

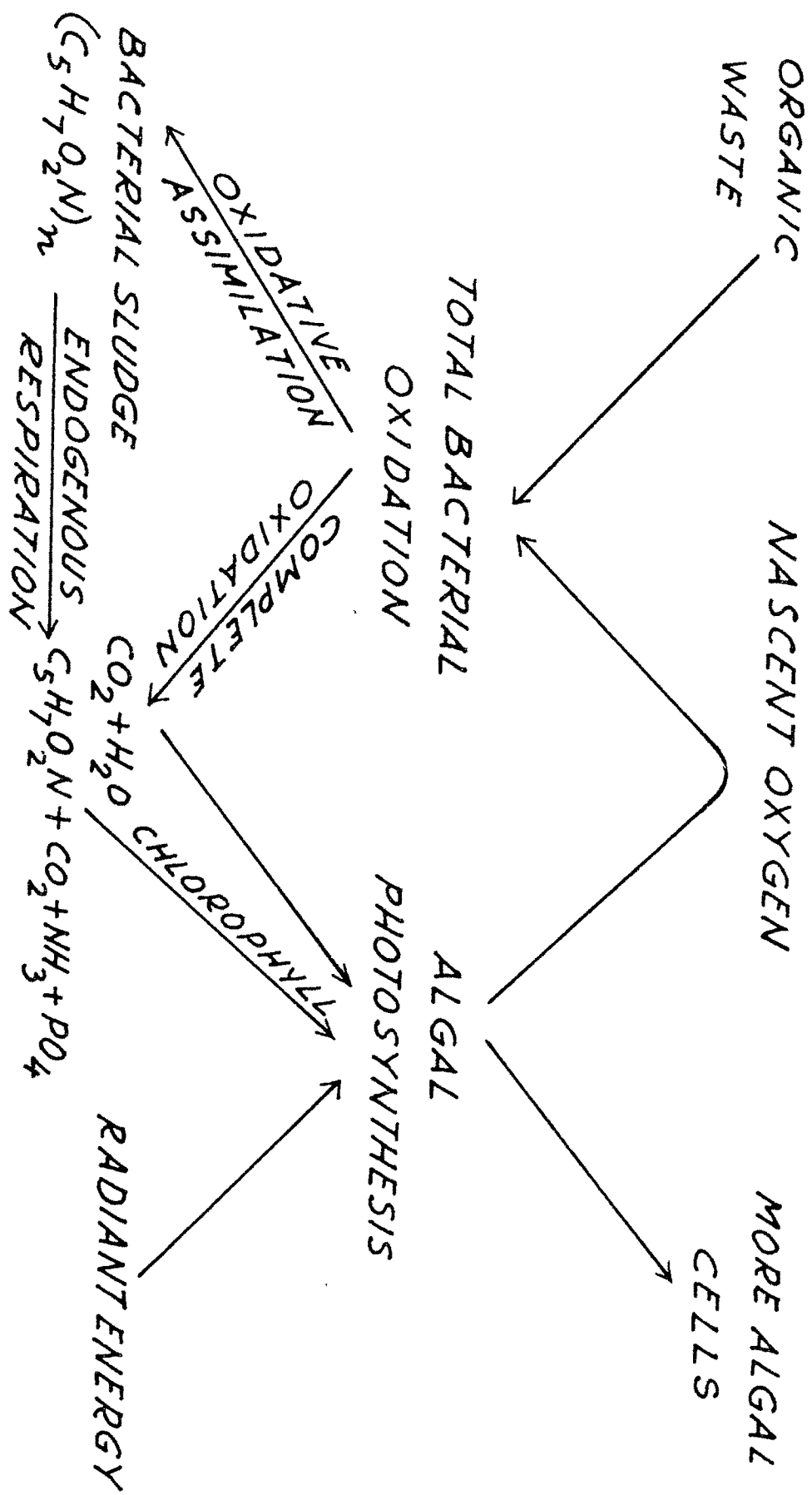
## CHAPTER - V

### DISCUSSION OF EXPERIMENTAL RESULTS

Dissection of the mechanism of algal-bacterial symbiosis into its two component parts : (a) bacterial oxidation and (b) algal photosynthesis is necessary to evaluate quantitatively how one has helped the other in our experiments with the two different algal specimens. For this purpose it is necessary to know the quantity of  $\text{CO}_2$  liberated during the total bacterial oxidation of sewage organic matter for production of each of the two algal bio-masses; and in turn also how much of the photosynthetic  $\text{O}_2$  is liberated by each of the algal biomass during its photosynthesis for total bacterial oxidation of sewage organic matter. But it is not possible to estimate directly either of the two gases in the ecosystem during algal- bacterial symbiosis, for they are not phased metabolic reactions (i.e. one taking place after the other), but they are considered to be not only simultaneous or concurrent but are also stated to be utilized as soon as they are liberated in the closed aquatic ecosystem (Oswald, 1960). The two metabolic processes are illustrated in Fig. 7.

Fig. 7

BIOCHEMISTRY OF ALGAL-BACTERIAL SYMBIOSIS IN HIGH RATE AEROBIC POND SYSTEM (MODIFIED AFTER OSWALD & GOTHAAS 1958)



So, attempts were made to estimate the quantities of the two gases indirectly by methods which are based upon certain well established equations and factors connecting  $\text{CO}_2$  production and  $\text{O}_2$  requirements for bacterial oxidation of sewage organic matter and photosynthetic  $\text{O}_2$  production and  $\text{CO}_2$  requirements for algal biomass formed. This is the first time that such an attempt has been made in the history of the oxidation pond literature for establishing new relations of facts from the two most indispensable parameters COD and algal biomass which we have actually estimated in our laboratory experiments.

(a) Bacterial oxidation:

Chief characteristics of raw settled sewage:

i) Theoretical aspects:

The liquid portion of sewage which has been freed from settleable solids, contains a gradation of particles varying in size from colloidal aggregates (finely divided suspended matter) to true colloids and organic material in molecular dispersion. The amount of true colloidal matter in sewage is small (Mills, 1932; Rudolfs and Gehm, 1939) and most of the non-settleable, non-soluble

material in sewage is in a state of colloidal aggregation. This would be taken to indicate that the colloidal material in sewage has a natural tendency to form aggregates or that it is a transitory stage of becoming larger particles and material in solution.

Partial or intermediate process of treatment:

(Phase - I):

Sewage containing finely divided suspended matter and soluble organic matter undergoes first auto-flocculation and bio-flocculation, processes which are of common occurrence in nature. This natural phenomenon takes place in the created environmental conditions and the equipment to foster it. Aeration, mechanical agitation, and increasing the submerged surfaces are all efforts in this direction (Lackey and Smith 1956; Heukelekian, 1941).

In the case of the high-rate oxidation pond, increased submerged surfaces are created on account of the algal constituents introduced as seed material by re-circulation in the ratio of

8 ; 1 of the final effluent containing also dispersed growths of bacteria. The seed material has no sludge but contains predominant bacterial flora and algae in suspension. Jones and Travis (1926) have shown that inert foreign materials like clay, silt, cotton fibres, may be the nuclei for the organisms around which they build their colonial gelatinous growth. Butterfield (1935) has stressed their importance and Zobell and Anderson (1936) have also concluded that surface area increases the bacterial development in stored sea water.

So, solid surfaces are provided by the algae in the high-rate ponds which, besides furnishing photosynthetic O<sub>2</sub> for bacterial oxidation by algal photosynthesis also seem to act as resting places for bacteria and where nutrients also concentrate on their solid surfaces. The famous 1913 experiments at the Lawrence Experiment Station in U.S.A. with and without green growths of algae have shown that "the heavier the green growths on the sides of the bottles, the clearer were the effluents". So, increasing the internal

surfaces accelerate flocculation, for when they become coated with an active biological slime, the rate of removal of both dispersed and soluble material is increased. For these reasons it is reasonable to understand that in high-rate aerobic ponds, the naturally occurring auto-flocculation and bioflocculation processes are exploited to the maximum possible extent in the first phase of purification of settled raw sewage.

Surface aeration is another factor of equal importance playing a significant role especially at nights when photosynthesis is impossible. It helps to drive oxygen and  $\text{CO}_2$  from the air into the liquid as in the case of the Simplex Activated Sludge Process. The wind action across the huge pond surface of the shallow high-rate ponds causes the breaking of the liquid surface and mixing of the pond effluents and thus helps to keep the ecosystem aerobic.

These phenomena are taking place in the laboratory experimental flasks and the effect of their actions are more visible in the control flasks.

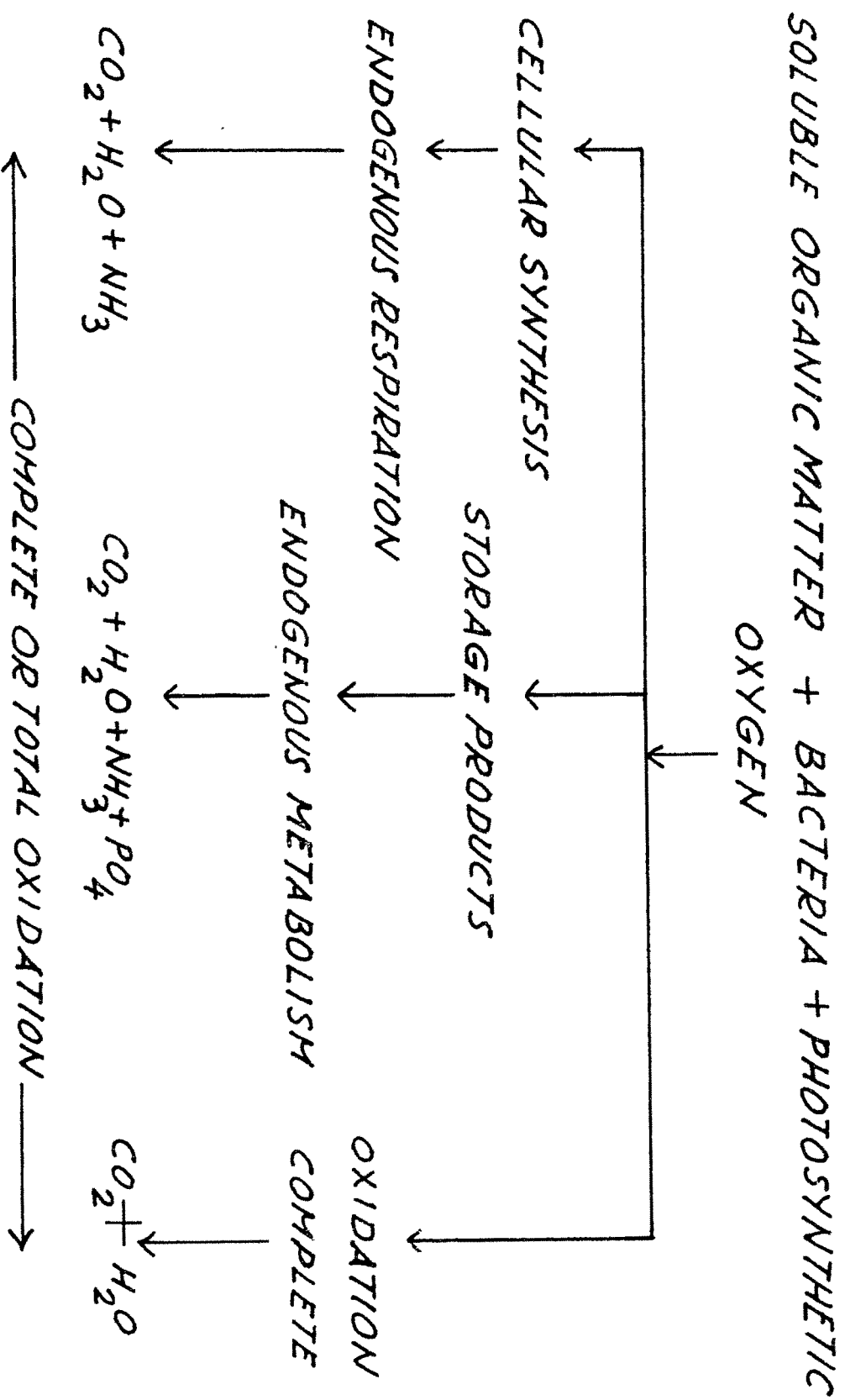
Bacterial oxidation of soluble organic matter:  
(Phase - II):

The biological growth or slime is important in furnishing adsorptive surfaces for the colloidal matter in sewage for the formation of an adsorption compound of the colloidal matter and the gelatinuous coating of the biological slime formed on inert surfaces. This is followed by oxidation or conversion of the adsorbed matter by bacteria using  $O_2$ . The biological slime contains aerobic bacteria of different species. Bacterial oxidation of organic matter is the result. This theory has been developed by Eckenfelder and Weston (1956) and Eckenfelder and O'Connor (1961); and the state of our knowledge on the bacterial oxidation of organic matter is based on their works. It is explained below:

All biological waste treatment systems operate on the same general bio-chemical principles, though the physical structures of the treatment systems may differ. The bacterial oxidation of

Fig. 8

TOTAL BACTERIAL OXIDATION OF ORGANIC MATTER (MODIFIED AFTER PORGES 1960)

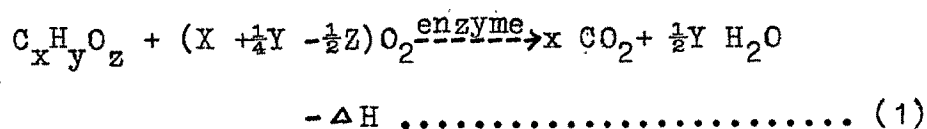




organic matter is a three phased process consisting of complete oxidation, cellular synthesis and oxidative assimilation of the remaining organic matter (incomplete oxidation) and endogenous metabolism of the bacterial solids. These principles are illustrated in Fig. 8.

Complete oxidation:

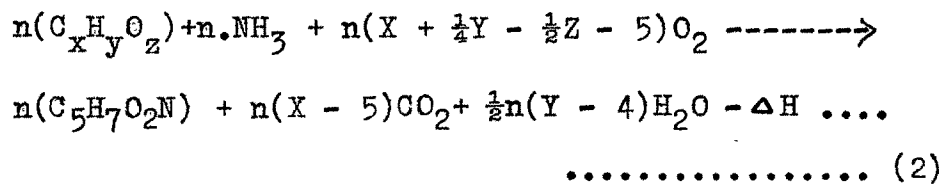
This results in the formation of  $\text{CO}_2, \text{H}_2\text{O}$  and energy, where numerous enzymes and intermediate products are involved besides electron transfer system. These reactions are shown by the following equations (Eckenfelder and Weston 1956, p. 10). It is the same as the conventional equation of combustion:-



Cellular synthesis and oxidative assimilation (incomplete oxidation):

Complete conversion of an organic substrate to  $\text{CO}_2$  does not take place but oxidised organic compounds accumulate in the medium as end products of respiratory metabolism. Biosynthesis and growth

accompany the decomposition of organic substrate and newly formed bacterial cells are a major ~~a~~ end product of this intermediate metabolism - or incomplete oxidation. The patterns of substrate utilization may be either concurrent or sequential. Eckenfelder and Weston (1956, p.18) have represented this metabolic reactions thus:-



The cellular material formed is represented by the empirical formula  $C_5H_7O_2N$  developed by Hoover and Porges and which represents the statistical average composition of the complex organic compounds constituting the bacterial cell material.

#### Endogenous metabolism:

Besides "complete" and "incomplete" oxidation of organic matter in biological waste treatment systems, another kind of metabolism is also taking place in high-rate aerobic ponds known as "endogenous metabolism". This fundamental concept

which is well known in the field of bacteriology has never been applied to a practical problem in waste treatment. Endogenous metabolism may be defined as the metabolic reactions that take place within the living microbial cells when they are held in the absence of compounds which may serve specially as exogenous substrates or these reactions may also continue in the presence of exogenous substrates. Endogenous metabolism takes place when the organic food material in solution is very low, so that bacteria derive their energy then from the destruction of their own storage products. At this stage the number of active organisms is low and most bacteria then lose their motility (Mckinney, 1956). Again according to Mckinney (1952, 1956) bacterial flocculation takes place when the microorganisms are in a state of endogenous respiration in the treatment system. If endogenous respiration is extensive there is no accumulation of sludge; if it is low, sludge accumulates (Hoover et al 1952). Gloyna (1965, p.5) has stated that for domestic wastes that have undergone primary treatment the amount of sludge accumulation

in a pond is almost negligible; and that even where ponds are used to treat excess activated sludge, the accumulation is almost negligible.

Endogenous substrates or storage products so far recognized in bacteria include carbohydrates (glycogen and polyglucose compounds), lipid, PHB, protein, peptides, amino acids, RNA and volutin according to Dawes and Ribbons (1962) and some of these substances have been identified in all over 200 bacterial isolates.

The concept of total bacterial oxidation of organic matter:

From the foregoing account of the three types of metabolic reactions taking place during bacterial oxidation of organic matter in a high-rate aerobic pond, it is possible to explain what "total oxidation" means. It means "absolute oxidation". In other words it includes "complete oxidation", "incomplete oxidation" and "endogenous respiration" in the system. The final result is that all the bio-degradable organic matter in influent sewage undergoes "absolute" or "total"

oxidation" to  $\text{CO}_2$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$ . How maximum amounts of these substances are made available as a result of "total oxidation" of organic matter during the phase of bacterial oxidation is schematically represented in Fig. 8 (Modified after Porges, 1960).

- ii) Application of the principles to an assessment of viable bacteria and related results in growth cycles of algal bacterial symbiosis with the two algae;

The main purpose of aerobic biological treatment for stabilization of organic matter is the removal of organic carbon in the organic substances of the waste water. This removal is brought about by reactions embodied in the three cardinal principles mentioned above. It is on account of these reactions the activated sludge process maintains and even increases itself (Symons and McKinney, 1958). The bio-chemical reactions mentioned above may be thus expressed in brief:

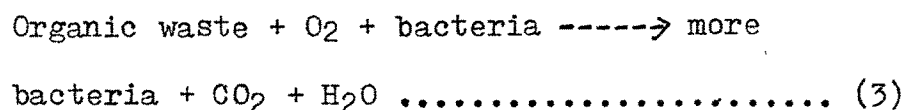
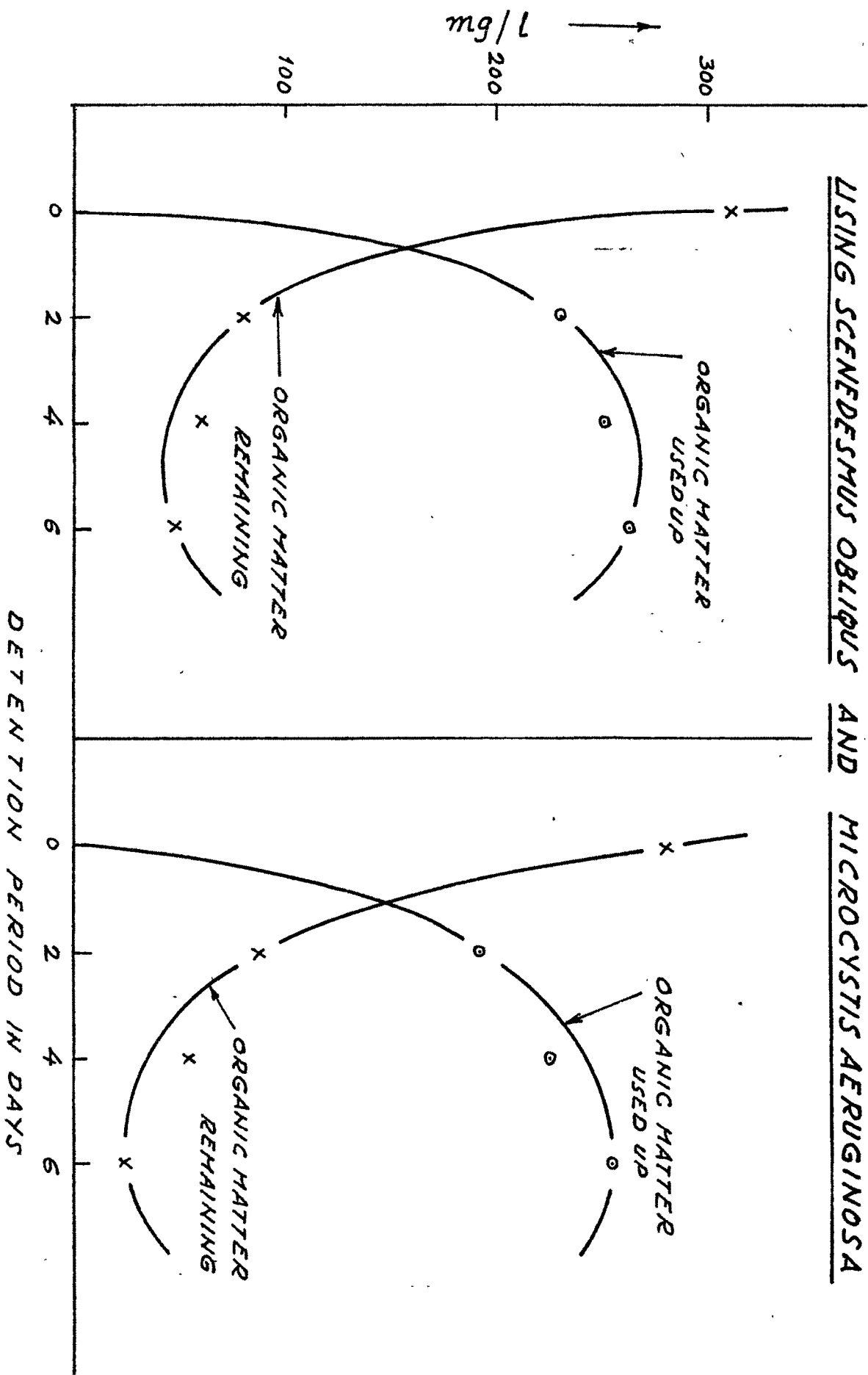


Fig. 9

BIO OXIDATION OF SEWAGE ORGANIC MATTER IN HIGH RATE

AEROBIC OXIDATION POND

USING SCENEDESMUS OBLIQUUS AND MICROCYSTIS AERUGINOSA



So, it will be evident that a very striking feature of microbial metabolism in waste water treatment system is the relatively enormous amount of new bacterial cells that is normally produced during the breakdown of organic matter. Rapid purification of a waste depends upon the unrestricted activities and reproduction of micro-organisms (Sawyer, 1956).

Removal of organic matter expressed as COD(mg/l)  
(Fig. 9).

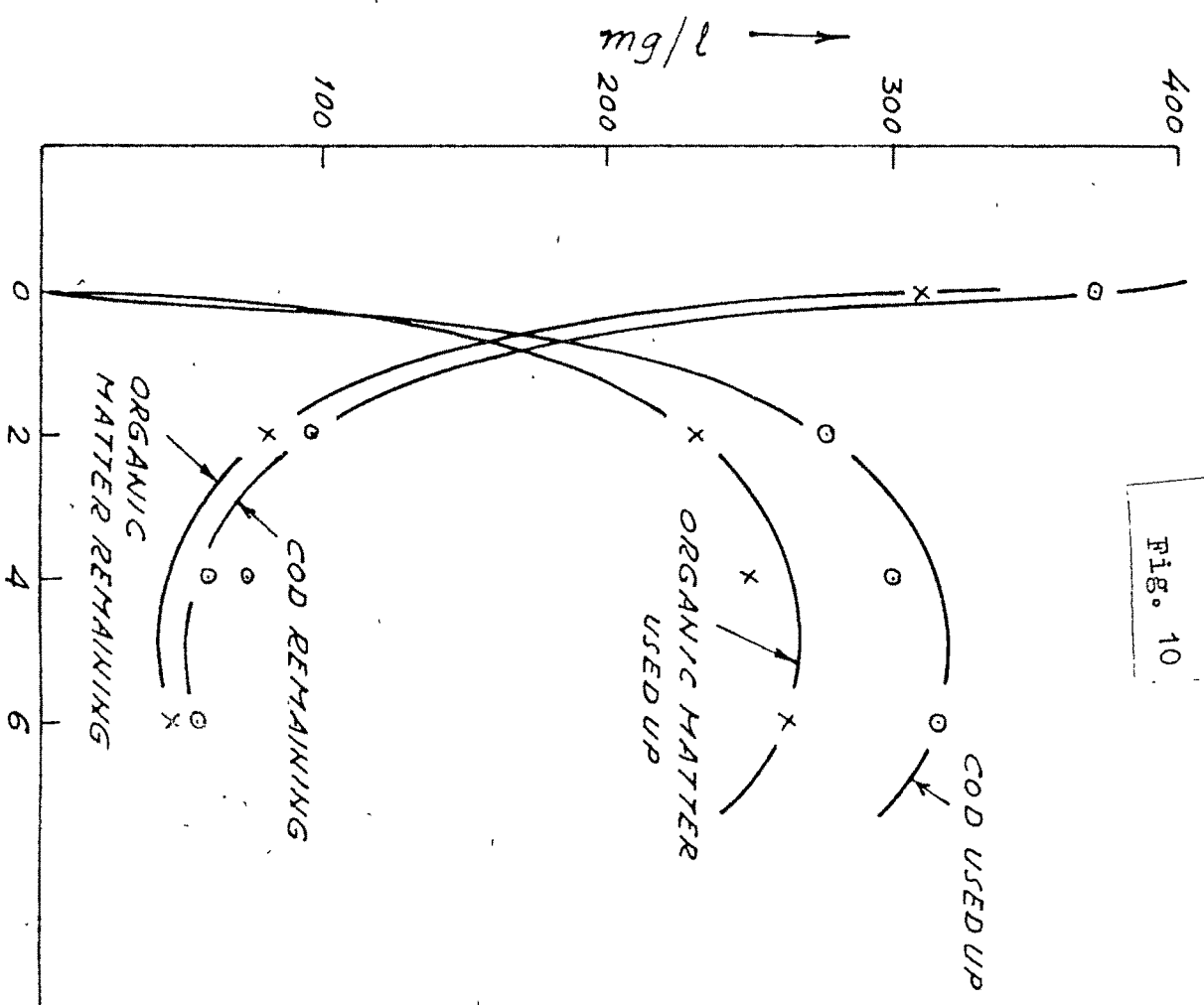
The organic matter used up during different detention periods in the two experiment and the algal biomass formed as a results are shown below:

Deten- tion time	Raw sewage		Raw sewage		Algal biomass	
	+		+		formed (mg/l)	
	<u>Scenedesmus</u>		<u>Microcystis</u>		<u>Scene-</u>	<u>Micro-</u>
	COD	COD	COD	COD	desmus	cystis
	used up		used up			
0 day	372	-	336	-	-	-
2 days	96	276	106	230	275	224
4 days	72	300	66	270	286	231
6 days	56	316	30	306	282	236

Conversion of used up COD values into organic  
matter values according to Porges (1960) and  
expressed in (mg/l) (Fig. 10 and 12)

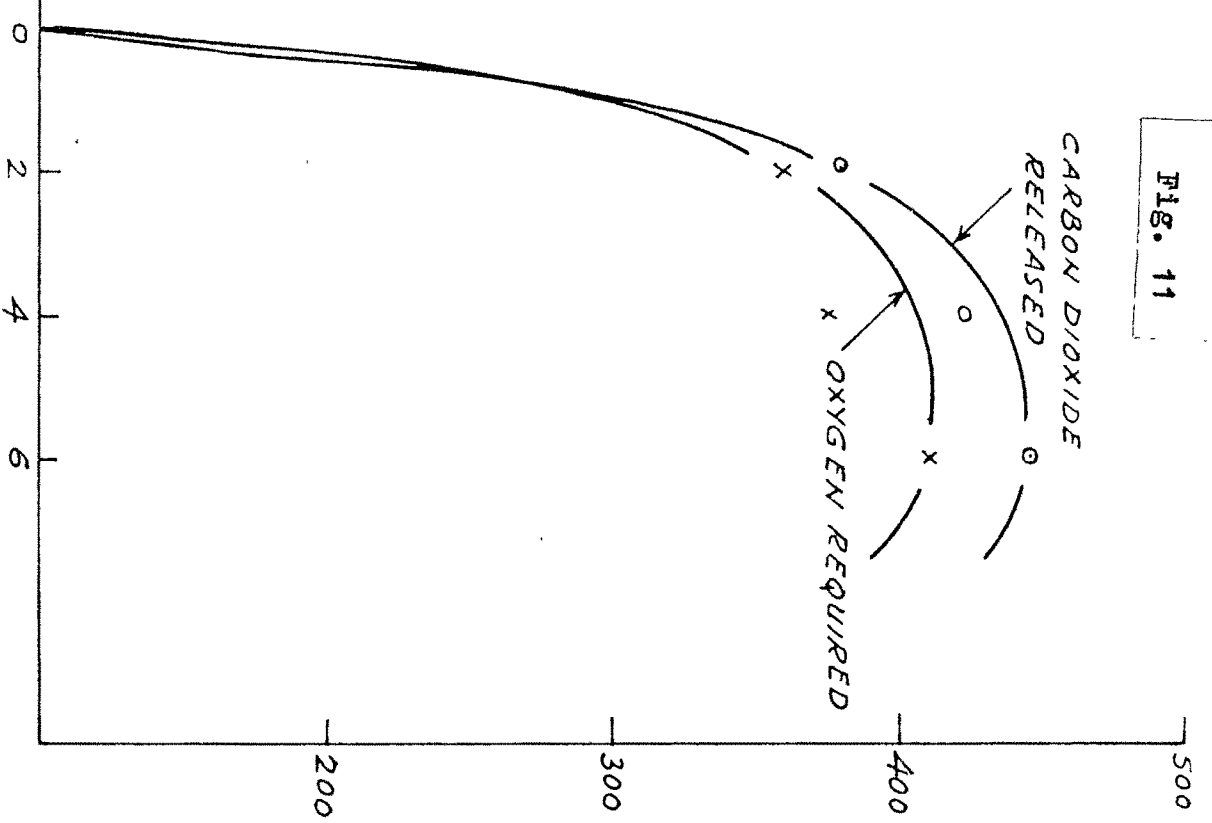
BACTERIAL OXIDATION OF ORGANIC  
MATTER USING SCENEDESMUS OBLIQUUS

Fig. 10



TOTAL BIO OXIDATION OF ORGANIC  
MATTER USING SCENEDESMUS OBLIQUUS

Fig. 11



DETENTION PERIOD IN DAYS



COD as we know, is a measure of the quantity of  $O_2$  required for total bio-oxidation of Sewage organic matter and not of the quantity of organic matter itself. But it is necessary to know the quantity of organic matter oxidized by bacteria; and Porges (1960) has furnished a method of estimating the same approximately from COD values. COD values divided by 1.2 give the corresponding values of the quantity of bio-degradable organic matter in any waste water containing organic matter. The results are shown under:

Deten- tion time	<u>Scenedesmus</u>		<u>Microcystis</u>	
	COD used up	Organic matter used up	COD used up	Organic matter used up
2 days	276	230	230	191.7
4 days	300	250	270	225.0
6 days	316	263	306	255.0

Thus reduction in COD values represents indirectly the organic matter removed by bacteria.

Calculation of bacterial biomass according to Sawyer  
(1956) and McKinney (1962):

It may be argued that "The quantity of bacterial sludge produced in the high-rate aerobic ponds cannot be compared to that produced in activated sludge as the two are different processes and would not be expected to yield the same quantity of sludge. In the activated sludge system, oxygen is sparged to support the development of heterotrophic aerobic microbes and the main consequence of such respiratory activity is the production of cell material. In the oxidation pond on the other hand, O<sub>2</sub> is provided primarily by algae growing in the surface layer, and aerobic heterotrophs are only one fraction of the physiological types of microbes present. Although the ponds are shallow, stratification may still occur and hence enrichment for micro-aerophilic and even anaerobic organisms occurs. Mineralization of organic compounds under these conditions would not be expected to give the same bacterial yield because (a) energy getting mechanisms are less efficient and crop yields are lower; and (b) algae may compete as heterotrophs for available carbon compounds".

In reply to the above points which may be raised, it has to be pointed out that the end results of all biological treatment processes are same and aerobic heterotrophs are also only one fraction of the physiological types of microbes present in an activated sludge system. Such being the case, the bacterial solids formed in 4-6 hours in the activated sludge process require 6 days in the high-rate aerobic pond so that the total % BOD removal is the same in both cases. So, it cannot be stated that the quantity of bacterial sludge will be considerably lower in the high-rate aerobic pond, if the efficiency of purification is the same in both the treatment systems. Oswald (1960) has also stated that "A healthy sludge comparable to activated sludge is maintained in the pond, provided mixing is carried out for about 3 hours a day". Therefore, the same quantity of bacterial yield may also be expected in 6 days (and not in 6 hours) in the high-rate oxidation pond.

So, an attempt was made to calculate the yield of bacterial cell material from the BOD or COD used up in 6 days applying the formulae of Sawyer (1956), McKinney (1962) and Oswald et al. (1958).

Bacterial growth and synthesis according  
to Sawyer (1956).

Nutrition and synthesis are immediately related factors. Synthesis and growth are essentially synonymous terms. Growth results from the marshalling of part of food supply and accessory mineral factors to produce new generations of the organisms with characteristic and chemical composition similar to the progenitors. A considerable fraction is used for energy purposes.

Gellman and Heukelekian (1953), Hoover and Porges (1952) and Weston and Eckenfelder (1955) have found that the yield of cell material during the rapid growth phase is slightly over 50% of the carbon utilised; and the remaining half is completely oxidized to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and energy in aerobic treatment systems. Sawyer (1956) and McKinney (1962) have furnished two different methods for calculating the cellular biomass representing about 50% of the carbon used up. Oswald et al. (1958) have furnished on the other hand, a formula for complete oxidation of sewage organic matter resulting in  $\text{CO}_2$ , water and ammonia. All the three methods of calculation are compared under.

Growth and synthesis can be predicted from 5-day BOD of a waste. Sawyer (1956) has found a formula to calculate total bacterial mass in activated sludge process which is given below

$$\text{Total bacterial growth} = 0.5 \times \text{BOD}_5(\text{used up}) \dots \dots \dots (4)$$

The above formula has been applied to our own results for the estimation of total bacterial solids in algal treated flasks only because we are only concerned with bacteria in algal-bacterial symbiosis.

Yield of bacterial cell material according to Sawyer (1956) in mg/l:

Deten- tion time	<u>Scenedesmus obliquus</u>		<u>Microcystis aeruginosa</u>	
	BOD <sub>5</sub> used up	Total mass	BOD <sub>5</sub> used up	Total mass
2 days	213	106.5	126	63.0
4 days	237	118.5	154	77.0
6 days	245	122.5	178	89.0

It can be seen from the above that bacterial growth increases as the detention time increases.

Total, active and decreasing bacterial  
biomass according to McKinney (1962):

Also, McKinney has given the following three formulae for calculating total bacterial solids, active bacterial mass and decrease in active mass due to endogenous metabolism in activated sludge process. The same formulae have been applied to our case also, assuming that the metabolic reactions are similar.

For total bacterial solids he has stated:

$F = 2.13 \times S$  where  $F$  = COD used up; and  $S$  = total bacterial mass (mg/l).

$$\therefore S = F/2.13 \dots\dots\dots (5)$$

$$\therefore \text{Total bacterial mass} = \frac{\text{COD used up (mg/l)}}{2.13}$$

For active bacterial mass:

$S = M_a + K_d \cdot M_a \cdot t$  Where  $t$  = time in hrs.,  $K_d = 0.006$ ;

$M_a$  = active bacterial mass (mg/l) and  $S$  = total bacterial mass.

$$\therefore S = M_a (1 + K_d t)$$

$$\therefore M_a = S/(1 + K_d t) \dots\dots\dots (6)$$

Decrease in active mass due to endogenous metabolism:

Total bacterial mass = active bacterial mass - decrease in active mass due to endogenous.

∴ Decreasing bacterial mass = Total bacterial mass  
- active bacterial mass

∴ Decreasing bacterial mass = S - Ma ..... (7)

Applying (5), (6) and (7) equations to our results we get the following data :-

Deten- tion time	Scenedesmus (mg/l)			Microcystis (mg/l)		
	Total mass	Active mass	Endoge- nous mass	Total mass	Active mass	Endogeno us mass
2 days	130.0	100.93	29.07	108.0	83.85	24.15
4 days	140.8	109.3	31.5	127.0	98.6	28.4
6 days	148.4	115.2	33.2	143.7	111.56	32.14

It can be seen from the above that endogenous mass (due to endogenous metabolism) increases from 2 to 6 days. The values for active mass calculated according to McKinney resembles approximately the total bacterial mass calculated according to Sawyer. From the data, it is also obvious that the total bacterial mass calculated according to Sawyer is about 2/3 of the corresponding values of total bacterial mass calculated according to McKinney.

Correlation between bacterial biomass and  
algal biomass:

All these values appear to be normal with domestic sewage. The 5-day BOD values represent 65 to 70% of the COD and assuming that 50% of the sewage organic matter are converted into total bacterial biomass according to McKinney's formula, the values about 2.0 representing the ratio of algal biomass to total bacterial biomass seems to be valid. In other words the quantity of algal biomass produced during algal-bacterial symbiosis in a high-rate aerobic pond seems to be nearly twice the quantity of bacterial biomass produced.

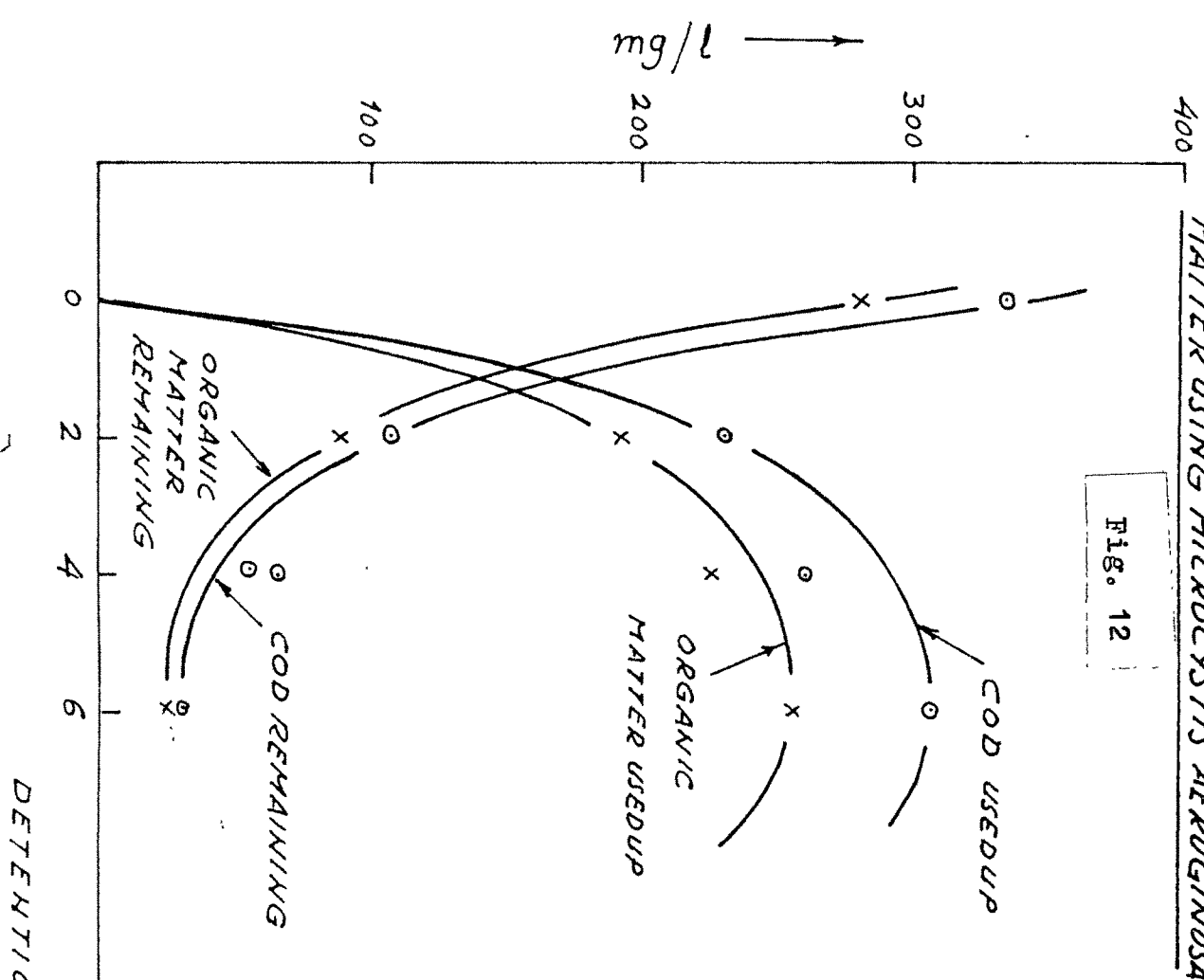
Deten- tion period	Total Biomass (mg/l)			
	Scene- desmus	Bacte- ria	Micro- cystis	Bacte- ria
2 days	275	130	224	108
4 days	286	140.8	231	127
6 days	282	148.4	236	143.7

From the above data, it is obvious that the bacterial mass calculated according to McKinney's formula is nearly half of the algal biomass actually determined.



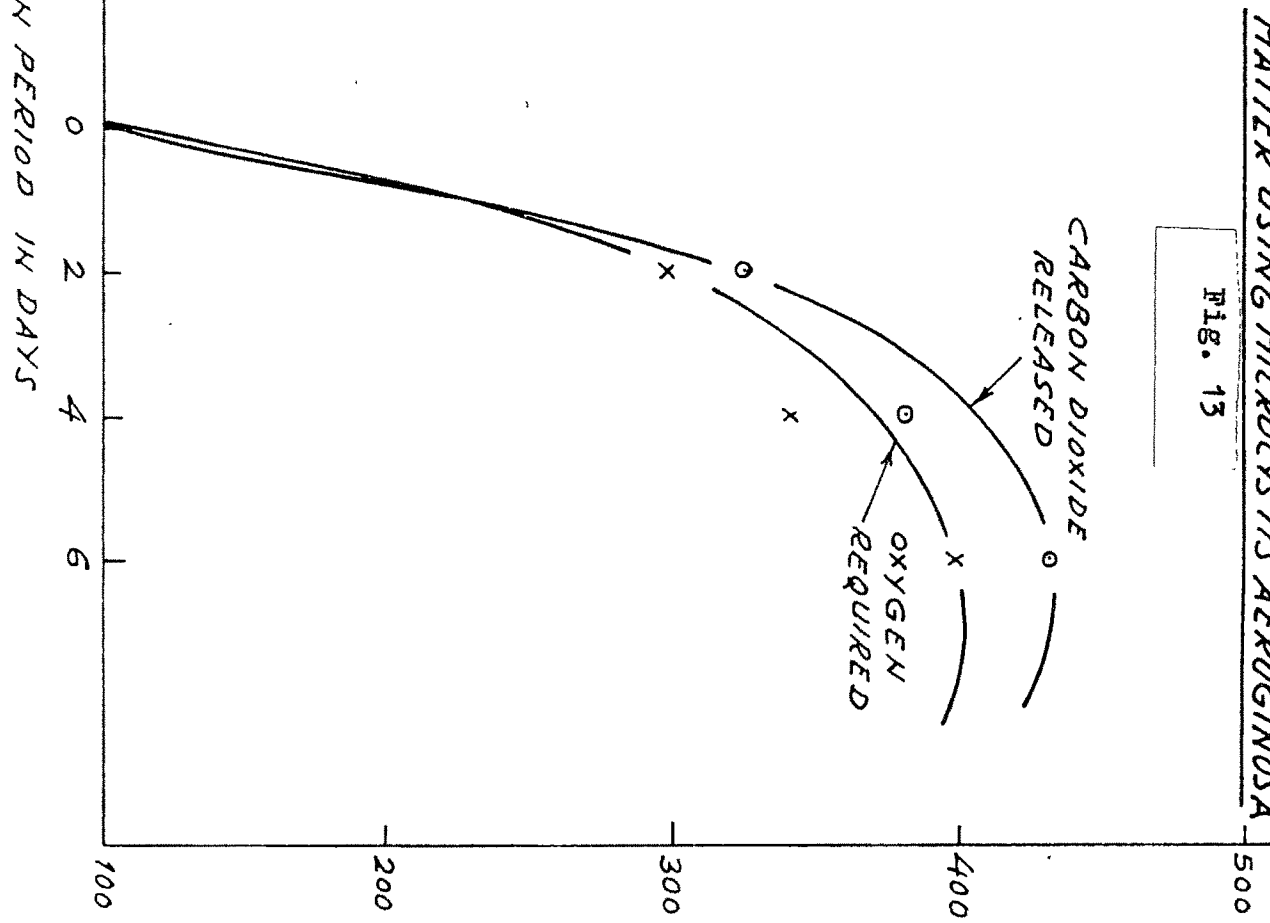
# BACTERIAL OXIDATION OF ORGANIC MATTER USING MICROCYSTIS AERUGINOSA

Fig. 12



# TOTAL BIO OXIDATION OF ORGANIC MATTER USING MICROCYSTIS AERUGINOSA

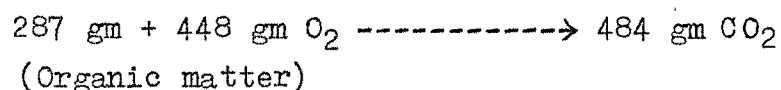
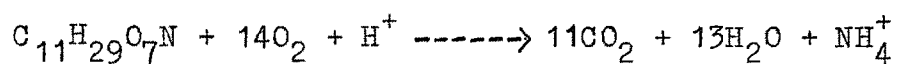
Fig. 13



Oxygen required and CO<sub>2</sub> released during the  
bio-oxidation of sewage organic matter according  
to Oswald et al (1958):

(Fig. 11 and 13).

Oswald et al. (1958) found experimentally in high-rate aerobic ponds the oxidation of sewage organic matter to follow the equation:



So, 1 gm of sewage organic matter will produce 1.69 gm of CO<sub>2</sub> and will require 1.56 gm of oxygen. Based on these two factors the following two tables have been prepared:

O<sub>2</sub> required for total bio-oxidation (mg/l):

Deten- tion time	Scenedesmus		Microcystis	
	Organic matter used up	O <sub>2</sub> required	Organic matter used up	O <sub>2</sub> required
2 days	230	358.8	191.7	299.0
4 days	250	390.0	225.0	351.0
6 days	263	410.3	255.0	397.8

CO<sub>2</sub> released during bio-oxidation of organic matter (mg/l):

Deten- tion time	Scenedesmus		Microcystis	
	Organic matter used up	CO <sub>2</sub> released	Organic matter used up	CO <sub>2</sub> released
2 days	230	388.7	191.7	324.0
4 days	250	422.5	225.0	380.2
6 days	263	444.5	255.0	430.9

It can be concluded from the above two tables that 398.0 to 410 mg/l of oxygen are required to degrade completely 255.0 to 263.0 mg. of organic matter within six days. At the same time 431.0 to 445.0 mg/l of CO<sub>2</sub> are released during the complete degradation of 255.0 to 263.0 mg/l of organic matter in six days.

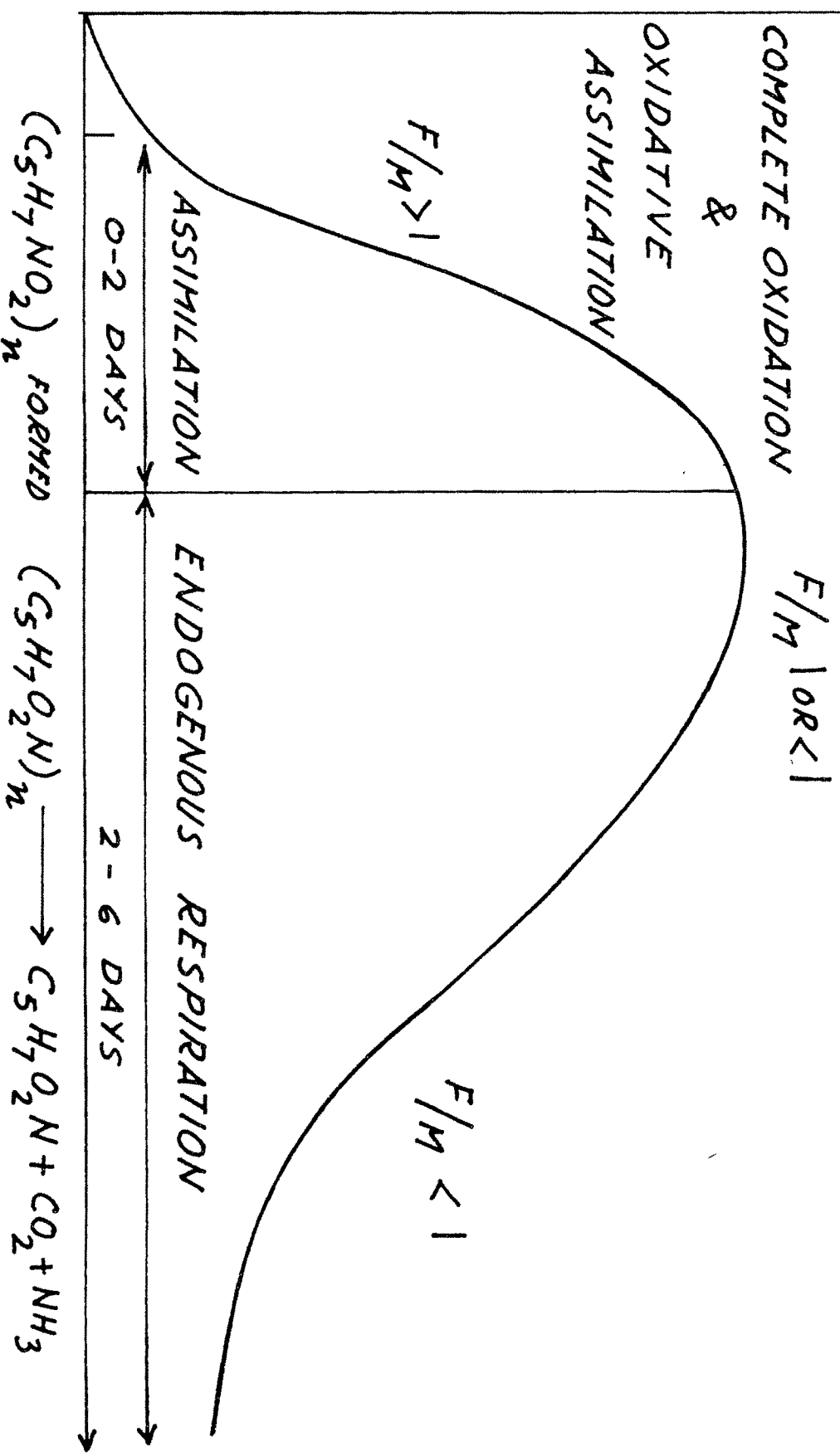
(b) Different growth phases of microbial metabolism in high-rate aerobic pond:

i. Theoretical considerations:

Biochemical principles of microbial metabolism of organic matter in biological oxidation

Fig. 14

# DIFFERENT PHASES OF BACTERIAL OXIDATION IN HIGH-RATE AEROBIC OXIDATION POND



processes, represent the kinetics of bacterial biosynthesis, growth and decay; and they are shown in the four growth phases in the ideal curve in Fig. 14. The lag phase is largely eliminated in the high-rate oxidation pond as it is inoculated with a fairly good amount of acclimated bacterial and algal seed at the beginning for starting the bacterial oxidation and algal photosynthesis.

The log growth phase may be defined as that period during which regular and maximum multiplication of bacterial solids take place. This maximum or logarithmic growth rate is dependent on the mean-generation time of the ecosystem. The generation time is defined as that interval during which one bacterium develops and completely divides itself into two cells. This results in geometric progression of bacterial growth. As the available food supply is exhausted, a negative accelerative phase exists where cellular division takes place at less frequent intervals.

A stationary phase will follow in which the rate of growth equals the rate of cell death and

destruction. When the rate of destruction exceeds the rate of growth, a death phase exists. This is probably the endogenous respiration phase, when the bacterial mass is oxidised to  $\text{CO}_2$ , water and ammonia.

"Endogenous metabolism can be defined as the sum of all chemical activities performed by organisms in the absence of utilizable extra-cellular materials serving as sources of energy and building stones for assimilation and growth. Water, molecular oxygen and non-carbonaceous and non-nitrogenous mineral salts may be present in external environment and precipitate in endogenous metabolism. In nutritional terms, endogenous metabolism can be viewed as encompassing all these chemical activities engaged in the starving cell".

An interesting and valuable application of this dual principle of assimilation i.e. (i) rapid temporary storage of intracellular polysachharide reserved glycogen in some cases, and (ii) subsequent slow oxidation of the store after the external metabolites have been utilized - has

been made by Porges et al (1953). By calculated adjustment of the alternating phase of feeding and starvation, a microbial population was enabled to dispose of large amounts of dairy wastes without proliferating so as to necessitate removal of the cell debris as sludge. In the words of Porges (1960) it should be possible theoretically, <sup>to have a system</sup> in which sludge or cell, do not accumulate. All that would be required is sufficient nutrients to produce enough cells to replace those being oxidized by endogenous respiration. This ideal state has been approached but not attained. But Oswald (1960) appears to have attained this stage in his high-rate aerobic pond. Maximum removal of organic matter as bacterial solids takes place during the log growth phase and long aeration period or when prolonged endogenous respiration is permitted as in high-rate aerobic pond, total or absolute oxidation takes place (Jenkins, 1931).

ii) Application to the connected results:

In our experimental studies of two algae i.e. Scenedesmus obliquus and Microcystis aeruginosa, it was shown that most of the nutrients were highly

used up during the detention period of 0 to 2 days, When the detention period increased from 2 to 4 and 4 to 6 days there was definite decrease in nutrients. It is obvious to conclude that 0 to 2 days detention will represent the assimilatory phase, while 2 to 4 and 4 to 6 days the endogenous phase. The results are shown in Table - 10.

(c) Algal Photosynthesis:

i). Theoretical considerations:

The possibility that green algae might be grown in sewage in order to provide  $O_2$  in the place and at the time when it is badly needed for biochemical stabilization of waste organic matter is a relatively new approach for which credit goes to Oswald, Gotaas and their colleagues whose investigations on shallow lagoons showed the existence of a complete biochemical cycle in which algae play a dominant role in furnishing  $O_2$  for bacterial oxidation of organic wastes and of reclaiming nutrients from the waste as algae.

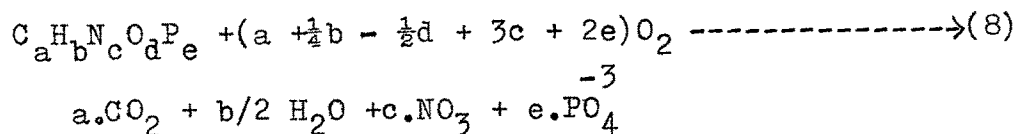
It was pointed out in the preceeding section that as a result of "total oxidation" of organic



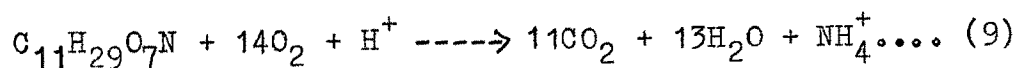
matter by bacteria,  $\text{CO}_2$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$  are released into the treatment system, which except for the additional requirement of light energy, are the essential needs of algal photosynthesis. In theory the bacterial oxidation of organic matter may take place at the same time as fresh algal cells are being synthesised in the presence of radiant energy. So, as a result of algal photosynthesis in high-rate aerobic oxidation ponds four benefits are likely to occur at the same time. They are: utilization of  $\text{CO}_2$  during photosynthesis, the concomitant release of nascent oxygen which may be immediately available for bacterial oxidation of organic matter, nutrient assimilation of nitrogen and phosphorus and large scale algal biomass production, a cheap form of vegetable protein for use by man and animals. Naturally several biochemical principles are involved and they are briefly considered below:

It is generally known that two broad groups of organisms i.e. heterotrophs and autotrophs are involved in algal bacterial symbiosis. The former are responsible for decomposing organic matter through a complex sequence of redox reactions and their over-all

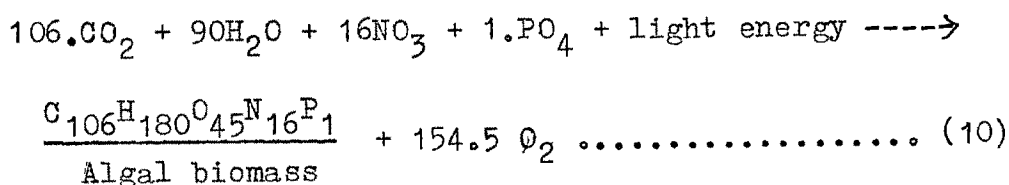
stoichiometry may be represented by the equation:



Soluble inorganic components are released into the ecosystem; Oswald, Hee and Gotaas (1958) found experimentally in high-rate aerobic ponds the oxidation of sewage organic matter to follow the reaction:

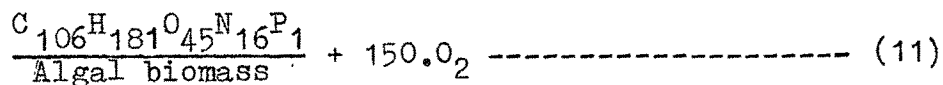
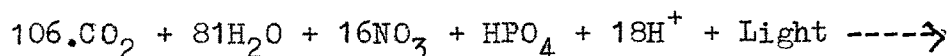


The autotrophs capturing solar energy are able to use  $CO_2$  and other inorganic nutrients (N and P) for the production of algal biomass. This production may be thus illustrated (Stumm 1968, Gloyna, 1971).

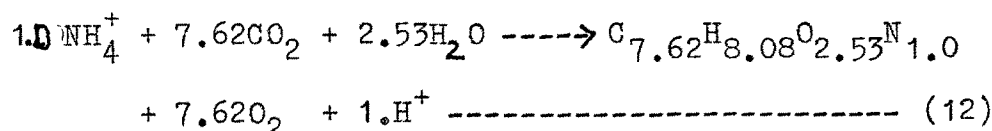


The stoichiometric relation between C, N and P in the algal biomass is 106 : 16 : 1 atoms.

But McCarty's (1969) equation is slightly different although the ratio of the main components in algae is the same;



Also, Oswald and Gotaas (1957, p.80) have developed an equation for algal cell production thus;



From the above equation they estimated that 153.56 g of algal cell material and 243.84g. of oxygen are formed during algal-bacterial symbiosis. Therefore, they stated that for every 1.0 g. algal cell material produced, 1.587 or 1.6g. of photosynthetic oxygen will be liberated in the system for bacterial oxidation.

#### Light :

Its penetration is directly affected by incident light, and inversely affected by depth and algal density. Optimum light intensities for maximum algal growth range from 200 to 400 foot-candles (ft.c) and the lower limit may be still lower at 100 ft.c. Allen (1955) found the growth of chlorella

in sewage was not affected by a reduction of the daily period of illumination until the extreme only 4 hours daily was reached. Similarly a reduction in light intensity from 400 to 20 ft.c. had little effect. So, light will not be a limiting factor.

Another possible method of increasing the availability of incident light to individual algal cells is to move the algae into the light path by induced mixing as in the high-rate aerobic pond.

#### Temperature:

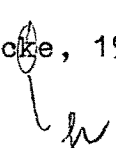
As in the case of all microorganisms, temperature affects the growth rate of algae also following the Vant Hoff rule according to which growth rate doubles for each 10°C rise in temperature within the range of temperature tolerance.

#### Carbon source:

It is usually the limiting element when algae are cultured in sewage. Algae normally use free CO<sub>2</sub> as an organic C source, though some algae are reported to use the bicarbonate ion. Use of artificially introduced CO<sub>2</sub> is neither essential nor

desirable when algae are cultured in sewage for photosynthetic oxygen production, because the culture may obtain the same from air. It is, established that air contains 0.03%  $\text{CO}_2$  normally; and this amount is adequate to sustain maximum photosynthetic efficiency if a sufficient volume of the mixture brought into contact with cell surface (Davis et al 1953).

Active photosynthesis causes the pH to increase to 10 or more according to the absorption of atmospheric  $\text{CO}_2$  by the culture. Under such conditions, this  $\text{CO}_2$  appears as a bicarbonate ion and becomes available to algae immediately. So, algae may compensate for a shortage of organic  $\text{CO}_2$  by increasing the  $\text{CO}_2$  absorbing properties of the liquid in which they grow i.e. by becoming more alkaline or by increase in pH. When all the available free  $\text{CO}_2$  is used up, the half-bound  $\text{CO}_2$  (bicarbonates) from 4/5 to 5/8 is then available and used up (Birge and Juday, 1911). When the first two sources are exhausted, the fully bound  $\text{CO}_3$  or mono-carbonates may also have to be used up (Schutow, 1926; Maucha, 1929; Neresheimer and Ruttnier, 1929; Juday, Birge, and Melocke, 1935).



This is an additional source of  $\text{CO}_2$  not connected with the metabolism of the wastes; and it can result in considerable excess of photosynthetic  $\text{O}_2$  to keep the system aerobic.

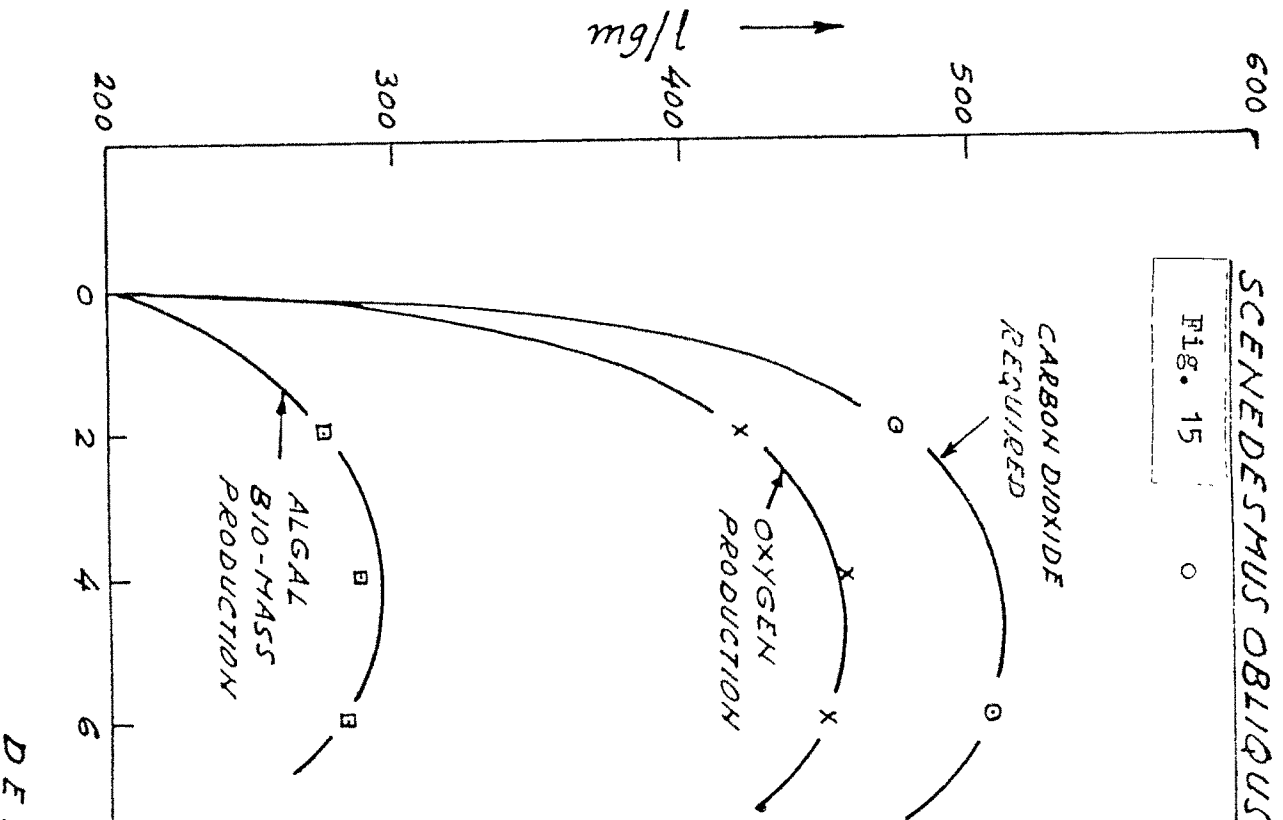
Inorganic nutrients:

Ammonia - nitrogen, nitrous - nitrogen, and nitric-nitrogen and orthophosphates are included under this head. Most algae are able to utilize either ammonia-nitrogen or nitrate-nitrogen and also nitrite nitrogen if the concentration is very low (about 0.001 molar) according to Fogg and Wolfe (1954), but they prefer  $\text{NH}_3\text{-N}$  to  $\text{NO}_3\text{-N}$  when both sources are provided in the same culture (Harvey, 1940; Schuler et al. 1953).

Algae use P as orthophosphate ( $\text{PO}_4^{-3}$ ). The ratio of N : P in a typical algal cell is about 10 : 1 and is essential to algal growth and without it no growth will take place. Sawyer (1952) found that N : P ratios in natural waters where algal blooms prevailed varied from 30 : 1 to 15 : 1 depending on the species of algae.

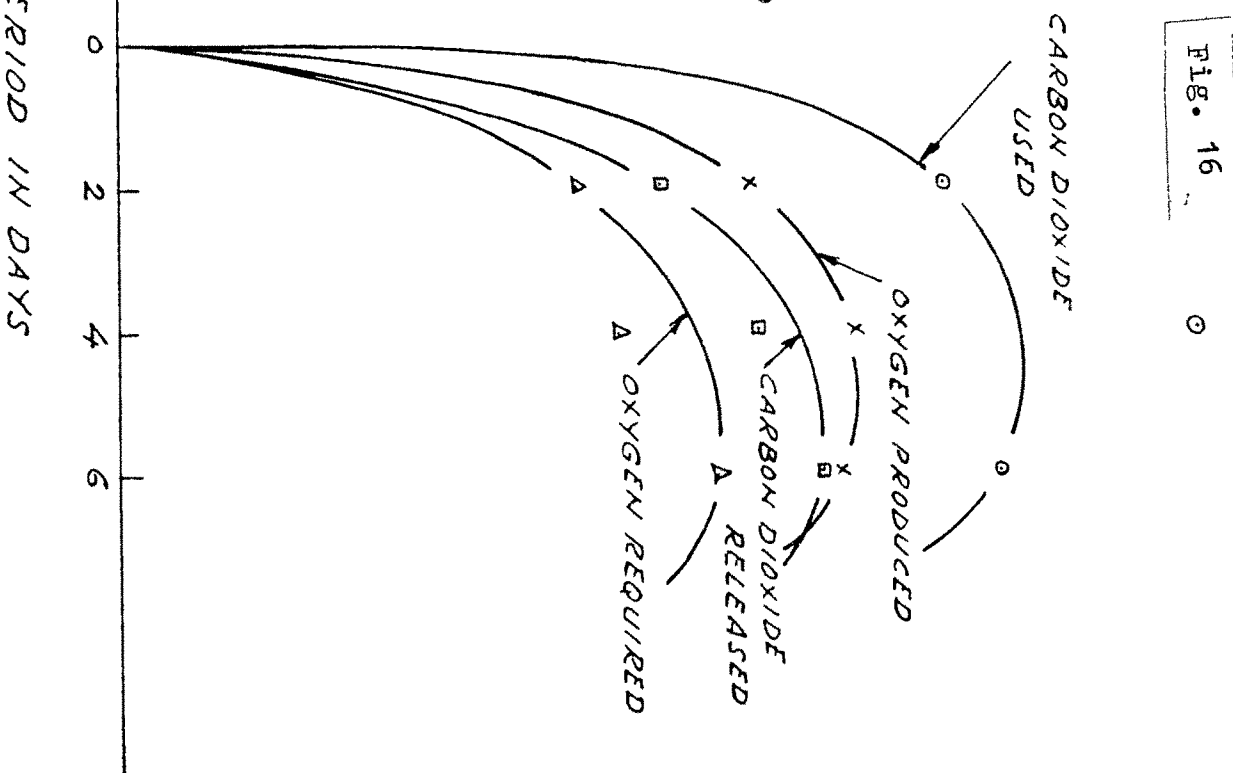
# ALGAL PHOTOSYNTHESIS USING SCENEDESMUS OBLIQUUS

Fig. 15



# ALGAL BACTERIAL SYMBIOSIS USING SCENEDESMUS OBLIQUUS

Fig. 16



ii) Application to connected results:

Myers (1962) has shown that for the production of 1.0mg. of dry algal matter, 1.8mg. of  $\text{CO}_2$  are required; and Oswald and Gotaas (1957) have shown that the production of 1.0 gm. of dry algal matter is accompanied by the liberation of 1.6 gm. of oxygen. These two factors have been used in the following calculations shown in the two tables.

$\text{CO}_2$  used up during algal biomass production  
(mg/l): (Fig. 15 & 17).

Deten- tion time	Scenedesmus		Microcystis	
	Algal biomass	$\text{CO}_2$ needed	Algal biomass	$\text{CO}_2$ needed
2 days	275	495.0	224	403.2
4 days	286	514.8	231	415.8
6 days	282	507.6	236	424.8

Photosynthetic oxygen released into the  
ecosystems(mg/l): (Fig. 15 & 17)

Deten- tion time	Scenedesmus		Microcystis	
	Algal biomass	$\text{CO}_2$ needed	Algal biomass	$\text{CO}_2$ needed
2 days	275	440.0	224	358.4
4 days	286	457.6	231	369.6
6 days	282	451.2	236	377.6

From the above results, it is concluded that 424.8 to 507.6 mg/l of  $\text{CO}_2$  are used up by the algae in photosynthesis during six days for the formation of 236 to 282 mg.



Fig. 17

ALGAL PHOTOSYNTHESIS USING  
MICROCYSTIS AERUGINOSA

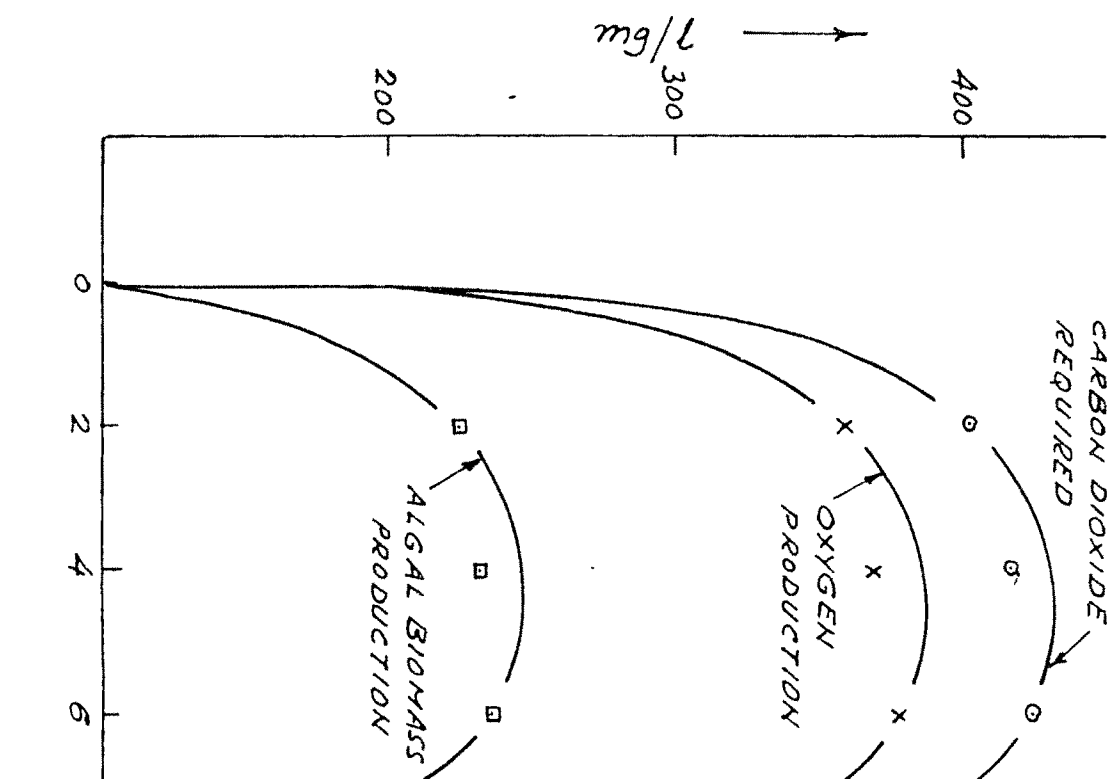
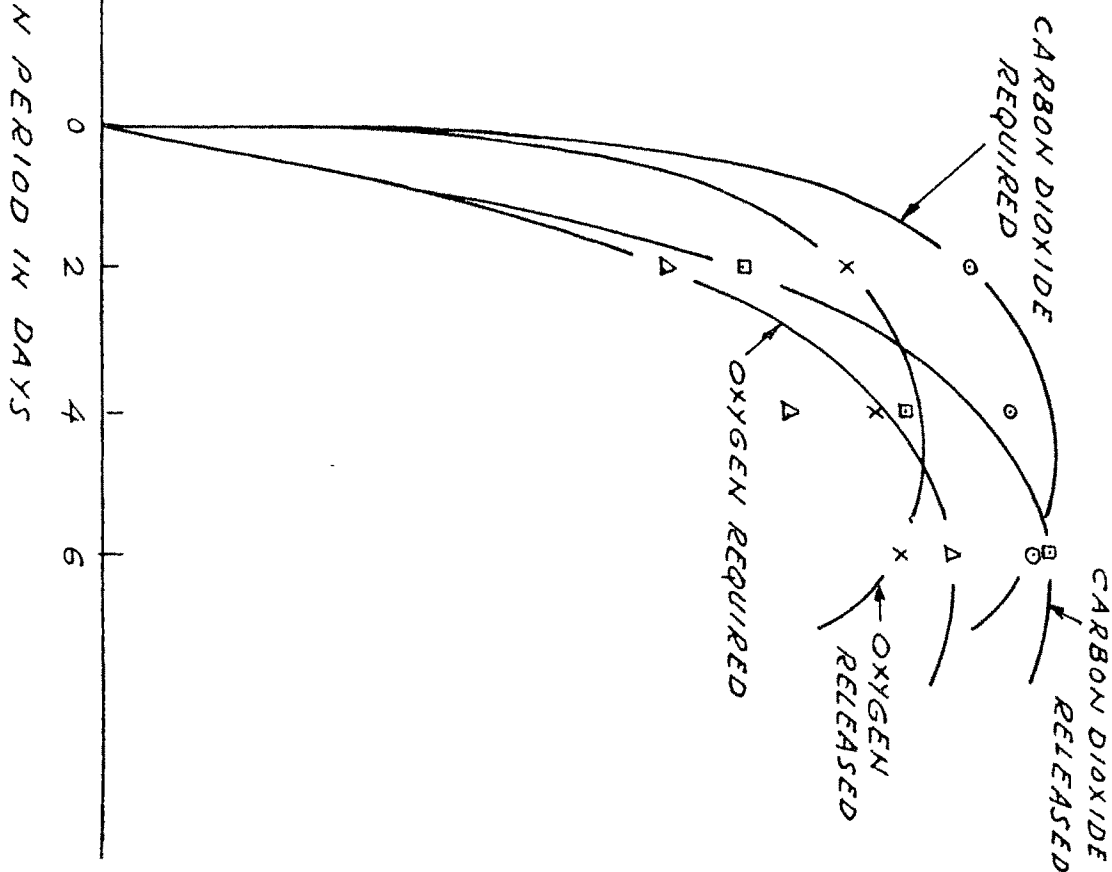


Fig. 18

ALGAL BACTERIAL SYMBIOSIS USING  
MICROCYSTIS AERUGINOSA



of algal biomass; and 377.6 to 451.2mg of photosynthetic  $O_2$  are liberated in the two ecosystems.

(d) Energy conversion efficiency by the two algae:

i) Theoretical considerations:

Gotaas and Oswald (1955) and Oswald and Gotaas (1957) have developed an input-output energy balance system for estimating the overall photosynthetic energy conversion efficiency in which a basic assumption is made that the system under study is a continuously stirred tank reactor (CSTR) with complete homogeneity of the algal cells in suspension (Beck et al, 1969). They have stated:- as in any continuously stirred tank reactor, there is a finite volume,  $V$ , in litres and flow rate  $F$ , in litres per day. The mean hydraulic residence time  $\theta$  is then defined as

$$\theta = V/F \quad \dots \quad \dots \quad \dots \quad \dots \quad (13)$$

For a given mean residence time  $\theta$ , in days, the total solar energy input per litre of pond volume is equal to:

$$E_{in} = S.A. \theta \quad \dots \quad \dots \quad \dots \quad (14)$$

where  $E_{in}$  is the total energy input - in calories per litre;  $S$ , the daily solar energy input in calories per square cm. per day;  $A$ , the surface

area of one litre of pond volume receiving sunlight in  $\text{cm}^2$  per day and  $\Theta$  is the mean hydraulic residence time. For one litre of pond volume  $A = 1000/d$  where  $d$  is the determined depth in Cm.

$$\text{Therefore } E_{in} = \frac{1000 \times S \times \Theta}{d} \dots \dots \dots (15)$$

The energy output in the form of synthetised algae is defined as:

$$E_{out} = h \times Cc \dots \dots \dots (16)$$

where  $E_{out}$  is the total energy tied up in synthetised algae in calories per lite,  $h$  is the heat of combustion of algae in calories per milligram, and  $Cc$ , is the concentration of algae in mg/l. Equations (14) and (16) can be equated by assuming that only a portion of the energy input is converted to algal biomass, so that,

$$E_{in} \times e = E_{out} \dots \dots \dots (17)$$

where  $e$  is an efficiency factor. The equation (17) can be expanded thus:

$$\frac{1000 \times S \times \Theta \times 1}{d} = h \times Cc \dots \dots \dots (18)$$

$$\text{and } \therefore e = \frac{h \times Cc \times d}{1000 \times S \times \Theta} \dots \dots \dots (19)$$

Myers (1964) recommends a value of 5.5 calories per mg. as the heat of combustion h of algae.

ii) Application to connected results:

The above equation is used in calculating the overall photosynthetic energy conversion efficiency of the two algae used in these experiments. The pertinent data used in our calculations are shown for the alga Scenedesmus obliquus used in our laboratory experiments:-

Volume of the culture fluid i.e. raw  
sewage used = 1.5 litres.

Depth of the liquid volume = 3.5 cm.

Maximum quantity of algal biomass produced in  
6 days in 1.5 litres of sewage = 423 mg.

Average light energy available in the lighted  
room = 375 lux.

10.5 lux = 1 gram - calorie

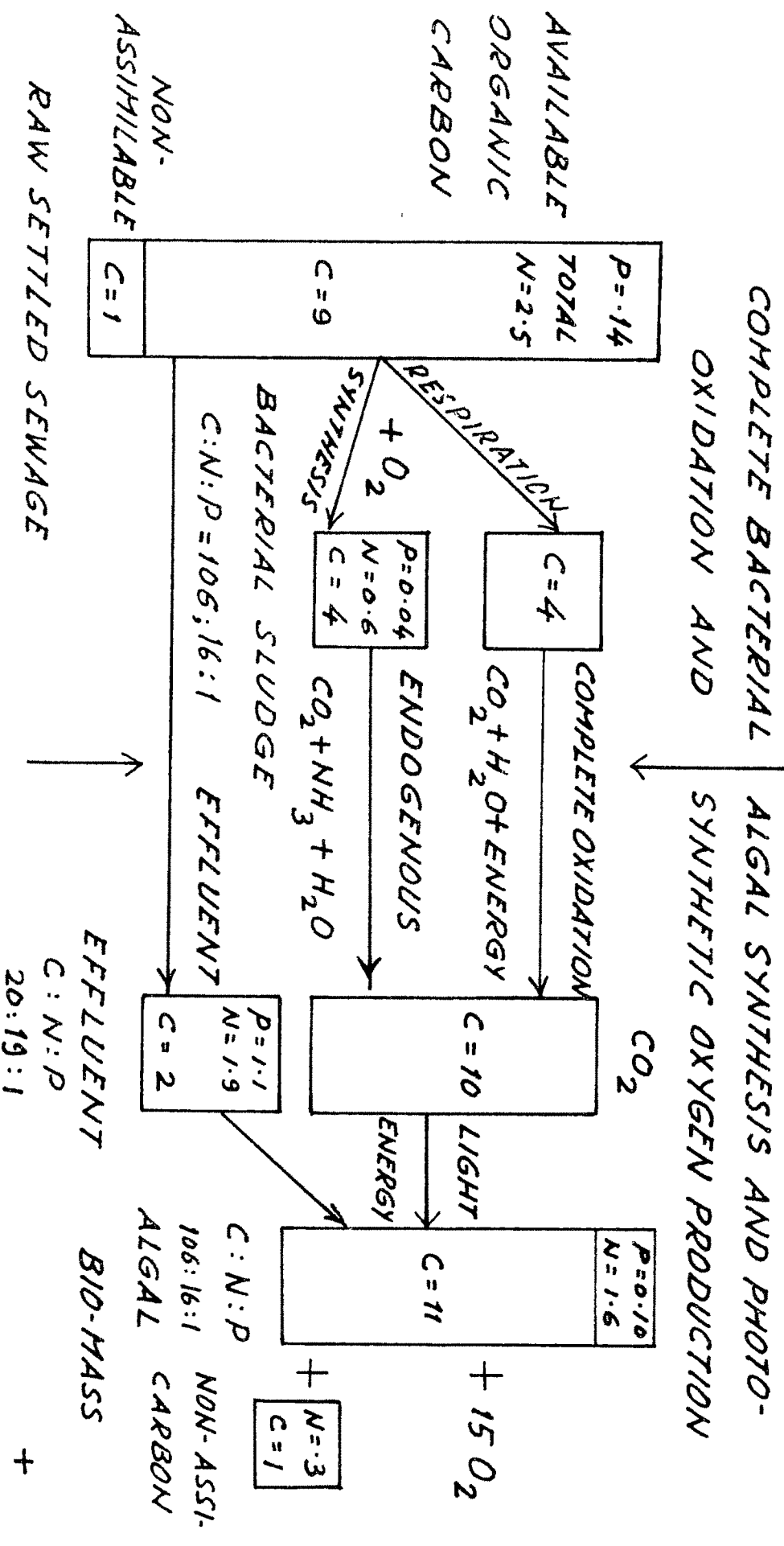
∴ 375 lux = 35.7 gram - calories

1 mg of algae contain 5.5 calories (heat of  
combustion).

Fig. 19

# BIOCHEMISTRY OF ALGAL-BACTERIAL SYMBIOSIS IN HIGH RATE AEROBIC POND SYSTEM(MODIFIED) AFTER STUMM 1968)

## A BALANCED SYSTEM OF



Applying these values in equation (19), we get

$$\begin{aligned} e &= 423 \times 5.5 \times 3.5/35.7 \times 1500 \times 6 \\ &= 0.0253 \\ &= 2.53\% \end{aligned}$$

Calculations were made in a similar way for the Microcystis aeruginosa; and the % of photosynthetic efficiency of both the algae are given in a tabular form below:

Name of the algae	%photosynthetic efficiency
(c) <u>Scenedesmus obliquus</u>	2.53
<u>Microcystis aeruginosa</u>	2.12

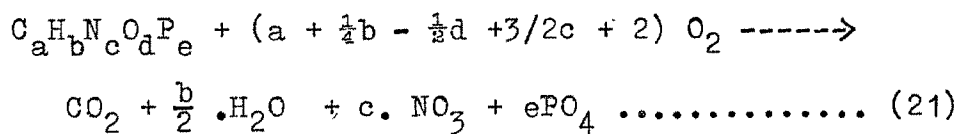
(e) Algal-bacterial symbiosis in high-rate oxidation ponds using Scenedesmus obliquus and Microcystis aeruginosa: (Fig. 19).

i) Theoretical considerations:

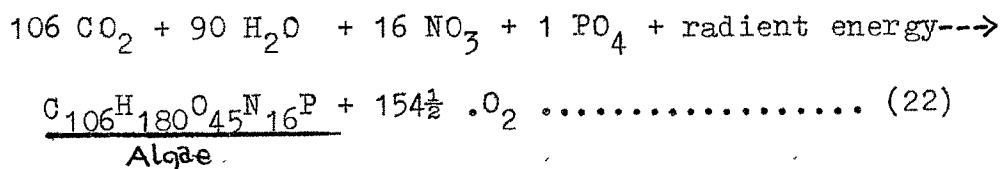
Two broad groups of organisms are mainly involved in the process. They are : heterotrophic organisms consisting of consumers i.e. protozoans and bacteria. The role of the latter which are found in billions is far more important than the former and therefore will be considered here. The autotrophs are the other



Oxidation of the ammonia to nitrate rarely occurs because ammonia is assimilated by algae, lost to the air or precipitated during periods of high pH before nitrification is established. The same reaction has been expressed by Stumm (1968) as follows:



The resultant products are soluble inorganic nutrients of biological significance which are returned to the ecosystem. These nutrients are used up by algae in the presence of radiant energy for further algal synthesis according to the equation (Stumm, 1968):



In short, the bacteria metabolise the organic components of the waste and release some substances utilisable by algae. During synthesis of fresh algal cells, algae release oxygen, which is utilised by bacteria for stabilization of organic matter.

The treatment effected by an oxidation pond results, from a complete symbiosis between bacteria



and algae (Ludwig, Oswald, Gotaas and Lynch, 1951).

ii) Application to connected results:

Quantity of photosynthetic  $O_2$  released during algal photosynthesis is compared with the  $O_2$  requirements for bacterial oxidation of sewage organic matter during algal-bacterial symbiosis in high-rate oxidation ponds in respect of the two algae investigated: (Fig. 16 & 18).

Scenedesmus obliquus ( $O_2$  : mg/l)

Deten- tion period	Released during algal photosynthesis	Required for bacterial oxidation	Difference (excess %)
2 days	440.0	358.8	81.2 (18.4)
4 days	457.6	390.0	67.6 (14.7)
6 days	451.2	410.3	40.9 (9.06)

Photosynthetic oxygen production is found greater than the quantity of oxygen needed for bacterial oxidation of sewage organic matter by 9 to 18% during algal bacterial symbiosis, so that the ecosystem is always kept under aerobic conditions.

Microcystis aeruginosa (O<sub>2</sub> ; mg/l)

Deten- tion period	Released during algal photosynthesis	Required for bacte- rial oxidation	Difference (excess %)
2 days	358.4	299.5	58.9(16.4)
4 days	369.6	351.0	18.6(5.03)
6 days	377.6	397.8	-20.2(-5.3)

In this case also, photosynthetic oxygen production is found to be greater than the quantity of oxygen needed for bacterial oxidation during the first 4 days but later there is slight reduction in quantity.

The increase in CO<sub>2</sub> released and the decrease in O<sub>2</sub> production on the 6th day in the case of Microcystis during bacterial oxidation may be due to the oxidation of the extra-cellular organic matter in the form of glycolate which may have been produced by algae according to Fogg and Watt, (1965). This aspect requires further investigation.

CO<sub>2</sub> released during total bacterial oxidation of Baroda sewage organic matter is compared with the quantity used up for algal biomass production in algal bacterial symbiosis:

(Fig. 16 & 18)

Scenedesmus obliquus (CO<sub>2</sub> : mg/l)

Deten- tion period	Used in algal Photo- synthesis	Released during total bacterial oxidation	Difference (excess %)
2 days	495.0	388.7	106.3(21.5)
4 days	514.8	422.5	92.3(17.9)
6 days	507.6	445.0	62.6(12.3)

Microcystis aeruginosa (CO<sub>2</sub> : mg/l)

Deten- tion period	Used in algal Photo- synthesis	Released during total bacterial oxidation	Difference (excess %)
2 days	403.2	324.0	79.2(19.6)
4 days	415.8	380.2	35.6( 8.5)
6 days	424.8	430.9	- 6.1(-1.4)

Explanation for the excess of CO<sub>2</sub> used up  
during algal-bacterial symbiosis:

From the above two tabular statements it will be found that sewage organic matter alone is not sufficient for the liberation of the required amount of CO<sub>2</sub> during algal-bacterial symbiosis in high-rate oxidation ponds. Other sources of CO<sub>2</sub> such as that form the atmosphere and from the bicarbonate-carbonate equilibrium system seem to have furnished the balance of CO<sub>2</sub>. This amount varies from 9 to 27% of that produced from sewage organic matter by bacterial oxidation. There is a slight excess of CO<sub>2</sub> on the 6th day from bacterial oxidation, which is slightly more than the required amount for algal photosynthesis.

(f) Regression analyses of nutrient strength expressed as COD and algal biomass and their correlation coefficients:

i) Theoretical considerations:

The nutrient strength of a waste is ordinarily expressed in terms of the Biochemical Oxygen Demand or BOD, which is a measure of the quantity of oxygen

required to carry out the aerobic bio-oxidation of the biologically available organic material in waste water under specific conditions of time and temperature (American Standard Methods, 1971). Oswald and Golueke (1960, p. 229) have found that in steady state continuous cultures under specific conditions of light intensity, temperature and other factors, there is an optimum BOD for algal growth, and that upto this optimum, algal growth increases almost linearly with increased BOD. They have also added that a decrease in concentration of algae occurs at BOD concentrations in excess of this optimum, probably because strong wastes contain excess colloidal material and bacteria which remain in suspension and thus decrease the energy available for algal growth.

ii) Application to connected results:

Our studies confirm the observations of Oswald and Golueke (1960). Under our laboratory batch-culture experimental conditions of light intensity, temperature and other factors and using Baroda, settled and strained ~~undisturbed~~ sewage, we find

a high degree of direct correlation between algal growths of the 2 different kinds of algae and their corresponding used up COD. Regression analyses relating algal growths in each case with the corresponding used up COD have been worked out for the individual alga. The correlation coefficient "r" in each case are also indicated in the subjoined tabular statement, alongwith the corresponding values for the two constants "m" and "b" in the regression equations. Regression analysis constants "m" and "b" and coefficient "r" can be calculated from the following formula, according to American Standard Methods (13th edition, 1971).

i) Regression analysis constant "m" and "b"

$$m = \frac{n \cdot \sum xy - \sum x \cdot \sum y}{n \cdot \sum y^2 - (\sum y)^2} \dots \dots \dots (23)$$

$$b = \frac{\sum y^2 \cdot \sum x - \sum y \cdot \sum xy}{n \cdot \sum y^2 - (\sum y)^2} \dots \dots \dots (24)$$

Where n = Number of observations.

x = Algal biomass

y = COD used up

ii) Correlation coefficient "r":

$$r = \frac{\sum x y}{\sqrt{\sum (x^2) \cdot \sum (y^2)}} \dots\dots\dots (25)$$

where x = Algal biomass

y = COD used up

Applying formula (23), (24) & (25) to our results the following calculations are made:

Name of the algae	<u>Regression analysis</u>		<u>Correlation</u>
	<u>constants</u>		<u>Coefficient</u>
	"m"	"b"	"r"
<u>Scenedesmus</u> <u>obliquus</u>	+ 0.197	+223.3	+0.710
<u>Microcystis</u> <u>aeruginosa</u>	+ 0.158	+187.8	+0.997

Regression analysis relating algal growths in each case with the corresponding used up COD have been worked out for the individual alga mentioned above. The correlation coefficient "r" in each case are also indicated in the above subjoined tabular statement along with the corresponding values of the

two constants "m" and "b" in the regression equations.

Our studies confirm the observations of Oswald and Golueke (1960). We find a high degree of direct correlation between algal growths and their corresponding used up COD values.

(g) Nutrient substances of biological significance in Baroda sewage and their removal during algal photosynthesis:

i) Theoretical considerations:

Two groups of nutrients are involved in algal-bacterial symbiosis, major and micro-nutrients. The former consists of C, N and P and the later of trace elements like Cu, Mn, Sb, As etc. The major nutrients are used up by heterotrophic bacteria and autotrophic algae, the two main partners in the game of algal-bacterial symbiosis.

Stumm (1968) has laboured to show that not only the main constitutional elements in both bacteria and algae are the same (C, N and P) but are also found in the same constant proportions of 106:16:1 atoms. Therefore, in the high-rate aerobic oxidation



pond method of waste water treatment the ratios of C:N and C:P are very important in view of their utilization in algal bacterial synthesis.

The major nutrients are C, N and P and the micro-nutrients are always present. The proportions in which the major nutrients present are (a) C:N ( $\text{NH}_3\text{-N}$ ). The reaction between C, COD and  $\text{BOD}_5$  is 12:32:21.9 for ordinary wastes (Porges, 1960, p. 81) and we get a ratio of 12:31:23.3. Stumm (1968) has observed that C is deficient in domestic sewage for utilizing all the N and P into algal cells in the above proportions.

ii) Application to connected results:

Nitrogen utilized as  $\text{NH}_3\text{-N}$ :

Nitrogen assimilation is calculated on the basis of the amount of  $\text{NH}_3\text{-N}$  used up from the ecosystem as a result of algal growth. This is an oversimplification of the real system found in the growth cultures where decomposition of organic matter, bacterial cellular synthesis and endogenous respiration and algal growth are taking place almost simultaneously and a constant flux of nitrogen

forms also take place. The  $\text{NH}_3\text{-N}$  consumed in the two algal growth cultures calculated at 7.92% of their biomass are shown below:

Scenedesmus obliquus (mg/l):

Deten- tion time	Algal bio- mass (mg/l)	$\text{NH}_3\text{-N}$ (mg/l)			N-con- tent of algal biomass @7.92%	%Utiliza- tion of N by algal biomass
		Found	Used	% Reduc- tion		
0 days	-	31.8	-	-	-	-
2 days	275	4.9	26.9	84.6	21.8	68.6
4 days	286	4.1	27.7	87.0	22.6	70.11
6 days	282	3.5	28.3	90.0	22.3	70.00

Microcystis aeruginosa (mg/l)

Deten- ten	Algal bio- mass (mg/l)	$\text{NH}_3\text{-N}$ (mg/l)			N-Con- tent of algal biomass mg/l @ 8.16%	%Utiliza- tion of N by algal biomass
		found	Used	%Reduc- tion		
0 day	-	33.0	-	-	-	-
2 days	224	11.4	21.4	65.4	18.3	55.4
4 days	231	7.2	25.8	78.2	18.8	57
6 days	236	4.4	28.6	86.6	19.2	58.2

87 to 90% of the  $\text{NH}_3\text{-N}$  has been utilized in 6 days by the Scenedesmus obliquus and Microcystis aeruginosa in the two experiments. The N content of Scenedesmus was estimated as 7.92% and Microcystis as 8.16%. The N content of Scenedesmus is about 22 mg/l and of Microcystis is 19.0 mg/l. So, the utilization of  $\text{NH}_3\text{-N}$  in the first case works out to 70% and in the second case to 58%, the average being 64%. So, 30 to 42% of the  $\text{NH}_3\text{-N}$  must have been utilized for other biochemical reactions or lost to the atmosphere on account of high pH values.

Algae require N either as  $\text{NH}_3\text{-N}$  or as  $\text{NO}_3\text{-N}$ , but they seem to prefer  $\text{NH}_3\text{-N}$  when both are provided together (Harvey, 1940; Schular et al. 1953). It is also reported that nitrate assimilation results in the production of (OH) ions which causes a rise in pH while  $\text{NH}_3\text{-N}$  assimilation lowers the pH by the formation of  $\text{H}^+$  ion.

#### Phosphorus used as $\text{PO}_4$ :-

Phosphorus is used as orthophosphate in algal-bacterial symbiosis. P content of Scenedesmus was found to be 0.96% and that of Microcystis as 1.06%

of the dry algal weight. The amount of phosphorus as P and as  $\text{PO}_4$  used up in the two experiments are shown under:

Scenedesmus obliquus (mg/l)

Deten- tion time	Algal bio- mass	PO <sub>4</sub> -P (mg/l) in the ecosystem				%Red- uction	P-con- tent of algal biomass (mg/l)@ 0.96%	%Utiliza- tion of P by algal biomass from the influent.
		Found		Used				
		PO <sub>4</sub>	P	PO <sub>4</sub>	P			
0 day	-	12.5	4.2	-	-	-	-	-
2 days	275	4.1	1.4	8.4	2.8	66.6	2.64	62.8
4 days	286	2.6	0.9	9.9	3.3	78.6	2.74	65.2
6 days	282	2.1	0.7	10.4	3.5	83.3	2.71	64.5

Microcystis aeruginosa (mg/l):

Deten- tion time	Algal bio- mass	PO <sub>4</sub> -P(mg/l) in the ecosystem					P-Con- tent of algal biomass (mg/l)@ 1.06%	%Utiliza- tion of P by algal biomass from the influent
		Found		Used		%red- etio n		
		PO <sub>4</sub>	P	PO <sub>4</sub>	P			
0 day	-	17.5	5.8	-	-	-	-	-
2 days	224	10.0	3.3	7.5	2.5	43.1	42.37	40.9
4 days	231	8.9	3.0	8.6	2.9	50.0	2.45	42.2
6 days	236	6.8	2.3	10.7	3.5	62.0	2.5	43.1

62 to 83% of the phosphate phosphorus have been used up in the two experiments.

The phosphorus content of Scenedesmus has been found to be about 2.7 mg/l and Microcystis is 2.5 mg/l. The utilization of  $PO_4$ -P in the case of Scenedesmus works out 65% and Microcystis 43.0%. The rest of the phosphate must have been precipitated as calcium phosphate and magnesium phosphate on account of higher pH in 6 days detention period.

(h) Biological conditions (Table-3, Appendix)

The results are tabulated in Table-3. The chief points arising out of these examinations are:

- i) the occurrence of dark to light brown filaments rods resembling the iron bacterium Leptothrix ochracea in the control and algae treated flasks; and brownish flocculant precipitates in suspension seen in the control flasks only.
- ii) Organic debris were seen intermixed with algal biomass in minute quantities.
- iii) the presence of rotifer Lucane sp. is an indication of a good condition of the effluent produced.

i) Biochemical conditions: (Table-9, Appendix).

The reduction of biochemical variables like amino acid nitrogen, protein, sugars and organic acids in the control flasks has to be attributed to metabolic activities of bacteria. Surface reaeration has helped in providing oxygen to bacteria for degradation of organic matter in the control flask. But the higher percentage of decrease in algal treated flasks has to be attributed to the greater availability of photosynthetic oxygen than from surface reaeration. They have been used up in bacterial synthesis.

(j) Bacteriological examination (Sanitary aspect) in high-rate aerobic oxidation pond using Scenedesmus obliquus.

The increase or decrease in the percentage reduction on different detention period is shown in the following table:

Deten- tion period in days	Control:Raw sewage		High-rate:Algae treated	
	Coli- form/ ml.	Total Colo nies count/ ml.	Coli- form/ ml.	Total Colonies count/ml
0	$16.09 \times 10^{10}$	$10.2 \times 10^{11}$	$16.09 \times 10^8$	$11.5 \times 10^{11}$
2	90.0	81.6	98.91	99.99
4	99.99	99.99	99.99	99.99
6	99.99	99.99	99.99	99.99

Control: The coliform group organisms show a reduction of about 90% on 2nd day and on the 4th and 6th day, the reduction is about 99.99%. But still at the end of 6th day  $46.0 \times 10^2$  / ml of sample coliform type of bacteria are present.

Total colonies count also decreases on 2nd day by about 81.6% and again the reduction on 4th and 6th day is about 99.99%. Still  $14.1 \times 10^6$  residual bacteria are present at the end of 6th day.

High-rate (Algae treated): Coliform organisms decrease on 2nd day by about 98.91% and again on the following 4th and 6th day the reduction is 99.99%, but still 120/ml of sample coliform bacteria remain present on the 6th day. The reduction in coliforms in 2, 4 and 6 days in algae treated flask is much higher than in the control.

Total colonies count decreases on 6th day by about 99.99%.

It is obvious from the above results that by using algae in the system, even on the 2nd day 98.9% reduction in coliform organisms takes place whereas in the case of control 90% reduction in coliform organisms takes place. So, it can be concluded that algae treated samples remove coliform organisms

from the medium more efficiently and the degree of purification is also greater.

Micro-organisms in action in high-rate aerobic pond:  
(Tables 7 and 8, Appendix).

The bacteria found in high-rate aerobic pond grown on domestic sewage belong predominantly to Gram negative, rods, affecting sugars and tentatively determined as belonging to the genera which varies in dominance on the different detention periods and the dominant genera are recorded in Table 5. It will be seen that the dominant genera on 2nd day were Achromobacter, Aeromonas, Bacillus, Proteus, Pseudomonas and Zoogloea. On 4th and 6th days, the dominant genera were Aerobacter, Alcaligens, Comamonas and Zoogloea. In brief Alcaligens, Aerobacter, Comamonas and Zoogloea were found on all the four detention periods and may be considered to be the main generic bacterial constituents of high-rate aerobic pond.