.21) certain variations were also observed. In (Fig. 6.12 and 6.13) an initial horizontal leg of the plot was obtained probably because unacclimated

domestic sewage was used as seed. Horizontal leg was also observed in (Fig.6.14) after initial equilibrium when 0.5 ml/l acclimated sewage seed was used. This seed was withdrawn for use from the tail end of the endogenous phase. Recorder plot of Fig.6.21(a) for 100 mg/l of glucose shows an initial horizontal leg when 5 ml/l sewage was used as seed. This seed was acclimated to glucose only.

After reaching a minimum D.O. value, there was an upward increasing trend for about an hour and a flat sag was observed in Fig.6.12, 6.13 and 6.19(a). This sag lasted for about six hours in the plot of Fig.6.19(a). After the initial rapid recovery period, there was a second sag (not shown in Fig.6.20 a) in the recorder plot of the experiment when nitrification was inhibited (Fig.6.20a,b).

Initial horizontal leg of the recorder plot corresponds to the lag phase and depicts zero order kinetics. Rapidly decreasing downward leg corresponds to log growth phase and rapidly increasing upward leg corresponding to declining growth phase. The position of downward and upward leg of the recorder plots with respect to log growth and declining growth phase depends on the rates of reaeration and deoxygenation in the respirometer. In Fig.6.11, the recorder plot levells off at the end of the log growth phase, since the reaeration rate is very high in comparison to the rate of BOD exertion. In the experiments of Issacs and Gaudy (1967), the rapidly increasing upward trend of the D.O. profile corresponded to the plateau because of low rates of

reaeration in the simulated stream (only 20% in comparison to the reaeration rate in the respirometer of the present investigations).

6.3.1.2 BOD Exertion

The exertion of BOD in the respirometer passes through the lag phase (if any), log phase followed by a declining growth phase. This was followed by a plateau which is discernible in Figs.6.10, 6.16, 6.17, 6.19(b), 6.20(b) and 6.21(b). Plateau has been masked in the other experiments. Further BOD exertion takes place at an ever declining rate of oxidation (endogenous phase). Thus the exertion of BOD in the respirometer takes place in two phases, initial high rate of oxidation (log and declining growth) and a slow ever decreasing rate of oxidation (endogenous phase). The diphasic nature of BOD exertion has been well recognised and attributed to sequential growth cycles, first of bacteria which utilize the original carbon source from the waste sample for cell replication, termed as bacterial growth phase, and second, of either protozoa or lysis-synthesis-lysis reactions of bacteria which grew during the first cycle.

Recorder plots of Figs.6.12 and 6.13 reveal similar trends in the initial horizontal legs representing similar trends in the two lag phases. These lag phases were observed when domestic sewage (without acclimation) was used as seed. As stated by Gaudy and Gaudy (1966), that when cells are placed in a new medium, initially substrate is used without replication and increase in weight occurs as carbon stores and metabolic pools are replenished and enzymes for balanced synthesis are formed. Further such a

system would be analogous to a non-proliferating system and would be expected to exhibit zero order kinetics until cell replication begins. Approximately uniform saturation deficit observed during the above two lag phases confirms zero order kinetics. The lag observed in Fig.6.14, when 0.5 ml/l acclimated sewage taken from the tail end of endogenous phase was the seed, was due to reduced quantity and poor quality of the seed. The apparent lag observed with acclimated sewage seed with glucose (Fig.6.21a) as substrate is not explainable on these grounds. During the lag period (4.5 hours) 8 mg/l of BOD was exerted but no change in total or filterate COD was obtained (Fig.6.21b). With 8 mg/l of BOD exertion, reduction of 4 mg/l in total COD and 8 mg/l in filterate COD was expected under normal growth conditions. Such a difference may not be registered in the standard COD test procedure as one drop (0.05 ml) of N/4 Ferrous Ammonium sulphate used for titration represents a COD value of 5 mg/l when 20 ml wastewater sample is taken for digestion. Notwithstanding these facts the observations of other workers in the field also throw some light on the apparent lag phase with glucose and acclimated sewage seed as observed in the present investigations.

Clifton (1963), as ref. by Gaudy and Gaudy (1966), has reported partial oxidation of glucose by E.coli. Bhatla and Gaudy (1965b) used a pure culture of E. freundii for BOD exertion of glucose. At the time all glucose was removed from the system, only half of the COD was removed. Two sequential bacterial growth phases were observed. Oxygen utilization during the second stage was accompained by metabolism of secondary extracellular carbon

source(s) produced by the cells during metabolism of the original extracellular carbon source (glucose). Volatile acids (primarily acetic acid) were released into the medium as products of partial oxidation during the course of initial BOD exertion. Cellular protein reached a peak corresponding to the point of maximum volatile acid prodction and attained another peak at the end of second stage of oxygen uptake.

Doelle et al. (1982) in reviewing 'Regulation of Glucose Metabolism by bacterial system' concluded that :

(a) majority of reports were those concerning the facultative anaerobic bacterium E.coli.

(b) studies on the effect of oxygen and glucose concentration on glucose metabolism indicate that the formation of organic end products is not unique to the anaerobic state. Such formation can lead to an oversupply of $NADH_2$ and can occur under aerobic and anaerobic conditions and is not necessarily accompanied by a decrease in ATP production. This means that microorganisms are able to produce organic end products under fully respiratory conditions in the presence of oxidative phosphorylation i.e. the carbon flow is via the Embden-Meyerhof - Parnas pathway.

(c) the organic end product formation occurs irrespective of the pathway used for glucose utilization to pyruvate.

In the present investigations, domestic sewage (acclimated) was used as seed for glucose as substrate (Fig.6.21a,b) and E.coli were reasonably expected to be present in the domestic

sewage seed. The apparently observed lag phase for glucose (Fig.6.21a) and also the concomitant difference in BOD and COD values (Fig.6.21b) may be explained on the basis of following postulates formulated on the findings of Bhatla and Gaudy (1965b) and others as reviewed by Doelle et al. (1982).

6.3.1.3 Postulates

(Reference Figs 6.21 a,b and Table-1, Appendix I)

(i) The two sequential bacterial growth phases as observed by Bhatla and Gaudy (1965b) merge into one bacterial growth phase represented by the sag in the recorder plot (4.5 to 15.0 hours).

(ii) At the end of apparent lag phase (4.5 hours), 8.3 mg/l BOD was exerted and an equivalent or partly less amount of organic end products are released into solution according to the stoichiometry of the reaction.

(iii) During the bacterial growth phase (4.5 to 15.0 hours), these released end products alongwith glucose are utilized.

At 10.5 hours, BOD exerted is 35.2 mg/l, total and filterate COD are 75 and 50 mg/l respectively and at the end of 15 hours BOD exerted is 43.2 mg/l, total and filterate COD are 60 and 15 respectively. As indicated by BOD and COD data, the organic end products were partially utilized between 4.5 to 10.5 hours and were fully utilized till 15 hours.

These facts are further authenticated by the peak in cellular protein at the end of the observed lag phase (4.5 hours) and another peak at the end of bacterial growth phase (15 hours). This

is in agreement with the results of Bhatla and Gaudy (1965b) in case the above postulates are accepted.

The present investigation is not conclusive with regard to the mechanism of occurence of apparent lag phase when acclimated sewage seed is used with glucose as substrate. Elaborate research efforts, with specific tests for the removal of substrate and organic end products, are needed to delineate the mechanism.

The release of organic end products into solution and its subsequent utilization during bacterial growth may well explain the extremely variable rates of BOD exertion and 5 day BOD values (Standard Methods) observed with glucose as substrate.

6.3.2 BOD Exertion In Respirometric BOD Bottle

Following the procedure outlined in Sec. 6.2.2., respirometric BOD (R.BOD) bottles were filled with the synthetic waste (20 to 40 R.BOD bottles were filled as required).

Samples were taken at 2 to 4 hour intervals for the first day and at 12 hour intervals subsequently, and analysed for dissolved oxygen by Winkler's method.

In the present investigations, a 1:1 mixture of glucose and glutamic acid was used as the substrate. In order to provide adequate replenishment of oxygen in the R.BOD bottle for biochemical activity rectangular single compartment R.BOD bottles were used for the study of 50 mg/l of synthetic waste and a waste strength of 100 mg/l was studied in rectangular, two compartments R.BOD bottles. A waste strength of 200 mg/l was studied in

rectangular, two compartments as well as three compartments R.BOD bottles. The results are presented in Figures 6.22 to 6.26. The following curves are shown on the figures.

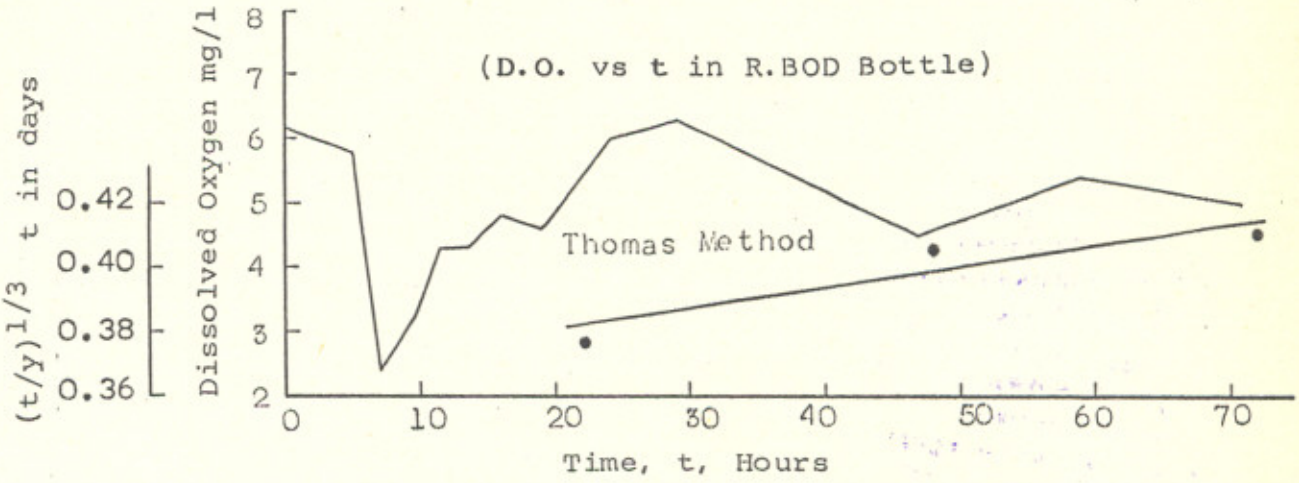
(a) the observed dissolved oxygen concentration (D.O. profile) in R.BOD bottles.

(b) the BOD exerted, for the synthetic wastewater, computed from the observed D.O. concentrations and respirometer constants for various R.BOD bottles (Table 6.7). The BOD calculations were made using the same method as discussed earlier and illustrated in Appendix I.

(c) the Figs. 6.22 to 6.26 show the curves of relative rate of growth Z [$=\ln (y_2/y_1)/\Delta t$] as a function of $\bar{y}$. Relative rate of growth was estimated by calculating values of BOD exerted at equal intervals of time as illustrated in Appendix I.

The contents of the respirometer were continuously stirred whereas the contents of the R.BOD bottle were not stirred in the present investigations. Due to replensihment of oxygen through the membrane, it was possible to study the BOD exertion of waste sample having a strength of 100 and 200 mg / l in the two respirometric systems respectively. No arrangement is needed for the absorption of carbon dioxide, since the respirometers are completely filled bioreactors and carbon dioxide is in equilibrium with the atmospheric carbon dioxide through the polyethylene membrane. Thus the two unique respirometric systems represent turbulent and quiescent bioreactors for the study of BOD exertion of wastewaters. The experimental results are discussed in the following sections.

Apparatus:
Respirometric BOD Bottle,
Rectangular, Single Compartment
Respirometer Constant r_1, Table 6.7
Substrate 1:1 Glucose
Glutamic Acid 50 mg/l
Acclimated Sewage Seed 5 ml/l
Temperature 20°C



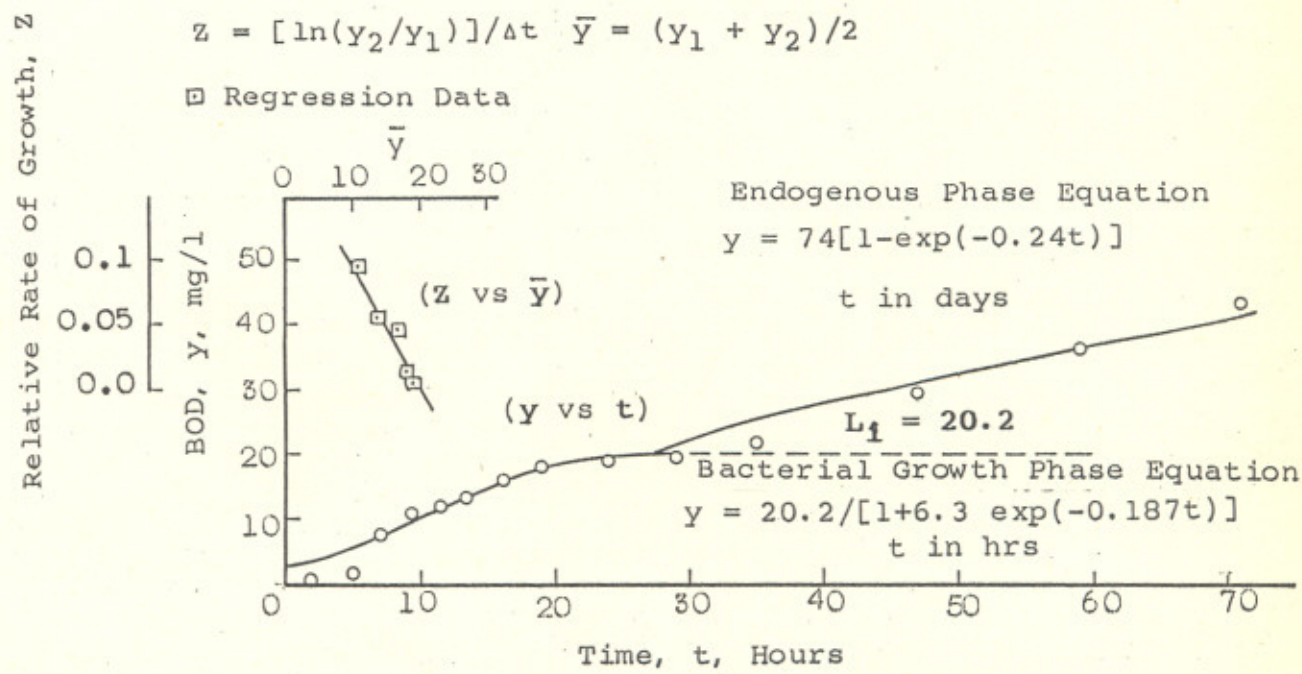
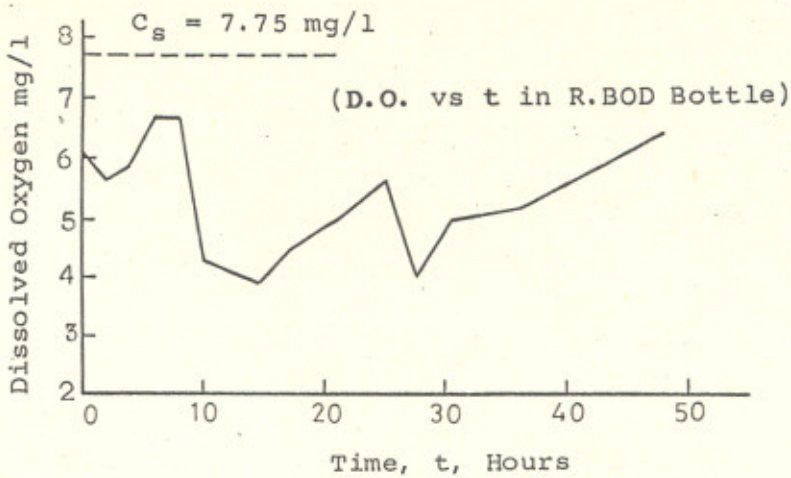
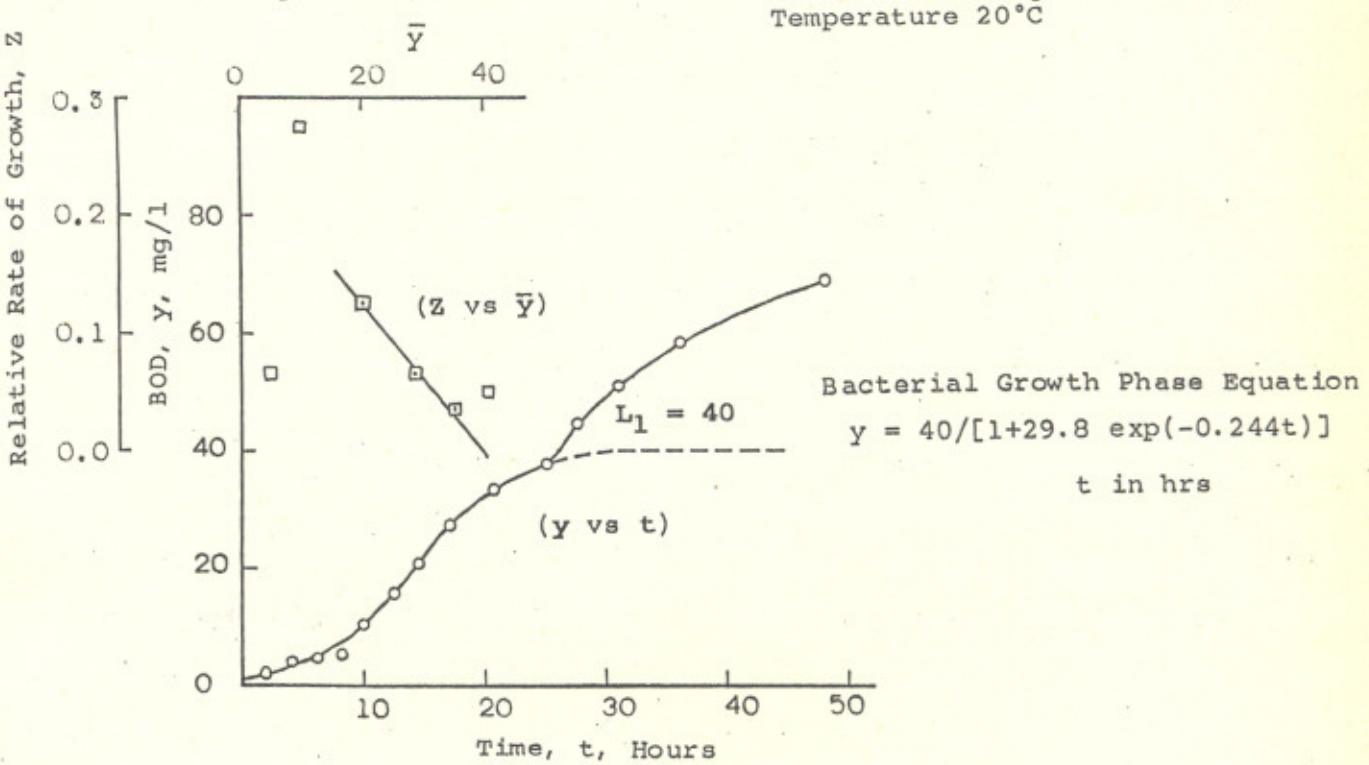


Fig. 6.22 Respirometric Study of Synthetic Waste



Apparatus:
Respirometric BOD Bottle,
Rectangular, Two Compartments
Respirometer Constant r_2, Table 6.7
Substrate 1:1 Glucose
Glutamic Acid 100 mg/l
Acclimated Sewage Seed 5 ml/l
Temperature 20°C

$$Z = [\ln(y_2/y_1)]/\Delta t \qquad \bar{y} = (y_1 + y_2)/2$$

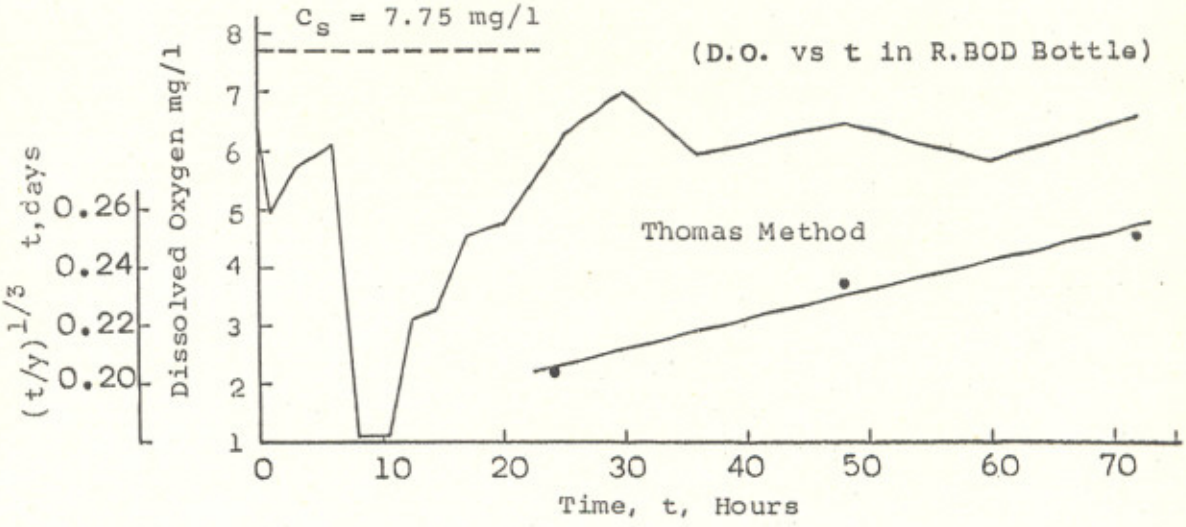


Bacterial Growth Phase Equation

$$y = 40/[1+29.8\ \exp(-0.244t)]$$

t in hrs

Fig. 6.23 Respirometric Study of Synthetic Waste



$Z = [\ln(y_2/y_1)]/\Delta t \quad \bar{y} = (y_1 + y_2)/2$

Apparatus:
Respirometric BOD Bottle,
Rectangular, Three Compartments
Respirometer Constant r_3, Table 6.7
Substrate 1:1 Glucose
Glutamic Acid 200 mg/l
Acclimated Sewage Seed 5 ml/l
Temperature 20°C

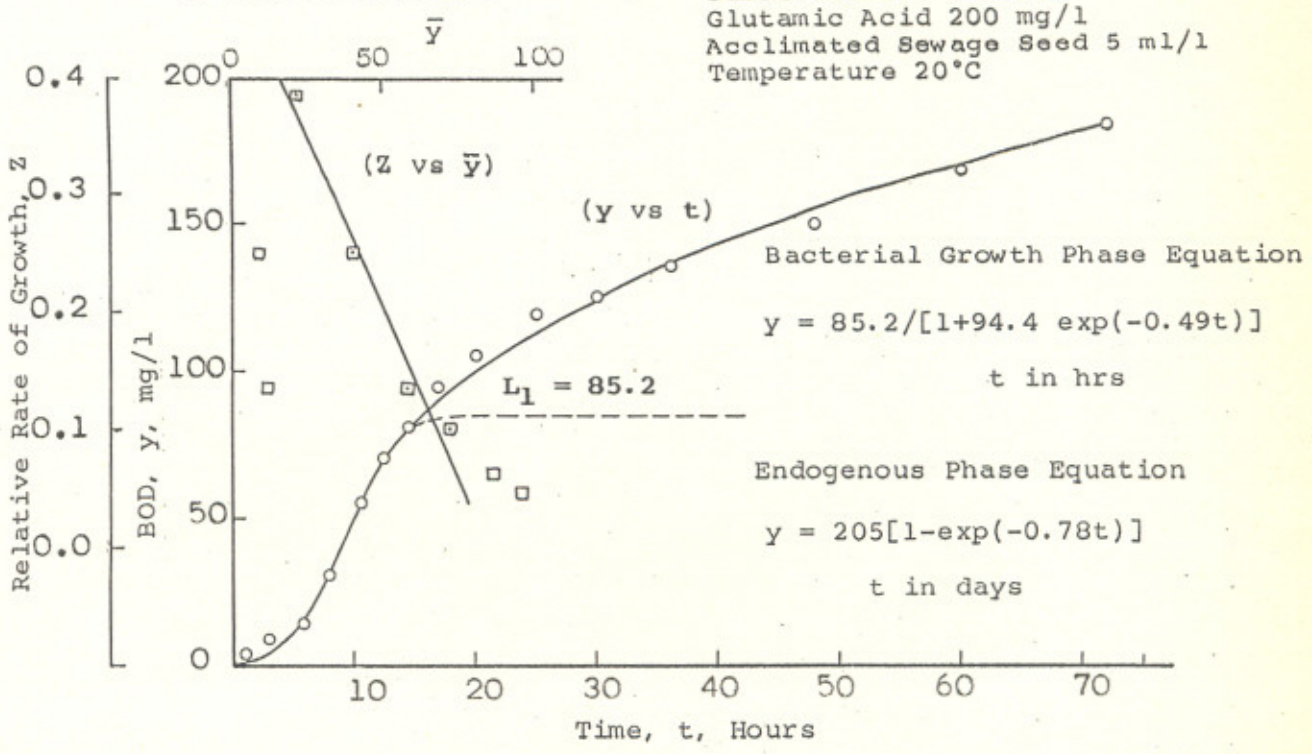


Fig. 6.24 Respirometric Study of Synthetic Waste

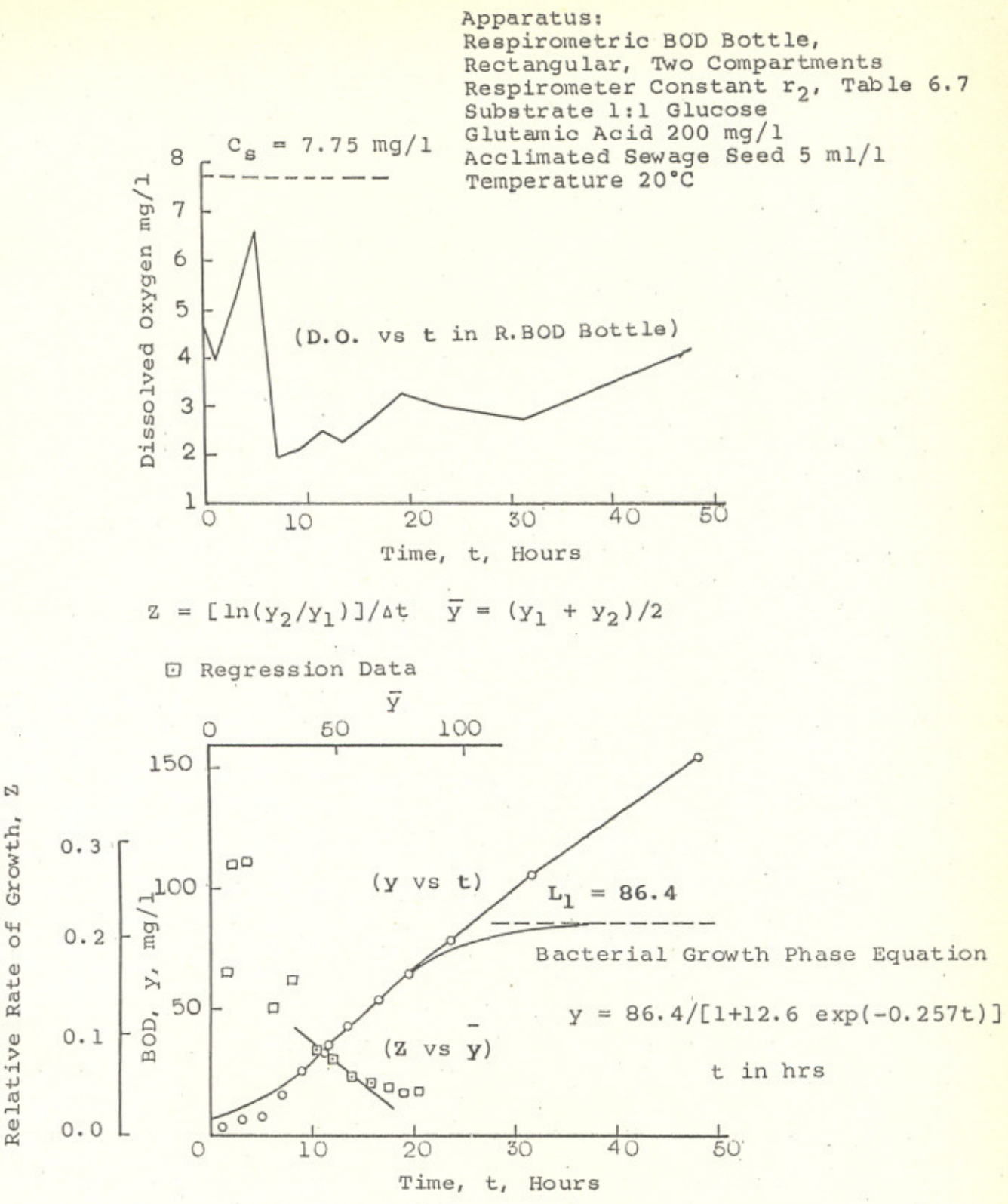
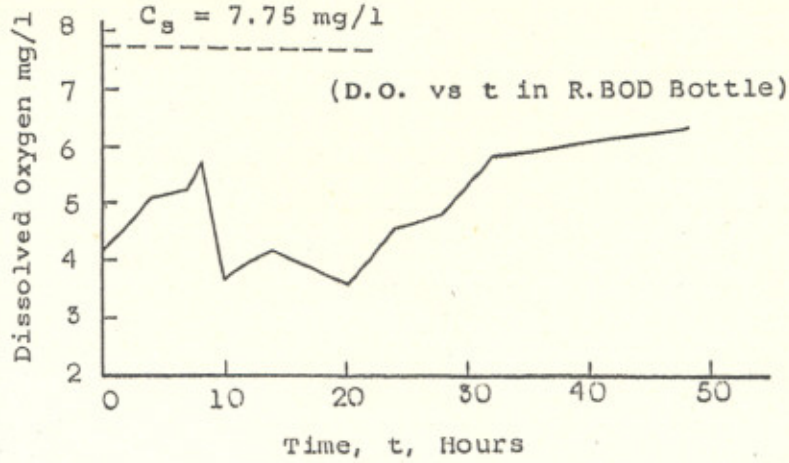


Fig. 6.25 Respirometric Study of Synthetic Waste



Apparatus:
Respirometric BOD Bottle,
Rectangular, Three Compartments
Respirometer Constant r_3, Table 6.7
Substrate 1:1 Glucose
Glutamic Acid 200 mg/l
Acclimated Sewage Seed 5 ml/l
Temperature 20°C

$$z = [\ln(y_2/y_1)]/\Delta t \quad \bar{y} = (y_1 + y_2)/2$$

⊡ Regression Data

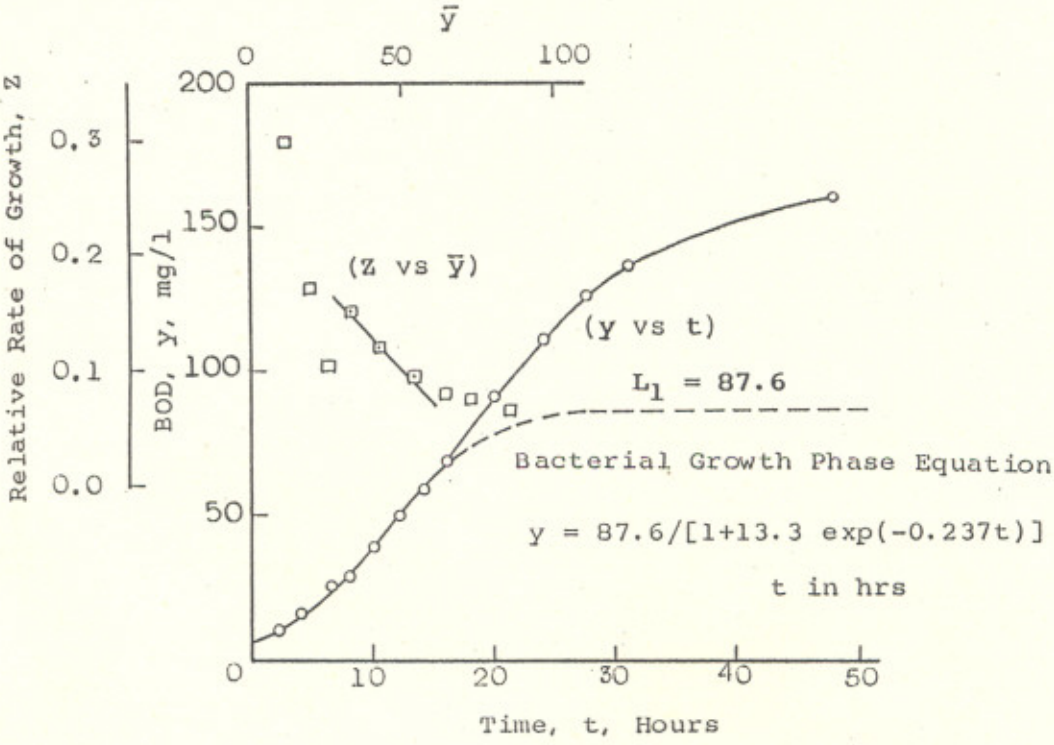


Fig. 6.26 Respirometric Study of Synthetic Waste

6.3.2.1 D.O. Profiles

The trend of D.O. profiles as seen in Figs. 6.22 to 6.26 is similar to the recorder plots for respirometer (Figs. 6.8 to 6.21) i.e. a rapidly decreasing downward leg followed by an increasing upward leg till the curves taper off. However following variations were observed.

The D.O. profile of Fig. 6.22 shows an initial low rate of oxygen uptake for 50 mg / l of glucose and glutamic acid waste. D.O. profiles of Figs. 6.23 to 6.26 show initial sag lasting for 5 to 8 hours for 100 and 200 mg / l of glucose and glutamic acid as substrate. In the D.O. prfiles of R.BOD bottles (Figs 6.22 to 6.26), sags can be seen in cycles.

6.3.2.2 BOD Exertion

The BOD exertion curves (Figs. 6.22 to 6.26 show that a lag of variable length occurs which is followed by a rapid first stage of oxygen uptake followed by a plateau (Figs 6.22 and 6.23).

Lag was not observed in experiments conducted in the respirometer with glucose glutamic acid when acclimated sewage was used as seed. In R.BOD bottle experiments initial lag was observed (Figs. 6.22 to 6.26) although acclimated sewage was used as seed. Sags observed during lag phase in R.BOD bottle experiments (Figs. 6.23 to 6.26) are not explainable with the only exception that lag in these experiments is due to the quiescent conditions existing in the R.BOD bottles.

Plateau was not discernible at 200 mg/l of Glucose Glutamic acid (Figs. 6.24 to 6.26). Bhatla and Gaudy (1964) have also

reported disappearance of the plateau as the concentration of substrate is increased.

Maximum BOD exerted (30%) during log growth phase in the quiescent R.BOD system is less than the maximum BOD exerted (38%) during log growth phase in the turbulent respirometric system. Due to diffusional resistance to mass transfer of the substrate, in the quiescent R.BOD system establishes a concentration gradient limiting the availability of substrate at the cell surface. This in turn resulting in early termination of the log growth phase in the R.BOD system.

Sags observed in cycles after the bacterial growth phase are due to lysis-synthesis-lysis reactions of biomass i.e. cyclic degradation of biomass due to extreme limitations on food supply caused by the quiescent conditions in the R.BOD bottle.

6.4 KINETICS OF BOD EXERTION

The most prevalently used kinetic model for BOD exertion is the first order decreasing rate characterized by the first stage ultimate BOD and a rate constant K_1. These constants are estimated by finding daily BOD exertions for 5 days. In doing so, the logarithmic growth phase of bacteria (sometimes considered as a lag phase) and the plateau between the bacterial and protozoan phases of oxygen uptake are neglected. First order decreasing rate law represents the occurences in the BOD bottle from the end of first day to end of fifth day of incubation but the happenings in the BOD bottle during the first 24 hours are not truly represented by the law.

The food to microorganism ratio, in the BOD bottle, permits the development of a log growth phase. In all probability the log growth phase is over within 24 to 48 hours. Log growth phase may signify only 25% of the ultimate BOD, but it is important to explore it to know the depletions in D.O. in the receiving body of water. The data from the growth curve is needed for designing aerobic wastewater treatment systems.

The course of BOD exertion needs to be traced almost continuously for the study of events taking place during the first 24 hours. Even though the intial events taking place in the BOD bottle are traced and analysed, the analytical information, derived from the dilute environment existing in the BOD bottle, will be of little value as direct input to various phases of water pollution control.

Concentration of substrate is considerably high in the respirometer and R.BOD bottle. In order to formulate the course of BOD exertion, the data (BOD exerted) generated by the recorder plots of Fig.6.19(a) was analysed by the Thomas Method. It can be seen from the trend of $(t/y)^{1/3}$ vs t Fig.6.27, that the BOD curve consists of three portions, initial downward leg of the Thomas plots representing the first order increasing rate, the slowly increasing last leg representing the first order decreasing rate and the portion interconnecting the two legs as the declining growth phase and plateau. In case the BOD curve is formulated as intial first order increasing rate representing the log growth phase, and the last leg by first order decreasing rate, the

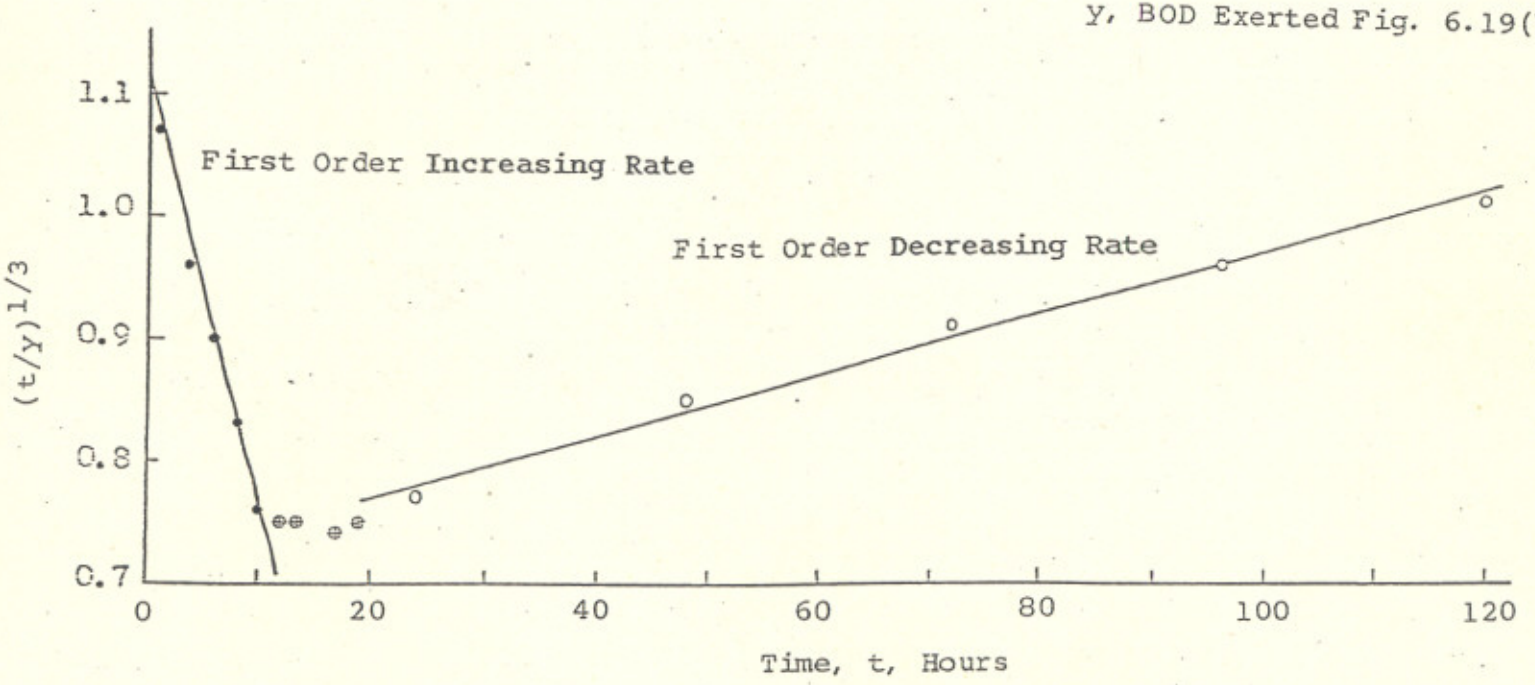


Fig. 6.27 Thomas Plots for BOD Exertion in Respirometer

plateau portion will still be neglected. In order to suitably formulate the discontinuous BOD curve, recourse was taken to the stoichiometery of the system i.e. the initial bacterial growth occuring through lag (if any), log growth, declining growth and the plateau marking the end point of the exogenous BOD reaction and the subsequent endogenous phase. During bacterial growth, exogenous substrate is used as food whereas in the endogenous phase lysis-synthesis-lysis and/or protozoan growth takes place at the expense of bacteria that had grown during the bacterial growth phase. Accordingly, the discontinuous BOD exertion curve was bifurcated into two portions and formulated separately.

6.4.1 Kinetics Of Bacterial Growth Phase

During bacterial growth phase, active cell growth takes place during logarithmic and declining growth phases. Cell population remains static during the plateau and the lag, however, increase in cell mass takes place during the lag. Zero order kinetics are observed during lag (if any) in the growth of bacteria. In order to formulate the kinetics of bacterial growth phase, recourse was taken to the various growth curves (Sec. 3.3.2). The last growth curve (logistic curve) of equations (3.5) was selected to represent the kinetics of bacterial growth phase. A portion of the substrate is channelled into cell mass and the remaining utilized for respiration. A constant ratio of synthesis to respiration has been assumed during the entire bacterial growth phase and the size of cell population reached is represented by the BOD exerted and the size of cell population remaining to be reached is represented by the BOD remaining to be exterted till the saturation population of

the cells is reached. With these considerations, the logistic curve (differential form) is (equation 3.6)

$$(dy/dt) = K_s\, y\, (L_1 - y) \qquad \ldots.(3.6)$$

Where (dy/dt) = rate of BOD exertion.

K_s = second order rate constant of logistic curve in the differential form.

y = BOD exerted.

$(L_1 - y)$ = BOD remaining to be exerted till the saturation population of cells is attained.

L_1 = BOD exerted till the attainment of saturation population of cells and exogenous substrate is exhausted.

How best the bacterial growth phase is represented by the logistic curve is seen from the following,

(i) when y is small in comparison to L_1, $L_1 - y \simeq L_1$, and $(dy/dt) = K_s y$ representing the log growth phase.

(ii) when y is large but less than L_1, the rate goes on decreasing (due to decreasing value of $L_1 - y$) thus representing the declining growth phase.

(iii) when $y \simeq L_1$, $L_1 - y \simeq 0$, the rate decreases to a bare minimum thus representing the plateau.

However the zero order kinetics of lag phase can only be approximately represented if y is extremely less and remains constant. However BOD exerted y is not constant during lag phase.

The logistic curve has two asymptotes ($y=0$ and $y=L_1$). The upper asymptote represents the plateau in BOD exertion, and the value of BOD exerted (L_1) is termed as **limiting growth BOD.**

The logistic curve (equation 3.6) upon integration becomes

$$y = L_1/[1+m \exp(-nt)] \quad \ldots.(3.5)$$

Where $n = K_s L_1$ and $m = (L_1-y_o)/y_o$ and $y=y_o$ when $t=o$

The parameters of the logistic curve (L_1, m and n) are estimated as explained in sec. 3.3.4 and illustrated in Appendix I.

The results of the kinetics of bacterial growth phase during the course of BOD exertion, in the respirometer and R.BOD bottle, are presented in Figs.6.8 to 6.26 and are discussed with reference to the following :

6.4.1.1 Relative Rate of Growth (Z)

The relative rate of growth is the logarithmic differential co-efficient dervied from equation (3.6)

$$\Delta \ln(y)/\Delta t = [\ln (y_2/y_1)]/\Delta t$$

and is denoted by $Z = [\ln (y_2/y_1)]/\Delta t = K_s(L_1-y)$ $\quad \ldots.(3.7)$

Here (y_2/y_1) is the proportional increase in BOD exerted during the time interval $\Delta t = t_2-t_1$ and y_1, y_2 are the BOD exerted at time t_1 and t_2 respectively.

During cell growth and hence BOD exertion physiological changes occur in the cells in going from the log phase, declining

growth to stationary growth. Metabolic acclimation or population selection will affect the growth process and hence the BOD exertion. Release of toxic end products during growth will also affect the BOD exertion curve.

A special problem arises in the estimation of parameters of the logistic curve because the observations are usually not stochastically independent due to the various influences listed above. In order to estimate the constants of the logistic (growth) curve, relative rates of growth (Z) or successive rates of growth, (in the instance BOD exerted) are considered to be stochastically independent.

In Figs. 6.12, 6.13, 6.14 and 6.22 to 6.26, initial relative rates of growth (Z) have a different trend due to the metabolic acclimation needed before the cells enter the log phase. Fairly uniform trend in Z is observed in Z vs $\bar{y}$ curves in Figs.6.9, 6.10, 6.16, 6.17, 6.19(b), 6.20(b), 6.21(b). Approximately uniform trend in Z was observed in Fig.6.21(b) (glucose with acclimated seed) inspite of the apparent lag phase observed in the recorder plot 6.21(a). Trend in Z was not uniform in Figs.6.8, 6.11,6.15, 6.18, 6.24, 6.25 and 6.26 that may be due to any one or more of the causes listed above.

6.4.1.2 Limiting Growth BOD (L_1)

Limiting growth BOD although is synonymous with plateau BOD but differs in a sense that it is the BOD exerted till the total available oxygen demand is partitioned into synthesis and respiration. Thus it is mathematically estimated value of BOD

exerted whereas plateau BOD is experimentally estimated. Limiting growth BOD, as expected is slightly more than the plateau BOD.

L_1 is estimated from Z vs $\bar{y}$ curve as explained in Appendix I. Variable values of y will be obtained when the plateau is not discernible. For example (Fig.6.26), slope of Z vs $\bar{y}$ line gradually decreases in the plateau region and if the next data point is also included in the regression analysis, value of L_1=98 and n=0.218. However the value of L_1 and n are not out of proportion but it remains to be seen whether the next data point should justifiably be included or not. With a little more analytical effort, that portion of Z vs $\bar{y}$ curve is tapped so that the computed y values are as close as possible to the observed y values.

Limiting growth BOD represents the energy oxygen required for cell synthesis and cell maintenance. Maintenance energy requirements are minimum during active cell replication (log and declining growth phases) but are maximum during lag and stationary phases. It is difficult to partition L_1 into energy oxygen for cell synthesis and for cell maintenance. It is assumed that L_1 is the energy oxygen for cell synthesis only. According to the stoichiometry of the BOD system, remaining fraction of theoretical oxygen demand ($1-L_1/TOD$) is converted to bacterial cells. Assuming bacterial cell formulation of Porges et al. (1956) as $C_5H_7NO_2$ for mass mixed cultures which have theoretical oxygen demand of 1.414 g /g and that pure bacteria are 90 per cent volatile, the yield co-efficient may be worked out by the equation,

yield co-efficient (y_c) = $(1-L_1/TOD)/(1.414)$ ....(6.1)

y_c = solids formed per gram of BOD removed.

In the respirometer experiments with glucose, glutamic acid as substrate (Figs.6.8 to 6.20), L_1 varied between 40 to 52% of the theoretical oxygen demand and the yield co-efficient varied between 34 to 42% of the theoretical oxygen demand.

In R.BOD bottle experiments, L_1 varied between 38.8 to 42.6% of TOD and y_c varied between 40.6 to 43.3% of TOD.

Yield co-efficient obtained in the present investigations is in close agreement with previous reported data of Servizi and Bogan (1963) and Eckenfelder and Weston (1956).

6.4.1.3 Relationship between Limiting Growth BOD and Theoretical Oxygen Demand (TOD)

Results of regression between L_1 and TOD are presented in Fig.6.28. Co-efficient of correlation of 0.98 shows that a fixed ratio of TOD is partitioned into synthesis and respiration. Maximum variations in L_1 were observed with 50 and 100 mg/l of glucose glutamic acid. Standard deviations of L_1 at 50, 100 and 200 mg / l are 4.3, 4.1 and 0.6 per cent of TOD. The standard deviations are within the limits of experimetal error.

A 1:1 mixture of glucose, glutamic acid and also glucose alone was used as the substrate for finding the limiting growth BOD. Direct experimental determination of cell yield is cumbersome. Cell yield and theoretical oxygen demand of a waste can be predicted from the limiting growth BOD.

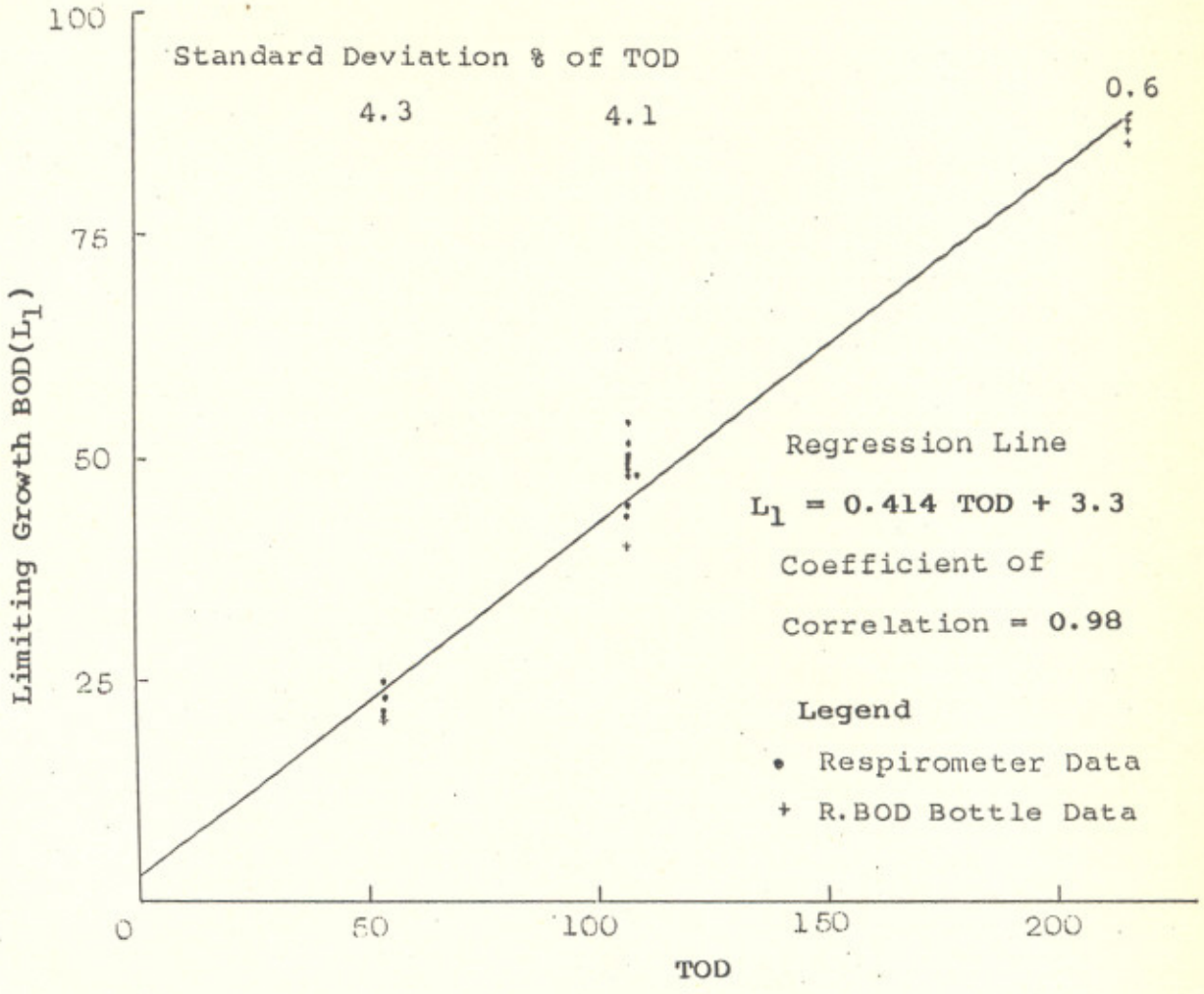


Fig. 6.28 Regression Between Limiting Growth BOD (L_1) and Theoretical Oxygen Demand

6.4.1.4 Parameters m and n of the Logistic Curve

With two fixed asymptotes (y=0 and $y=L_1$), the logistic curve can be traced for various values of m and n. When value of m is very high (Figs.6.13,6.14), the computed initial y values are less than the corresponding observed y values. This suggests metabolic changes in the cells from lag phase to log growth.

Values of n observed in the present investigations varied from 0.187 to 0.49. More work needs to be undertaken to explain the observed variations in n.

6.4.2 BOD Exertion During Endogenous Phase

Endogenous phase was formulated as a first order decreasing rate law and the results are shown in Figs. 6.19(b), 6.20(b), 6.22 and 6.24. K_1 and L were calculated by Thomas and Moments methods (Figs.6.19b, 6.20b). Ultimate BOD values agreed well but variations in K_1 were observed. In Fig.6.19(b), three day sequence K_1 value was less than the 5-day sequence K_1 value whereas reverse of this was noted in Fig.6.20(b).

In Fig.6.19(b), nitrogenous BOD was exerted thereby raising the 5-day sequence K_1 value. In Fig.6.20(b), the curve tapers off to a decreasing slope due to absence of nitrification which depresses the 5-day sequence K_1 value.

Except in Fig.6.22, K_1 values observed in the present investigations are high than the reported K_1 values in BOD bottle and other respirometric studies by various workers. This is possibly due to the reasons that,

(i) substrate concentration in the respirometer and R.BOD bottle is not dilute as in the BOD bottle.

(ii) carbon dioxide produced due to biological activity is in equilibrium with the atmospheric carbon dioxide through the polyethylene membrane.

6.5 ULTIMATE BOD IN THE RESPIROMETER

Ultimate BOD(L) is mathematically calculated from the first order decreasing rate model. Also 20 day BOD value in the standard BOD method represents L value. The experiments (Figs.6.19,6.20) were carried out continuously for 5-days in the respirometer with 100 mg/l (Glucose glutamic acid as substrate). The D.O. probe was calibrated initially and also checked at the end of the experiment. D.O. was determined by siphoning out a sample from the respirometer at the end of the experiment.

Ultimate BOD was exerted (Fig.6.19b) in 3.75 days and the D.O. deficit in the respirometer was 0.7 mg/l. At the end of 5th day BOD exerted was 117.4 mg/l and D.O. deficit was 0.3 mg/l. At the end of 5th day, BOD exerted was more than the theoretical oxygen demand. To find the cause of this another experiment (Fig.6.20a,b) was carried out in which nitrification was inhibited (with 0.1 M NH_4-N) and at the end of 4th day 94.8 mg/l of BOD was exerted and the D.O deficit was 0.6 mg/l. At the end of 5th day 103 mg/l of BOD was exerted and the D.O. deficit was 0.3 mg/l.

Thus in Fig.6.19(b) ultimate carbonaceous as well as nitrogenous BOD was exerted at the end of 5th day and in

Fig.6.20(b), ultimate carbonaceous BOD was exerted at the end of 5th day.

At the end of 24 hours about 50 per cent of ultimate BOD is exerted.

This may be attributed to high rate of BOD exertion during the synthesis of cells and high K_1 values during the endogenous phase. However further work needs to be undertaken before deriving any definite conclusions.

6.6 RELATIONSHIP BETWEEN 2-DAY RESPIROMETRIC BOD $(BOD_2)_R$ AND 5-DAY BOD BY THE DILUTION METHOD $(BOD)_5$

Results of regression between $(BOD_2)_R$ and $(BOD)_5$ are presented in Fig.6.29 along with the regression lines. The results of regression show a direct correlation between $(BOD_2)_R$ and $(BOD)_5$. Thus the 5-day BOD may be predicted from the two day BOD in the respirometer and R.BOD bottle.

6.7 SUMMARY AND CONCLUSIONS

As the solubility of oxygen in water is limited, the wastewater sample needs to be diluted for BOD determination. The dilute environment of the BOD technique represents neither the environment of the receiving stream nor that of a wastewater treatment plant. Moreover, different results of BOD are obtained with different dilutions of the wastewater in the standard BOD procedure.

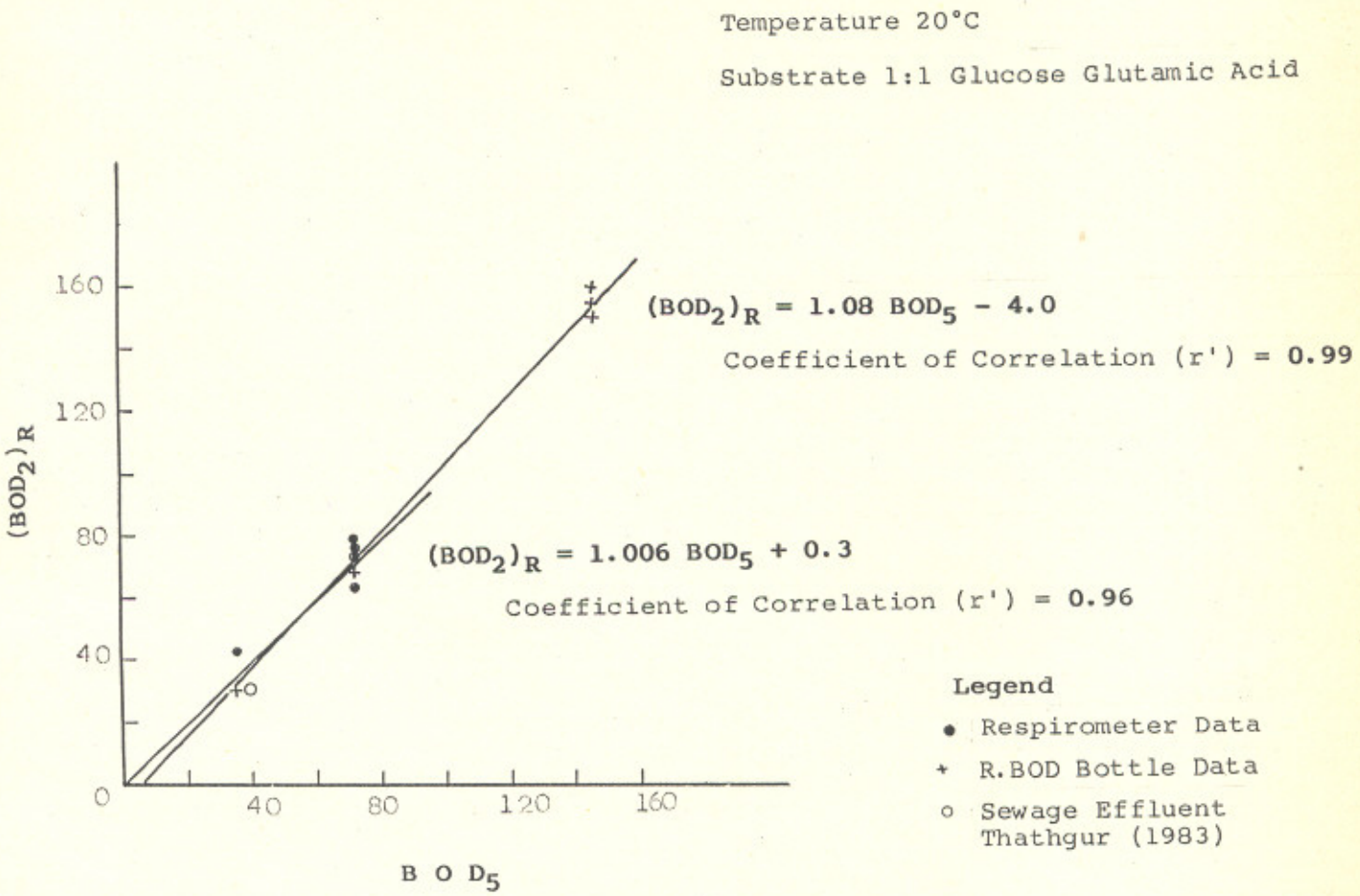


Fig. 6.29 Regression between Two Day Respirometric BOD and Standard Five Day BOD

To overcome this limitation of the BOD test, improvised respirometric BOD systems were fabricated. The property, that polyethylene films are permeable to oxygen, was exploited in the fabrication of a respirometer and disposable respirometric BOD bottles (R.BOD bottles). The contents of the respirometer were continuously stirred to increase the effective area to volume ratio. In R.BOD bottles area to volume ratio was increased by the particular construction and a system of compartments in the R.BOD bottle. The inward movement of the four sides of the polyethylene chamber was prevented by stiffening frame(s), thereby allowing only part inflation of the chamber. With this arrangement, the volumetric capacity of the chamber was considerably decreased which resulted in increase in area to volume ratio. R.BOD bottles having different area to volume ratio were fabricated. Increase in area to volume ratio resulted in increase in the rate of oxygen transfer in the respirometric systems. The respirometric system were also standardized.

Stagnant film model was applied to the mass transfer in the respirometer. Mass transfer in the R.BOD bottle was modelled on the Higbie penetration model. To account for the resistance of the membrane, an analogy was drawn between conduction of heat through infinite solids having surface and internal resistance. Analogous conclusions were drawn viz. the average saturation deficit lags behind the interface saturation deficit and the average saturation deficit and the interface saturation deficit behave as if the contents of the bottle are stirred. Transfer lag for various R.BOD bottles was estimated. The diffusion data was segregated into

different phases and mass transfer co-efficients were estimated. The respirometer constants increased with decreasing thickness of polyethylene membranes and by increasing area to volume ratio of the R.BOD bottle. Branched low density (0.916) polyethylene films of 60 and 100 gauge thickness were used in the present investigations. Variance in D.O. concentrations of replicate samples from R.BOD bottles were calculated (Appendix II). The standard deviation varies in the range of 0.12 to 0.22 mg/l of D.O. for various types of R.BOD bottles. Temperature dependence of respirometer constants and solubility constants were also studied. Respirometer constants increased with increase in temperature whereas the solubility constants decreased with rise in temperature.

Study of BOD exertion of glucose and glutamic acid in 1:1 ratio (50 to 100 mg/l) was carried out in the respirometer and D.O. was continuously recorded. The sag curves reveal the happenings during the course of BOD exertion. The lag phase (if any), log growth phase, declining growth phase are clearly discernible in the recorder plots. An apparent lag phase was observed with glucose although acclimated sewage seed was used in the study.

BOD exertion in the respirometer and R.BOD bottles shows an initial rapid rate of oxidation followed by endogenous respiration. The two phases are separated by a plateau. The plateau is not discernible in substrate concentrations of 200 mg/l in R.BOD bottle experiments. Lysis-synthesis-lysis reactions are clearly indicated in the cyclic nature of the sags observed in R.BOD bottle experiments.

Kinetics of BOD exertion during bacterial growth phase were represented by the logistic curve. The upper asymptote ($y=L_1$) of the logistic curve represented the stoichiometric end point of the BOD reaction and L_1 was defined as the BOD exerted till the exogenous substrate is exhausted. BGP lasted for about 24 hours.

L_1 varied between 40 to 52% of TOD. L_1 value was slightly less in the case of R.BOD bottle experiments due to the quiescent conditions prevailing in the respirometric system. Yield co-efficients calculated from L_1 agreed well with the data reported by previous workers.

Regression between L_1 and TOD showed a co-efficient of correlation of 0.98. The two day respirometric BOD and five day standard BOD agreed well. A co-efficient of correlation of 0.99 was obtained.

The following conclusions are drawn from the results presented herein :

(i) BOD test can be performed in the respirometer and disposable respirometric BOD bottles. The wastewater sample need not be diluted since oxygen diffuses through the LDPF as it is consumed due to biological activity. The rate of diffusion of oxygen is proportional to the D.O. saturation deficit in the respirometer and R.BOD bottle.

Further the system would afford study of the biodegradability of wastes.

(ii) The exertion of BOD in the two respirometric BOD systems presents a more detailed information. The state of happenings during the course of BOD exertion can be continuously traced and valuable information regarding the existence of lag phase, the extent of log and declining growth and the plateau can be completely pictured in the two respirometric BOD systems.

(iii) The carbon dioxide and oxygen levels in the respirometer and R.BOD bottles are in equilibrium, through the LDPF, with the atmospheric air. In addition, the wastewater concentration is not dilute and it would provide simulated conditions as existing in a receiving stream or wastewater treatment plant.

(iv) Simulated stream studies can be carried out in the respirometer and the D.O. levels in a stretch of a stream can be determined experimentally in the laboratory.

(v) The effect of turbulance, on the course of happenings during BOD exertion, can be separated, since BOD exertion study can be carried out under quiescent as well as turbulent conditions and that too without diluting the wastewater sample.

(vi) The logistic curve depicts the log growth, declining growth and plateau and fits well to the bacterial growth phase.

(vii) A new term viz. limiting growth BOD (L_1) is introduced into the literature. L_1 marks the stoichiometric end point of the BOD reaction and is mathematically estimated by fitting a logistic curve to the bacterial growth phase.

(viii) An estimate of yield co-efficient can be obtained from L_1 and an assumed formulation of the cells.

(ix) Theoretical oxygen demand of a wastewater can be predicted from the limiting growth BOD.

(x) The 5-day BOD of a wastewater as obtained in the dilution method can be predicted from the 2-day BOD value of the wastewater in the respirometer and R.BOD system.

A P P E N D I C E S

APPENDIX I

Calculations of BOD Exertion

(Reference Fig.1 and Table 1)

The recorder plot (D.O. vs. t plot) was discretized into segments having approximately uniform slopes. The BOD exerted was calculated by the integration of basic differential equation of the sag curve (Issacs and Gaudy, 1967)

$$\pm (dD/dt) + rD = (dy/dt) \qquad \ldots.(1)$$

Where dD/dt represents the change in D.O. deficit due to the combined effects of reaeration and deoxygenation.

r = the respirometer constant.

(dy/dt) = rate of BOD exertion.

Calculations are shown in Table 1 and are self explanatory.

Estimation of the Parameters of the Logistic Curve

(Reference Table 2)

Estimation of L_1

Value of BOD exerted, y, are calculated at equal intervals of time t from (Table 1). Z (equation 3.6) and $\bar{y}$ are estimated. When Z vs $\bar{y}$ curve shows approximately a horizontal trend, plateau is reached (Fig.2). Data prior to the plateau is utilized for regression between Z vs $\bar{y}$, and L_1 is estimated.

Estimation of m and n

By regression between $\ln (L_1-y)/y$ vs t, m and n are estimated (sec. 3.3.4.2). Values of y are computed (Table 2) and the BOD exertion curve is graphed (Fig.2).

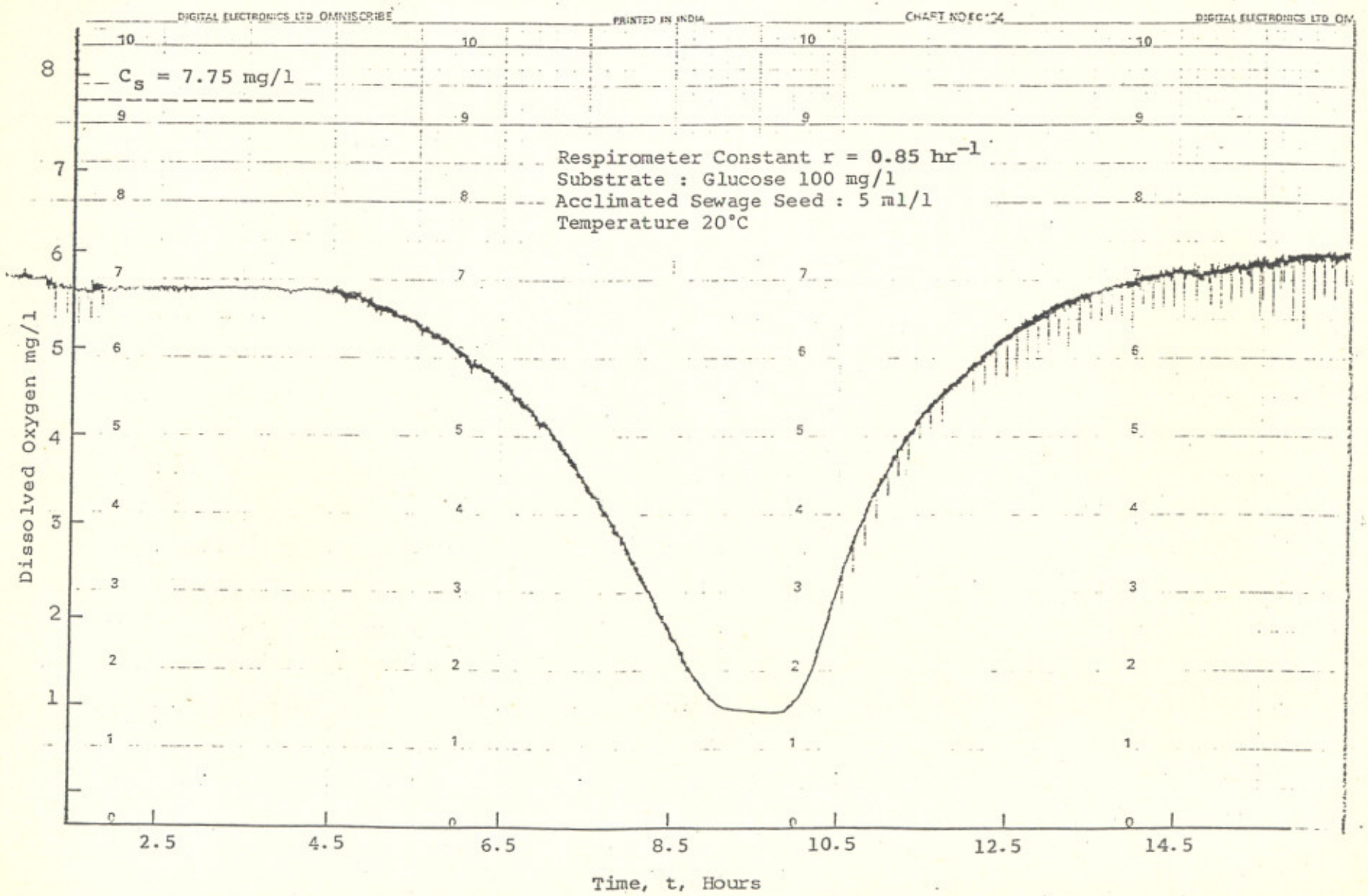


Fig. 1 Respirometric Study of Synthetic Waste (Recorder Plot)

Respirometer Constant r = **0.85 hr^{-1}**
Substrate : Glucose 100 mg/l
Acclimated Sewage Seed : 5 ml/l
Temperature 20°C

$$Z = [\ln(y_2/y_1)]/\Delta t \qquad \bar{y} = (y_1 + y_2)/2$$

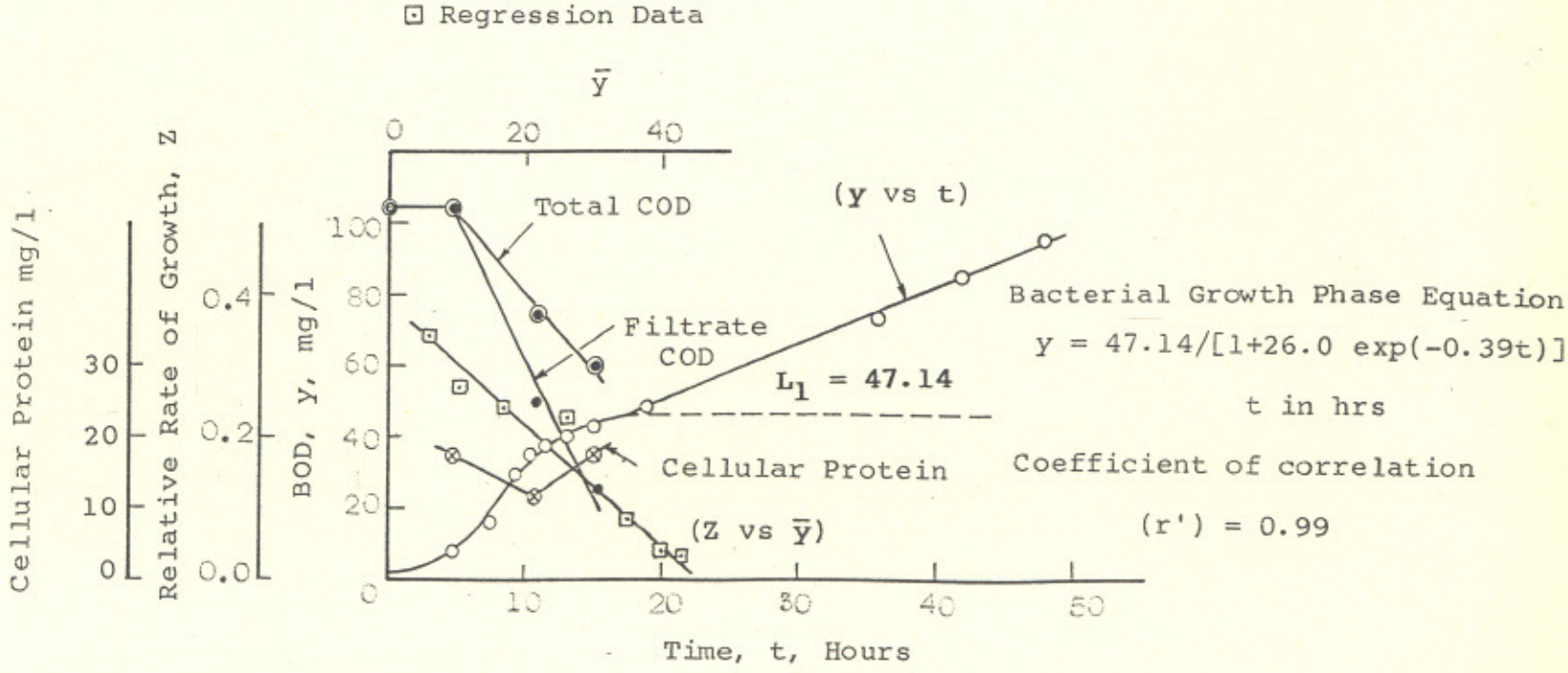


Fig. 2 Respirometric Study of Synthetic Waste

TABLE 1

Respirometric BOD of Synthetic Waste

Temperature : 20°C
Substrate : Glucose 100 mg/l
Seed : Acclimated domestic Sewage 5 ml/l
Respirometer 300 ml capacity (Fig.4.1)
Respirometer constant r = 0.85 hr^{-1}

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)			
Time (t) hrs	Δt hrs	Graph Reading	D.O.Conc. mg/l C	Saturation Deficit(D) (7.75-C)	rD	$\overline{rD}$	$\overline{rD}\,\Delta t$	ΔD	Δy	$\Delta y/\Delta t$	y mg/l	COD mg/l Total	COD mg/l Filt-erate	Protein mg/l
0.0	-	6.80	5.57	2.18	1.85	-	-	-	-	-	-	105	105	-
4.5	4.5	6.80	5.57	2.18	1.85	1.85	8.33	0.00	8.33	1.85	8.33	105	105	17.6
7.3	2.8	5.20	4.18	3.57	3.03	2.44	6.83	+1.39	8.22	2.94	16.55	-	-	-
9.5	2.2	1.50	0.97	6.78	5.76	4.39	9.67	+3.21	12.88	5.85	29.43	-	-	-
10.5	1.0	1.50	0.97	6.78	5.76	5.76	5.76	0.00	5.76	5.76	35.19	75	50	11.8
11.5	1.0	4.00	3.14	4.61	3.92	4.85	4.84	-2.17	2.67	2.67	38.86	-	-	-
12.0	0.5	5.30	4.27	3.48	2.96	3.44	1.72	-1.13	0.59	1.18	39.45	-	-	-
13.0	1.0	6.30	5.13	2.62	2.23	2.59	2.59	-0.86	1.73	1.76	40.18	-	-	-
15.0	2.0	7.20	5.91	1.84	1.56	1.90	3.80	-0.78	3.02	1.51	43.20	60	15	17.6
19.0	4.0	7.40	6.09	1.66	1.41	1.48	5.92	-0.18	5.74	1.44	48.94	-	-	-
36.0	17.0	7.40	6.09	1.66	1.41	1.41	23.97	0.00	23.97	1.41	72.91	-	-	-
42.0	6.0	6.60	5.39	2.36	2.01	1.71	10.26	+0.70	10.96	1.83	83.87	-	-	-
48.0	6.0	7.30	6.00	1.75	1.49	1.75	10.50	-0.61	9.89	1.65	93.76	-	-	-

TABLE 2

Estimation of Kinetic Constants for data of Table 1

Bacterial Growth Phase

Estimation of L_1				::	Calculation of m and n				
Time t hrs	BOD y mg/l	$\bar{y}$	$Z=\frac{\ln(y_2/y_1)}{\Delta t}$	::	Time t hrs	BOD y mg/l	$(L_1-y)/y$	Computed $[(L_1-y)/y]'$	Values $[y]'$
				::					
0.0	0.00	-	-	::	0.0	0.00	-	26.00	1.74
2.0	3.70	-	-	::	4.5	8.33	4.66	4.50	8.57
4.0	7.40	5.55	0.347	::	7.3	16.55	1.85	1.51	18.78
6.0	12.73	10.06	0.271	::	9.5	29.43	0.60	0.64	28.72
8.0	20.62	16.67	0.241	::	10.5	35.09	0.34	0.43	32.86
10.0	32.31	26.46	0.224	::	11.5	37.76	0.25	0.29	36.42
12.0	38.35	35.33	0.086	::	12.0	38.35	0.23	0.24	37.95
14.0	41.58	39.97	0.041	::	13.0	40.08	0.18	0.16	40.50
16.0	44.54	43.07	0.034	::	15.0	43.10	0.09	0.07	44.06
18.0	47.40	45.97	0.031	::					

Regression : Z vs $\bar{y}$

Slope $= K_s = -0.0081$

y - Intercept $= K_s L_1 = 0.382$

$L_1 = 47.14$

Co-efficient of correlation (r') = 0.97

Regression : $\ln (L_1-y)/y$ vs t

Slope $= n = -0.390$

Antilog (y - Intercept) $= m = 26$

(r') = 0.99

Check

y - Intercept (Z vs $\bar{y}$) = slope $\ln (L_1-y)/y$ vs t

or 0.382 - 0.390

Bacterial Growth Phase Equation

$y = [47.14]/[1+26.0 \exp(-0.39t)]$

APPENDIX II

Estimates of Variances

(Reference to Tables 6.3 to 6.6)

Variances inbetween respirometric BOD bottles (variance of D.O. concentration of the three replicate R.BOD bottles tested at each time) at various times were calculated. Homogeneity of the variances, for each type of R.BOD bottle, was tested by the Bartlett Test. Chi square was the test static.

Computed χ^2 was not significant for P=0.05 except in the case of circular respirometric BOD bottle (Table 6.6). A close examination of the data shows that a single reading of D.O. concentration at S.No. 6 (Table 6.6) is out of proportion. After rejecting this D.O. value, computed χ^2 was not significant.

The variances for D.O. values inbetween R.BOD bottles were pooled. The results are summarized in Table 1.

TABLE 1

Variances and Standard Deviations

Type of R.BOD Bottle	Variance	Standard Deviation	n	N
Rectangular, Single Compartment	0.024	0.15	3	30
Rectangular, Two Compartments	0.015	0.12	3	30
Rectangular, Three Compartments	0.042	0.20	3	27
Circular, Single Compartment	0.05	0.22	3	24

REFERENCES

1. Arthur, R.M. (1964). "An Automated BOD Respirometer" Proc. 19th Ind. Waste Conf. Purdue University., pp.628-637.

2. Astarita, G. (1967) "Mass Transfer with Chemical Reaction" Elsevier Publishing Co. New York,

3. Barrer, R.M. (1941) "Diffusion in and through solids" Cambridge University Press.

4. Bhatla, M. N. and A.F. Gaudy, Jr. (1964) "Studies on the causation of Phasic Oxygen uptake in High Energy systems" Proceedings, 19th Industrial Waste Conference Purdue University, Lafayette.

5. Bhatla, M.N. and A.F. Gaudy, Jr. (1965a) " Role of Protozoa in the Diphasic Exertion of BOD" Jr. Sanit.Eng. Div. Amer. Soc. Civil Eng. Vol.91, SA3, pp.63-87,

6. Bhatla, M.N. and A.F. Gaudy (1965b) "Studies on the Plateau in Oxygen uptake during exertion of BOD by Pure cultures of Bacteria" Biotechnology and Bioengineering" Vol.7, pp387-404,

7. Bogan, R.H. (1958) "Biochemical Degradation Products - A new Dimension in Stream Pollution" Sewage and Ind. Wastes, Vol.30, p.208.

8. Busch, A.W. (1958) "BOD Progression in Soluble Substrates" Sewage and Industrial Wastes, Vol.30, p.1336,

9. Busch,A.W. (1959) "Theory and Design of Bench Scale Units for Biological Oxidation Studies" Water and Sewage Works, Vol.106, No.6, p.254,

10. Busch,A.W. and N. Myrick (1961) "Aerobic Bacterial Degradation of Glucose". J. Water Pollution control Fed. Vol.33, p.897,

11. Buswell, A.M., I. Van Meter, and J.R. Gereke (1950) "Study of Nitrification Phase of the BOD Test" Sewage and Ind.Wastes, Vol.22, p.508,

12. Caldwell, D. H. and W.F. Langelier (1948). "Manometric Measurement of the Biochemical Oxygen Demand of Sewage" Sewage Works J, Vol.20, pp.202.

13. Canale, R.P. and F.Y. Cheng (1974) "Oxygen Utilization in Bacterial-Protozoan Community" Journ. of Env. Engg. Div., Proc. ASCE, Vol. 100, pp.171-187,

14. Clark, J.W. (1960) "Continuous Recording BOD Determination" Water and Sewage Works Vol.107, R-55.

15. Crank, J. and G.S. Park (1968) "Diffusion in Polymers" Academic Press, London,

16. Doelle, H. W., K. N. Ewings and N. W. Hollywood (1982) "Regulation of Glucose Metabolism in Bacterial Systems" in Microbial Reactions in Advances in Biochemical Engg. Ed. Fiechter Vol.23 A, pp.1-35.

17. Downing, A. (1966) "Advances in Water Quality Improvement". Vol.1, University of Texas Press, Austin. Texas.,

18. Eckenfelder, Jr. W.W. and R.F. Weston (1956) "Kinetics of Biological Oxidation" Reinhold Publishing Corporation.

19. Eckenfelder, Jr. W.W. (1970) "Water Quality Engineering for Practising Engineers" Canner Books Boston. Massachusettes,

20. Eldridge, E.F. (1933) "More About BOD Determinations" Sewage Works Journ. Vol.5, p.788,

21. Eye, J.D., L.H. Reuter and K. Keshavan (1961) "New Platinum Electrode System Measures DO and BOD" Wat. Sewage Wks. Vol.108, pp.R-55.

22. Eye, J.D. and C.C. Ritchie (1966) "Measuring BOD with a Membrane Electrode System". J.Wat Pollut. Control Fed. Vol.36, pp.1430-1440.

23. Fair,G.M. (1936) "The Log Difference Method of Estimating the Constants of the First Stage BOD Curve" Sewage Works Journ., Vol.8, pp.430-434,

24. Fair,G.M., J.C. Geyer and D.A. Okun (1981) "Water and Waste Water Engineering". John Wiley and sons New York, London,

25. Frisch, H.L. (1965) "Mechanism for Fickian Diffusion of Penetrants in Polymers" J. Polymer Science B. Vol.3, pp.13-16,

26. Garg, R.P. (1965) "Electrometric Method for the Estimation of Dissolved Oxygen in Water". M.E. Thesis Submitted to University of Roorkee, Roorkee

27. Gates,W.F. and S. Ghosh. (1971) "Biokinetic Evaluation of BOD Concepts and Data". Journ. San. Engg. Div. Proc. ASCE, Vol.97, pp.287-309,

28. Gaudy, A.F. Jr., K. Komolrit and M.N. Bhatla (1963) "Sequential Substrate Removal in Heterogenous Populations" Journ. Water Pollution Control Fed., Vol.35, p.903,

29. Gaudy,A.F. Jr., M.N. Bhatla R.H. Follett, and F.Abu-Niaaj (1965) "Factors affecting the Existence of Plateau during the exertion of BOD" J. Water Pollution Control Fed. Vol.37, p.444,

30. Gaudy,A.F. Jr. and E.T. Gaudy (1966) "Microbiology of Waste Waters" Annual Rev. of Microbiology Vol.20, p.319,

31. Gaudy,A.F. Jr. (1972) "Biochemical Oxygen Demand" in Water Pollution Microbiology Ed. Ralph Mitchell, Wiley Interscience N.Y. London., pp.305,

32. Gellman, I. and H. Heukelekian (1951) "Studies of Biochemical Oxidation by Direct Methods-I Direct Method for Determining BOD" Sewage and Ind. Wastes, Vol.23, pp.1267-1281,

33. Giese, A.C. (1962). Cell Physiology W.B. Saunders Co. Philadephia pp.119

34. Grady, C.P.L. and A.W. Busch (1963) " Further Developments in the Total Biochemical Oxygen Demand Tests" Proceedings 14th Oklahoma Industrial Waste Conference Stilwater, Okla. p.16.

35. Grewal, N.S. (1969) "Studies of the Exertion of BOD in the Carbonaceous Phase" M.E. Thesis, University of Roorkee, Roorkee (India).,

36. Gunsalus, I.C. and C.W. Shuster (1961) "Energy yielding metabolism in bacteria". The Bacteria, Vol.2, pp.1-58, Academic Press, New York,

37. Hach Chemical Company (1973) "Laboratory Instrumentation Manual for Hach BOD apparatus" Ames Iowa U.S.A.

38. Hald, A. (1952) "Statistical Theory with Engineering Applications". pp. 658, Wiley Toppan, Japan.

39. Hartman, L. and P. Wilderer (1968) "Physical and Biochemical Aspects of BOD Kinetics" Water Research, Vol.2, paper 14.

40. Helmers, E.N., J.D. Frame, A.F. Greenberg and C.N. Sawyer (1951) "Nutritional requirements in the biological stabilization of Industrial Wastes" Sewage & Industrial Wastes Vol. 23, pp.884-899,

41. Herbert, D., P.J. Phipps and R.E. Strange (1971) "Chemical Analysis of Microbial cells". Methods in Microbiology (Eds) Norris J.R and Ribbons D.W., Academic Press N.Y. London Vol. 5B, pp.209-349,

42. Heukelekian, H., H.E. Orford and R. Manganelli (1951) "Factors Affecting the Quantity of Sludge Production in the Activiated Sludge Process". Sewage and Industrial Wastes. Vol.23, pp.945-958,

43. Hiser, L.L. and A.W. Busch (1964) "An 8-hour Biological Oxygen Demand Test Using Mass Culture Aeration and COD".J.Water Pollut. Control Fed., Vol.36, p.505,

44. Hoover, S.R., L. Jasewicz, J.B. Pepinsky and N. Porges (1951) "Assimilation of dairy wastes by activated sludge". Sewage and Idustr. Wastes, Vol.23, pp.167-173,

45. Hoover, S.R., L. Jasewicz, and N. Porges (1953) "An Interpretation of the BOD test in terms of Endogenous Respiration of Bacteria". Sewage and Industrial Wastes. Vol.25, No.10, p.1163,

46. Issacs, W.P. and A.F. Gaudy, Jr. (1967) "Comparison of BOD Exertion in a Simulated Stream and in Standard BOD Bottles". Purdue Univ. Engg. Bull. Extension Service. Vol.52, p.165,

47. Issacs, W.P. and A.F. Gaudy, Jr. (1968) "Atmospheric Oxygenation in a Simulated Stream". J Sanit. Eng. Div. Amer. Soc. Civil Eng., Vol.94, No.SA2, pp.319-344,

48. Jenkins, D. (1960) "The use of manometric Methods in the Study of Sewage and Trade Wastes". In Waste Treatment Ed. P.C.G. Issac, pp.99,

49. Karube, I., T. Matsunago, S. Mitsuda and S. Suzuki (1977) "Microbial Electrode BOD Sensors" Biotechnology and Bioengineering. Vol.19, pp.1535,

50. Kumins, C.A. and T.K. Kwei (1968) "Free Volume and other Theories" in Diffusion in Polymers. Ed. Crank and Park.

51. Lamb, J.C.,W.C. Westgarth, J.L. Rockers, and Vernimmen A.P. (1964) "A technique for evaluating the Biological Treatability of Industrial Wastes" J. Water Pollut. Control Fed. Vol.36, pp.1263-1284,

52. Le Blanc, P.J.(1974) "Rewiew of Rapid BOD Test Methods". J. Water Pollution Control Federation. Vol.46, p.2202,

53. Lee, E.W. and W.J.Oswald (1954) "Comparative Studies of the Dilution and Warburg Methods for Determining BOD" Sewage Ind. Wastes, Vol.26, pp.1097-1108,

54. Lenhard, G and L. D. Nourse and H.M. Schwartz (1964) "The Measurement of Dehydrogenase activity of Activated Sludge" Advances in Water Pollution Research. Proceedings of the Second Inter. conf. Tokyo Pergamon Press, Vol.2, p.105,

55. Lewis, J.W. and A.W. Busch (1964) "BOD Progression in soluble substrate VII - The Quantitative Error due to Nitrate as a Nitrogen Source". Proc. 19th Ind. Waste Conf., Purdue University, p.847,

56. Luria, S.E. (1960) "The Bacterial Protoplasm" composition and organisations", in I.C. Gunsalas and R.Y. Stanier (Eds.) 'The Bacteria',Vol.1, Academic Press, Inc. New York.

57. McCarty, P.L. (1964) "Thermodynamics of Biological Synthesis and Growth". Proc. 2nd Intern. Water Pollution Research Conf. Tokyo Japan., Vol.2, p.169,

58. McKinney, R.E. (1962) "Mathematics of Complete Mixing Activated Sludge". Proc. ASCE., Vol.88, No.SA3, pp.87-113,

59. McWhorter, T.R. and H. Heukelekian (1964) "Growth and Endogenous phases in the oxidation of glucose". Proc. Ist Inter. Conf.on Water Poll. Control, London, Pergamon Press Oxford.

60. Mears, P. (1965) "Polymers Structure and Bulk Properties". D.Van Nostrand London.

61. Michaels, A.S. and R.B. Parker, Jr (1959) "Sorption and Flow of gases in Polyethylene". J. Polymer Sci., Vol.41, p.53,

62. Michaels, A.S. and H.J. Bixler (1961) "Flow of Gases through Polyethylene". J. Polymer Sci., Vol.50, p.413,

63. Montgomery, H.A.C. (1967) "The Determination of Biochemical Oxygen Demand By Respirometric Methods". Water Research Vol.1, pp.631, Pergamon Press England.

64. Moore, E.W., H.A. Thomas,Jr., and W.B.Snow. (1950) "Simplified Methods for Analysis of BOD Data". Sewage and Ind. Wastes, Vol.22, pp.1343-1355,

65. Myrick, N. and A.W. Busch (1960) "The Selective Stimulation of Respiration in Mixed Cultures of Bacteria and Protozoa" J.Wat. Pollut. Control Fed., Vol.32, pp.741-754,

66. Orford, H.E. and W.T. Ingram (1953) "Deoxygenation of Sewage II, The Logarithmic Forumla as Applied to Sewage" Sewage and Industrial Wastes., Vol.25, No.4, p.424,

67. Paynes, W.J. (1970) "Energy Yields and Growth of Heterotrophs" Annual Rev. of Microbiology. Vol.24, pp.8-52.

68. Phelps, E.B. (1953). Stream Sanitation. Wiley, New York.

69. Placak, O.R. and C.C. Ruchhoft (1947) "Studies of Sewage Purification - XVII. The Utilization of organic Substrates by Activiate Sludge" Sewage Works J., Vol.19, pp.423

70. Porges, N., L. Jasewicz and S.R. Hoover (1956) "Principles of Biological Oxidation" in B.J. McCabe and W.W. Eckenfelder, Jr. (Eds.) "Biological Treatment of Sewage and Industrial Wastes" Reinhold N.Y., Vol.1, pp.35-48,

71. Porges, N., L. Jasewicz and S.R. Hoover (1963) "Biochemical Oxidation of Dairy Wastes VII Purification, Oxidation, Synthesis and Storage" Proceeding, 18th Industrial Waste Conference, Purdue Universty Lafayette, p.135.

72. Rao, B.S. and A.F. Gaudy, Jr. (1966) "Effect of Sludge Concentration on various Aspects of Biological Activity in Activated Sludge" J. Water Pollution Control Fed., Vol.38, p.794,

73. Revelle, C.S., W.R. Lynn and M.A. Rivera (1965) "Bioxidation Kinetics and a Second Order Equation, Describing the BOD Reaction". J. Water Pollution Control Fed., Vol.37, No.12, pp. 1679,

74. Sawyer, C.N. (1956) "Bacterial Nutrition and Synthesis" Biological Treatment of Sewage and Industrial Wastes Vol.1, pp.3-17, Reinhold N.Y.

75. Schneider, P.J. (1955) "Conduction Heat Transfer". Addison-Wesley Publishing Co. Cambridge 42, Mass, p.256,

76. Schroeder, E.D. (1968) "Importance of the BOD Plateau". Water Res.,Vol.2, p.803,

77. Schroeder, E.D. (1977) "Water and Wastewater Treatment" McGraw Hill Book Co. New York.

78. Schroepfer, G.T. et al. (1960) "Reappraisal of Deoxygenation Rate of Raw Sewage-Effect of Receiving Waters" J. Water Pollution Control Fed. Vol.32, p.1212.

79. Servizi, J. A. and R.A. Bogan (1963) "Free Energy As a Parameter in Biological Oxidation" J.Sanit. Eng. Div. Amer. Soc. Civil Eng., Vol.89, p.17.

80. Sherrard, J.H. and E.D. Schroeder (1972) "Importance of Cell Growth Rate and Stoichiometry to the Removal of Phosphorus from the Activated Sludge Process" Water Res.,Vol.6, p.1951,

81. Sherrarrd, J.H. and E.D. Schroeder (1973) "Cell Yield and Growth Rate in Activated Sludge". Water Pollut. Control Fed. Vol.45, p.1889,

82. Sherwood, T.K., R.L. Pigford and C.R. Wilke (1975) "Mass Tranfer". McGraw-Hill Kogakusha Ltd. Tokyo.,

83. Shrivastava, A.K. (1982) "Analytical and Experimental Investigations of BOD Kinetics in an Aquatic Eco-systems" Ph.D. Thesis submitted to University of Roorkee, Roorkee.

84. Siddiqui, R.H., R.E. Speece, R.S. Engelbrecht, and J.W. Schmidt (1967) "Elimination of Nitrification in the BOD Determination with 0.1 M Ammonia Nitrogen" J. Water Pollution Control Federation. Vol.39, p.579,

85. Sierp, F. (1928) " A New Method for Determining Biochemical Oxygen Demand" Ind. Engrg. Chem., Vol.28, pp.247,

86. Simpson, J.R. (1966) "Technical Bases for assessing the strength, charges for treatment and Biological Treatability of Trade Waste". J. Appl. Chem. Lond., Vol.16, pp.272-280,

87. Skelland, A.H.P. (1974) "Diffusinal Mass Tranfer". John Wiley and Sons. New York., pp.83.

88. Snaddon, X.V.M. and N. Harkness (1959) "A manometric Apparatus for the measurement of Oxygen uptake by Activated sludges, Sewage effluents and Trade Wastes" Wat. Waste Treat. J., Vol.7, pp.250-253,

89. Snaddon, X.V.M. and S.H. Jenkins (1964) "Biological Oxidation in a Self Operating Respirometer". Advances in Water Pollution Research, Vol.2., pp.289-303, (Ed. by Baars. J.K.) Pergamon Press Oxford.

90. Stack, V.T. (1972) "Biochemical Oxygen Demand Measurement" in Water and Water Pollution Hand Book Edited by L.L. Ciaccio. Marcel Dekker., pp.801,

91. "Standard Methods for the Examination of Water and Wastewater" 11th and 12th Eds. American Public Health Association,New York (1960) and 1965.

92. Stones, T. (1979) "A Critical Examination of the uses of the BOD Test". Effluent + Water Treatment J. Vol. 19.,pp.250.

93. Thathgur, I.S. (1983) "Respirometric Study of the Kinetics of Biochemical Oxyen Demand of Sewage". M.E. Thesis Submitted to Punjabi University,Patiala.

94. Thomas, Jr. H.A. (1937) "The Slope Method of Evaluating the Constants of the First Stage BOD Curve" Sewage Works Journ., Vol.9., p.425,

95. Thomas, Jr. H.A. (1950) "Determination of BOD Constants". Water and Sewage Works, Vol.97, pp.123-124,

96. Vernimmen, A.P., E.R. Henken and J.C. Lamb (1967) "A short term Biochemical Oxygen Demand Test". J. Water Pollut. Control Fed., Vol.39, pp.1006-1020,

97. Walters, F. Charles, Richard S. Engelbrecht and Richard E. Speece (1968) "Microbial subtrate Storage in Activated Sludge". Jr. of the sanitary Engg. Div. Proc. of the American Soc. of Civil Engineers, April 1968., p.25.

98. Wilson, I.S. and M.E. Harrison (1960) "The Biochemical Treatment of Chemical Wastes". J. Institute of Sewage Purification., Vol. 3, p.261,

99. Woodword, R.L. (1953) "Doxygenation of Sewage II, A Discussion". Sewage and Industrial Wastes. Vol.25, p.948,

100. Wuhrmann, K. (1956) "Factors Affecting Efficiency and Solids Production in the Activated Sludge Process" in B.J. McCabe and W.W. Eckenfelder Jr. (eds), Biological Treatment of Sewage and Industrial Wastes Reinhold Publishing Corporation, New York,

101. Young, J.C. and J.W. Clark (1965) "Second Order Equation For BOD". Journ. San. Engg. Div. Proc. ASCE, Vol.91, pp.43-57,

102. Young , J.C. (1973) "Chemical Methods for Nitrification Control" J.Water Pollution Control Fed. Vol.45, p.637.

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