CHAPTER - V

DISCUSSION OF THE EXPERIMENTAL RESULTS

An Experimental evaluation of "algal-bacterial Symbiosis"

In order to evaluate "algal-bacterial symbiosis" (or how one helps the other) in our experiments with the three different types of algae, it is necessary to know the quantity of carbondioxide liberated during total bio-oxidation of sewage organic matter for the production of each of the three algal biomasses; and in turn also how much of the photosynthetic oxygen is liberated by each of the algal biomass during 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 two reasons: (i) They are not phased metabolic processes (i.e. one taking place after the other) but they are considered to be not only almost simultaneous or concurrent but are also stated to be utilised as soon as they are liberated in the closed aquatic ecosystem (Oswald 1960). The two metabolic processes are illustrated in Fig. 7, (ii) Even granting that it is possible to estimate them

directly by means of isotopes, there are no facilities for such work in our laboratory. Further, according to Fogg (1970, p.102) "the radio carbon method suffers from more uncertainties." Therefore the method suggested in this thesis appears to be more appropriate than isotope method for the conditions existing in the ecosystem. So we were forced to resort to short-cut methods, which could be verified, if needed be, when facilities for work with labelled compounds are available in other laboratories.

So attempts were made to estimate the quantities of the two gases indirectly by methods which are based upon certain well-established factors and equations connecting carbon dioxide production and oxygen requirements for oxidation of sewage organic matter and photosynthetic oxygen production and carbon dioxide requirements for the algal biomasses 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.

The main metabolic reactions taking place in oxidation pond is "algal-bacterial symbiosis" and therefore an attempt has been made to dissect the mechanism of "algal-bacterial symbiosis" into its two component parts: (a) bacterial oxidation and (b) algal photosynthesis; and to evaluate quantitatively how one has helped the other. So the results of our experiments have been discussed in four parts:

A. Bacterial Oxidation

B. Algal Photosynthesis,

C. "Algal-bacterial Symbiosis",

and D. Removal of biostimulatory nutrients.

A. Bacterial Oxidation

Sewage contains nearly 99.9% water and 0.1% solids. The sewage solids are about 14% settleable, 11% supra-colloidal, 6% colloidal and 69% soluble (Rickert and Hunter 1967). The soluble constituents are mainly carbohydrates, proteins and fatty substances.

Purification of sewage:

Purification of sewage is affected in two phases. I: Partial or intermediate process of treatment and II: Bacterial oxidation of soluble organic matter.

They are discussed below.

Phase -I : Partial or intermediary process of treatment:-

The phenomena of autoflocculation, bioflocculation, bioprecipitation and surface reaeration are of common occurence in nature and act as partial and intermediary process of purification (Heukelekian 1941). Sewage containing finely divided suspended matter and soluble organic matter undergoes first autoflocculation and bioflocculation, since colloidal material in sewage has a natural tendency to form aggregates or it is a transitory stage of becoming larger particles and materials in solution. The process of autoflocculation is a coalescence of finely divided suspended matter under the influence of physical forces, while bioflocculation is also coalescence of finely divided suspended matter but acting under the influence of biological agencies. In the case of high-rate oxidation pond solid surfaces are provided by alga, which besides furnishing photosynthetic oxygen for bacterial oxidation by algal photosynthesis also seem to act as resting places for bacteria and where nutrients also concentrate on their solid surfaces. So the naturally occuring autoflocculation and bioflocculation processes

are exploited to the maximum possible extent in the first phase of purification of settled raw sewage. This phase is followed by bioprecipitation, in which soluble substrates of sewage are converted into cellular protoplasm by bacteria. Surface reaeration is another factors of equal importance playing a significant role especially at nights when photosynthesis is impossible. It helps to drive oxygen and carbon dioxide from the air into the liquid as in the case of Simplex Activated Sludge Process. The wind action across the huge pond surface of 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. Thus, these phenomena are taking place in the laboratory experimental flasks and the effect of their actions are more visible in the control flasks.

Phase-II: Bacterial oxidation of soluble organic matter:

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 gelatinous coating of

the biological slime formed on inert surfaces. This is followed by oxidation or conversion of the adsorbed matter by bacteria using oxygen. The biological slime contains aerobic bacteria of different species. Bacterial oxidation of the organic matter is the result (Eckenfelder and Weston 1956, Eckenfelder and O'connor 1961). Bacteria oxidise the sewage organic matter in three ways:

(i) Complete oxidation:

The oxidative decomposition of any organic compound follows a metabolic pathway that is characteristic for the compound and for the organism that oxidises it. The respiratory oxidation of organic compounds by most bacteria results in the formation of carbon dioxide, water and energy as a principal waste products of metabolism, where a great number of enzymes and intermediate products are involved besides the electron -transfer system. Such oxidations are said to be complete.

(ii) Incomplete oxidation:

There are cases, however, in which the organism does not possess all the enzymes necessary for complete conversion of a substrate to carbon dioxide. In such

cases, complete conversion of an organic substrate to carbon dioxide does not take place but oxidised organic compounds accumulate in the medium and/or in the cellular bodies as end products of respiratory metabolism. These oxidations are said to be incomplete. The reactions of the acetic acid bacteria are classical examples.

In addition, the early enzymatic steps in the oxidation of a compound may sometimes be more rapid than subsequent steps. When this happens, incompletely oxidised products of intermediary metabolism accumulate in the medium until the substrate has disappeared. This is specially true when abnormally high concentrations of substrates are furnished to the bacterial cells.

A very striking aspect of bacterial metabolism is the relatively enormous amounts of new cell material that is normally produced during the break-down of organic nutrients. Thus growth accompanies the decomposition of the substrate and newly formed cells are a major end product of intermediary metabolism. This process is known as "cellular synthesis" and "oxidative assimilation". The patterns of substrate utilization may be either concurrent or sequential.

Thus, in incomplete oxidation a part of the organic matter is used up as reserve materials or storage products and the rest is assimilated for synthesis of new cells. These types of metabolism seem to be taking place in the trickling filter and in the activated sludge process.

(iii) Endogenous metabolism:

Endogenous metabolism takes place when the organic material in solution is very low so that bacteria derive energy from the destruction of their own storage products. At this stage the number of active organisms is low and most bacteria then loose their motility. Endogenous respiration occurs, because substrate to bacterial mass ratio and maintenance energy of bacteria differ at different situations of a treatment system. When bacteria grow at low substrate level, cells increase in size but do not divide and bacterial counts do not show this type of growth (Van Gil/s 1964).

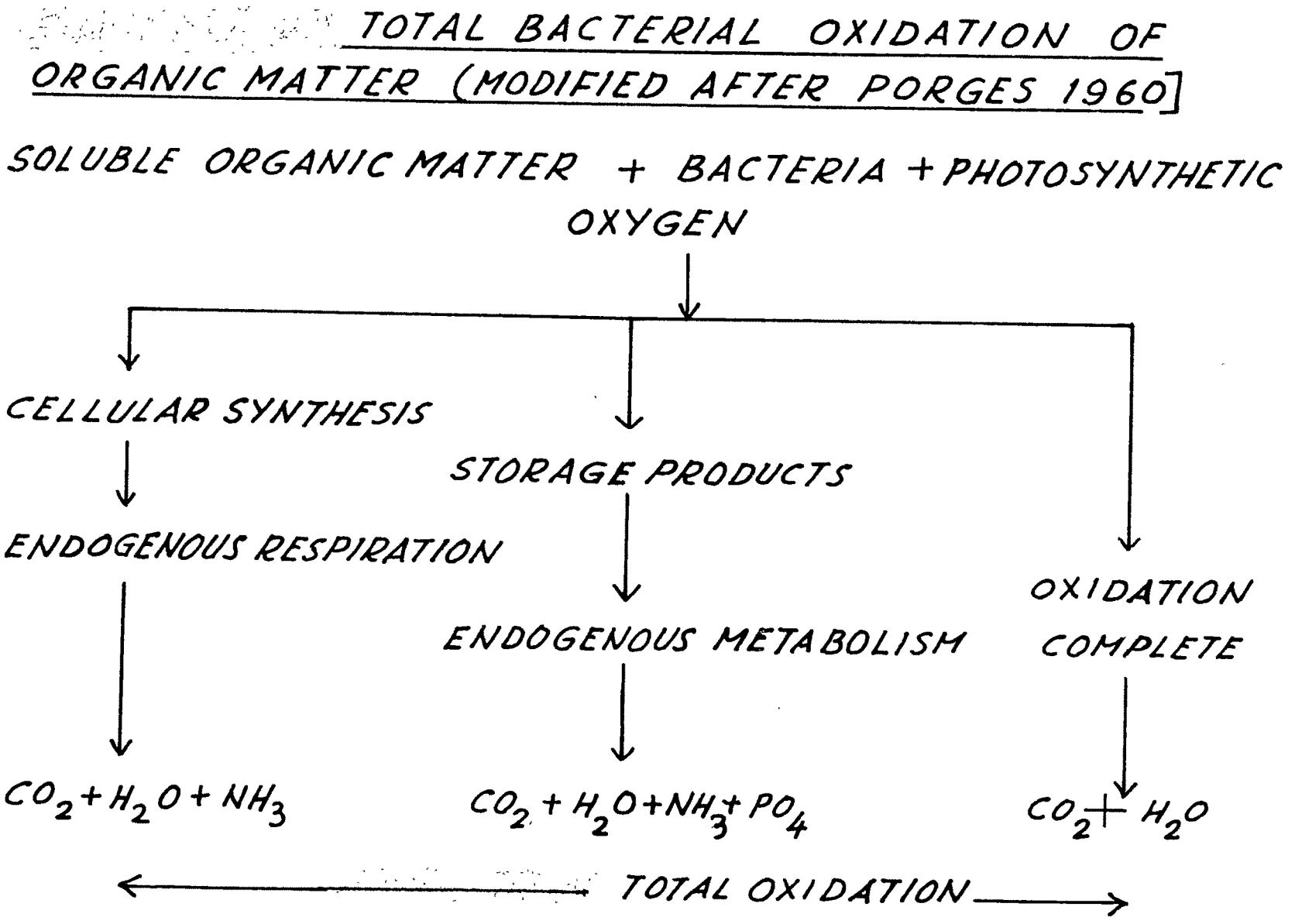
Endogenous respiration may take place, especially when the time of treatment i.e. the contact period between substrate and bacteria, is extended; which leads to low substrate concentration with more bacterial growth. And as there is no substrate left behind,

bacteria use their storage products and auto-oxidation of bacteria takes place resulting in low accumulation of bacterial sludge.

According to Lamana Carl (1963); "Endogenous metabolism can be defined as the sum total 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".

"Depriving organisms of nutrients causes them immediately to be dependent exclusively upon material resources for maintaining their lives. At the moment of deprivation of nutrients the cell finds itself with an acquired compliment of enzymes that can continue to act as long as no barriers are interposed to access to endogenous substrates. Thus the metabolic activity of the starving cell could be the expression of an unreasonable but natural compulsion

Fig. 8

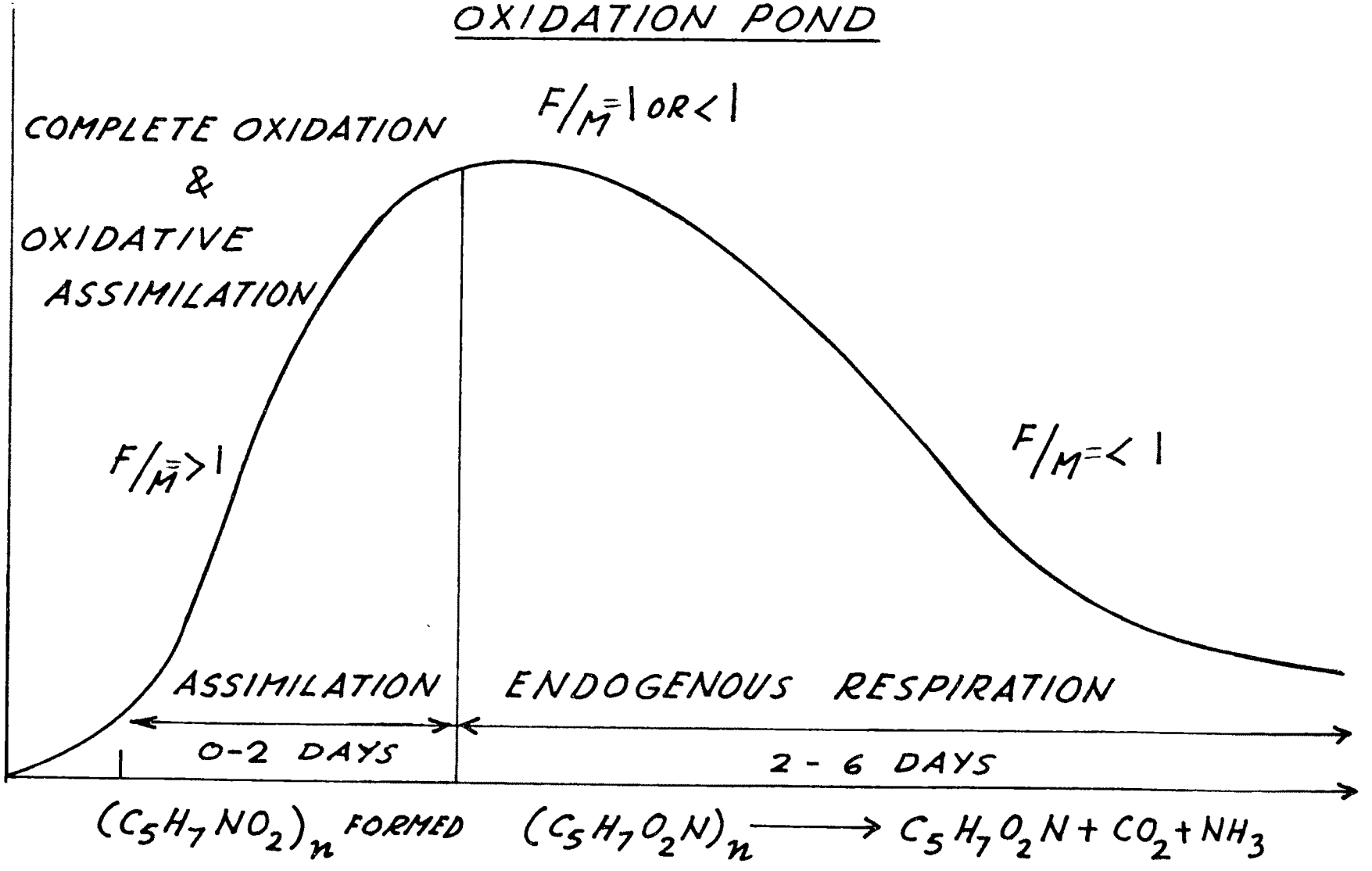

TOTAL BACTERIAL OXIDATION OF ORGANIC MATTER (MODIFIED AFTER PORGES 1960]
SOLUBLE ORGANIC MATTER + BACTERIA + PHOTOSYNTHETIC OXYGEN
CELLULAR SYNTHESIS
STORAGE PRODUCTS
ENDOGENOUS RESPIRATION
OXIDATION COMPLETE
ENDOGENOUS METABOLISM
$CO_2 + H_2O + NH_3$
$CO_2 + H_2O + NH_3 + PO_4$
$CO_2 + H_2O$
TOTAL OXIDATION

of an enzyme to work with no necessary production end in sight. In fact, a starving cell is losing its substance and must eventually waste away and die. It would be much more efficient if the cell deprived of external resources could automatically stop its endogenous metabolism and rest in a state of suspended animation".

If endogenous respiration is extensive there is no accumulation of sludge; if it is low, sludge accumulates (Hoover et al. 1952). In oxidation ponds, the absence of considerable microbial sludge would seem to show that all the bio-degradable organic matter in influent sewage undergoes "total oxidation". "Total oxidation" 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 are oxidised to carbondioxide, ammonia and water. How maximum amounts of these substances are made available as a result of "total oxidation" of organic matter during bacterial oxidation is schematically represented in Fig. 8 (modified after Porges 1960).

Fig.9

DIFFERENT PHASES OF BACTERIAL OXIDATION IN HIGH RATE AEROBIC OXIDATION POND



Different phases of microbial metabolism:

The above biochemical principles of microbial metabolism of organic matter in biological oxidation processes, represent the kinetics of bacterial biosynthesis, growth and decay; and they are shown below in the four growth phases in the ideal curve Fig. 9. The lag phase is largely eliminated in the high-rate oxidation pond as the pond is inoculated with a large amount of algal-biomass for starting the photosynthetic process.

The log growth phase may be defined as that period during which regular and maximum multiplication of bacterial sludge takes 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 into two cells. This results in geometric progression of of bacterial growth. As the available food supply is exhausted, a negative accelarative phase exists where cellular division occurs occasionally.

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

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 carbon dioxide, water and ammonia.

In our experimental studies of the three algae, it has been observed that all the inorganic and organic chemical and biochemical tests had high values during the detention period of 0 to 2 days. The nutrients, during these two days were mostly used up by bacteria and algae. When the detention period increased from 2 to 4 and from 4 to 6 days there were gradual decrease of nutrients. It is obvious to conclude that 0 to 2 days detention may be considered as assimilatory phase, while 2 to 4 and 4 to 6 days as endogenous phase. These results are shown accordingly in the Table 6 (Appendix). From the study of the table it will be seen that most of the data such as ammonia nitrogen, phosphate phosphorus, BOD_5, COD and algae formed confirmed to the above classification of assimilatory and endogenous phases. But in the case of biochemical results such as protein, amino acid nitrogen, free sugar, total sugar and organic acids, such a classification is possible only if we

take into consideration the assimilatory phase as zero to four days and endogenous phase as four to six days. These results seem to be intriguing and require further work.

Bacterial biomass formed in sewage treatment system:

Gellman and Heukelekian (1953), Hoover and Porges (1952) and Weston and Eckenfelder (1958) have found that the yield of cell material during the rapid growth phase is slightly over 50% of the carbon utilized and the remaining half is completely oxidised to carbon dioxide, water 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. The results of both the methods of calculations are compared under.

(a) According to Sawyer, total bacterial growth is calculated as: total bacterial growth = 0.5 x BOD_5 used up (mg/l).

An attempt is made to apply this formula to our results and the calculated results are shown below;

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis		Anabaena cylindrica		Mixed algae	
	BOD_5 used up	Total mass	BOD_5 used up	Total mass	BOD_5 used up	Total mass
(i) in the 3 controls						
2 days	83	41.5	45	22.5	56	28.0
4 days	95	47.5	68	34.0	82	41.0
6 days	136	68.0	89	44.5	95	47.5
(ii) in the algae treated flasks						
2 days	141	70.5	110	55.0	92	46.0
4 days	153	76.5	128	64.0	134	67.0
6 days	171	85.5	141	70.5	139	69.5

(b) But, McKinney (1962) has furnished different formulae for calculating total bacterial mass, active bacterial mass and decreasing bacterial mass. According to him.

(i) Total bacterial mass = COD used up(mg/l) / 2.13

(ii) Active bacterial mass = $S/(1+K_3.t)$

Where S = total bacterial mass

K_3 = 0.006

t = time in hours; and

(iii) Decreasing bacterial mass + total bacterial mass - active bacterial mass

Total bacterial mass, active bacterial mass and decreasing bacterial mass are calculated in the sub-joined tabular statement(i).

The total bacterial mass in the three algae treated samples are considerably more than in the respective controls.

The percentage of decrease in active bacterial mass would seem to range from 22 to 46% during 2 to 6 days of the detention periods in the case of the three algae.

From the data it is also obvious that the total bacterial mass calculated according to Sawyer is less than the total bacterial mass calculated according to McKinney. But it would appear that it is related more to the active mass of McKinney. So an attempt is made (see the subjoined tabular statement(ii)) to correlate the total bacterial mass of Sawyer with active bacterial mass of McKinney assuming that the metabolic activities of bacteria in the high-rate oxidation pond are similar to an activated sludge process.

(i)

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis			Anabaena cylindrica			Mixed algae		
	Total mass	Active mass	Decreasing bacterial mass	Total mass	Active mass	Decreasing bacterial mass	Total mass	Active mass	Decreasing bacterial mass.
(i) in the 3 controls									
2 days	39.9	31.0	8.9	28.1	21.8	6.3	50.7	39.4	11.3
4 days	70.9	45.0	25.9	54.0	34.3	19.7	72.3	45.9	26.4
6 days	102.3	54.9	47.4	65.7	35.2	30.5	75.1	40.3	34.8
(ii) in the algae treated samples:									
2 days	105.6	82.0	28.6	80.8	62.7	18.1	76.1	59.1	17.0
4 days	115.5	73.3	42.2	94.4	59.9	34.5	103.3	65.5	37.8
6 days	128.2	68.8	59.4	104.2	55.9	48.3	105.2	56.4	48.8

(ii)

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis			Anabaena cylindrica			Mixed algae		
	Total mass (Sawyer)	Active mass (McKinney)	% Diff.	Total mass (Sawyer)	Active mass (McKinney)	% Diff.	Total mass (Sawyer)	Active mass (McKinney)	% Diff.
2 days	70.5	82.0	+16.3	55.0	62.7	+14.0	46.0	59.1	+28.5
4 days	76.5	73.3	- 4.2	64.0	59.9	- 6.4	67.0	65.5	+ 2.2
6 days	85.5	68.8	-19.5	70.5	55.9	-20.7	69.5	56.4	-18.8

The values for active mass calculated according to McKinney approximates the total bacterial mass calculated according to Sawyer.

It has to be borne in mind that the formulae of Sawyer and McKinney are related to activated sludge process and not to high-rate aerobic oxidation pond and still a comparison has been made to indicate the progress of biochemical reactions in our case, in the absence of any formula for high-rate oxidation pond.

Further, total bacterial mass calculated according to Sawyer, total bacterial mass calculated according to McKinney and algal biomass actually estimated in our experiments are compared in the sub-joined tabular statement.

It will be seen from the sub-joined table that the total bacterial biomass calculated according to Sawyer is about 50 to 60% of the corresponding values calculated according to McKinney and McKinney's values are nearly one half of the corresponding values for algal biomass.

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis			Anabaena cylindrica			Mixed algae		
	Total bacterial mass (Sawyer)	Total bacterial mass (McKinney)	Algal biomass (estimated)	Total bacterial mass (Sawyer)	Total bacterial mass (McKinney)	Algal biomass (estimated)	Total bacterial mass (Sawyer)	Total bacterial mass (McKinney)	Algal biomass (estimated)
2 days	70.5	105.6	206	55.0	80.8	199	46.0	76.1	208
4 days	76.5	115.5	212	64.0	94.4	201	67.0	103.3	210
6 days	85.5	128.2	218	70.5	104.2	205	69.5	105.2	214

Removal of organic pollutants as COD:

The process of respiration demands oxygen from the environment. Thus sewage exerts a BOD i.e. Biological Oxygen Demand through the organisms living on it, so that these organisms can oxidise a part of of the organic matter into carbon dioxide, water and energy for their cellular growth.

But BOD_5 is a measure of the oxygen demand by bacteria for a period of 5 days only. While Chemical Oxygen Demand (COD) estimates oxygen demand exerted by bacteria for their life span of twenty days.

Thus reduction in COD values represents indirectly the organic matter removed by bacteria. The COD values indirectly give also an idea of the amount of organic matter present in sewage.

Percentage reduction of COD values in the three algal-treated experiments is summarised below:

Detention period	Percentage reduction of COD			
	Control raw sewage	Nostoc pyriformis	Anabaena cylindrica	Mixed algae
2 days	25 to 41	71	72	61
4 days	48 to 58	78	84	83
6 days	58 to 70	87	93	85

The COD removed in control within six days was 58 to 70 percent, while that removed in the algae-treated samples was nearly of the same amount within two days. The percentage removal of COD by the three algae in six days varied between 85 to 93%.

The reduction of COD in control flask has to be attributed to the phenomena of mechanical flocculation, bioflocculation, bioprecipitation and surface reaeration which are of common occurrence in nature (Heukelekian, 1941). In the case of the three algal samples the increased percentage reduction has to be ascribed to photosynthetic oxygen furnished to bacteria as a result of "algal-bacterial symbiotic" reactions in the algal growth cultures (Oswald, 1960).

Conversion of COD values into equivalent organic matter:

"COD" as we know is a measure of the quantity of oxygen required for total bio-oxidation of sewage organic matter and not of the quantity of organic matter itself. But we have to know the quantity of organic matter oxidised by bacteria; and Porges (1960) has furnished a method of estimating the same approximately from COD values using the conversion factor

Fig. 10

Fig. 11

BIO-OXIDATION OF SEWAGE ORGANIC MATTER IN HIGH-RATE OXIDATION POND USING

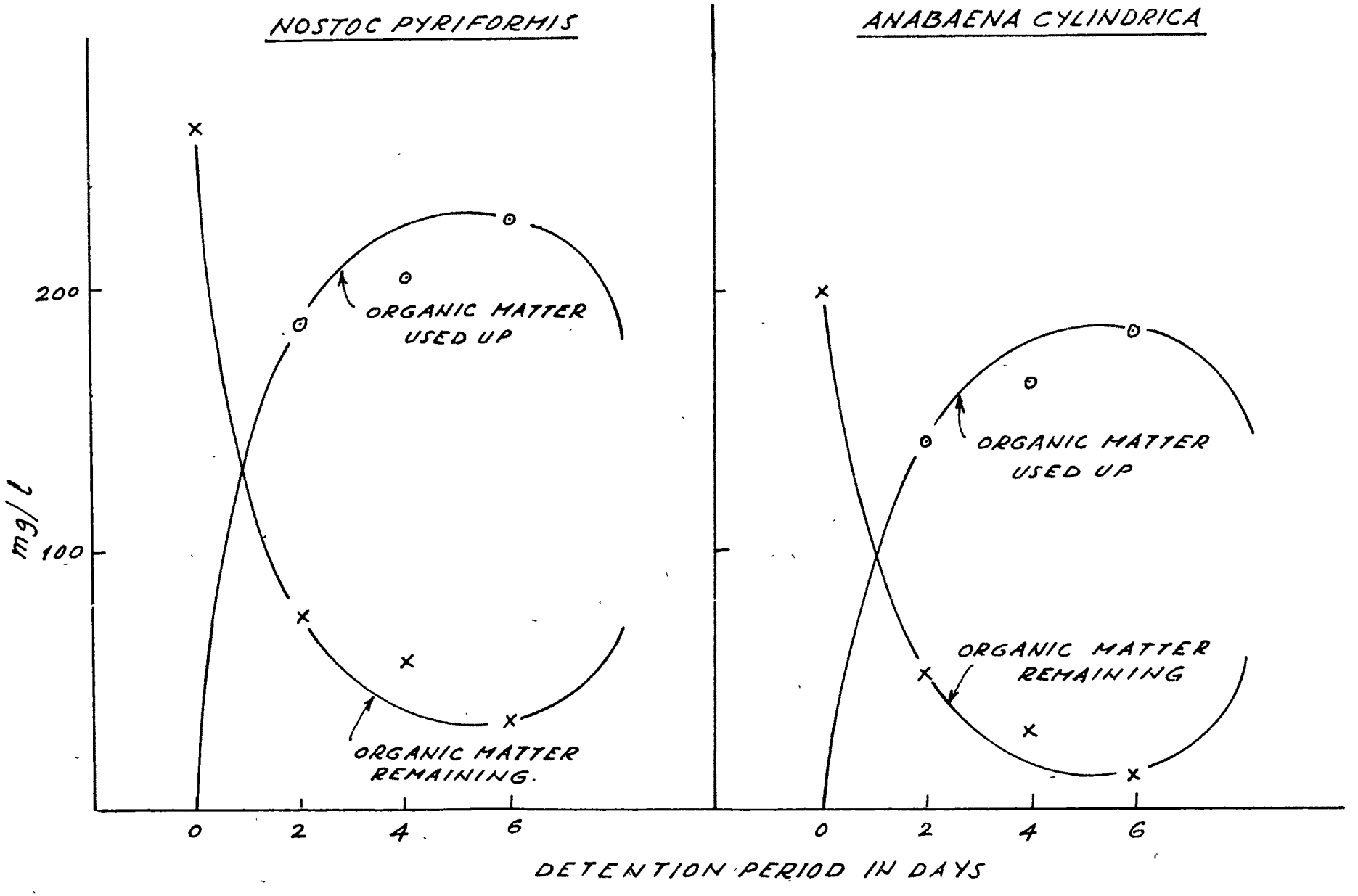
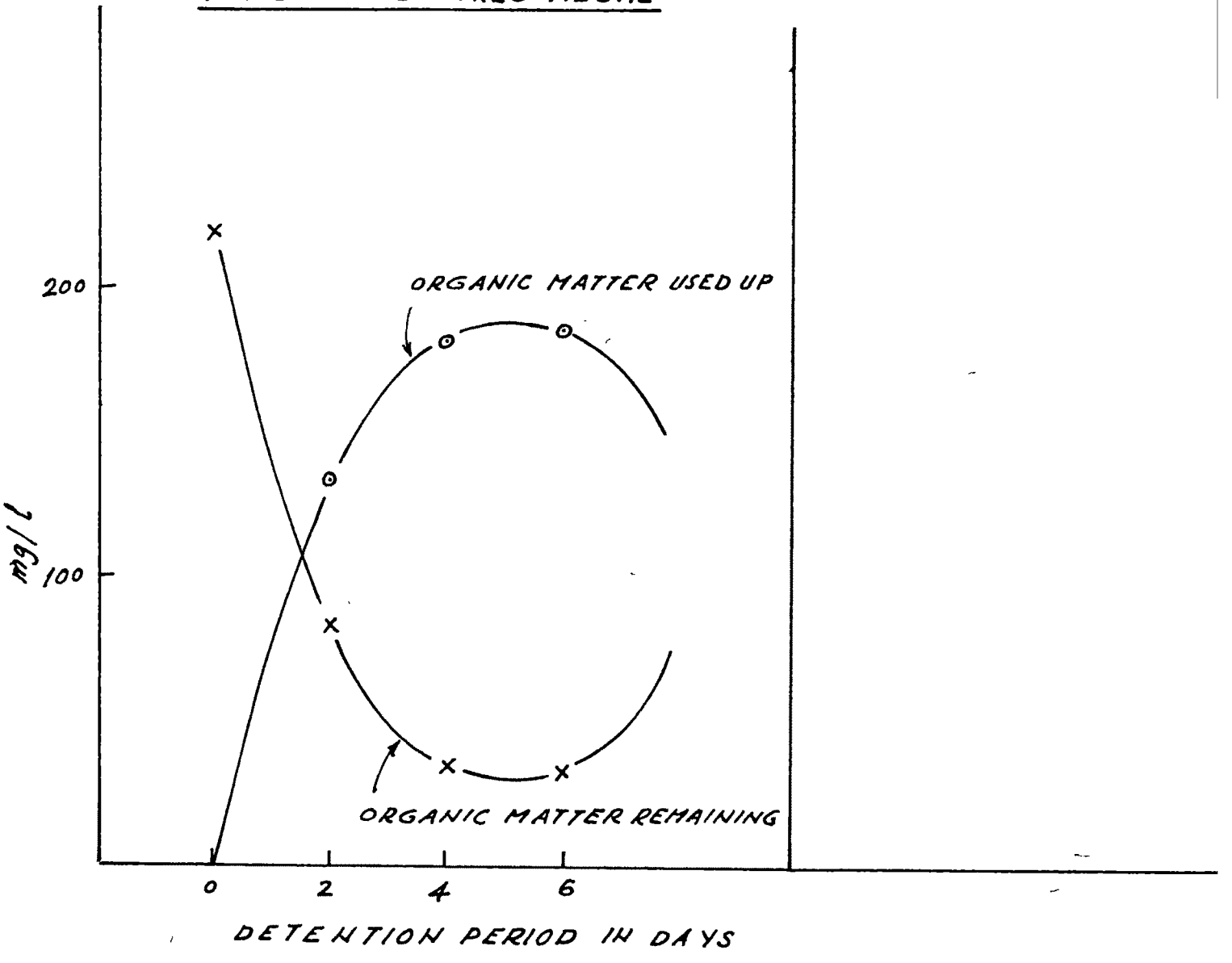


Fig. 12

BIO-OXIDATION OF SEWAGE ORGANIC MATTER IN HIGH-RATE OXIDATION POND USING MIXED ALGAE



1.2. COD values divided by 1.2 give approximately the corresponding values of the quantity of bio-degradable organic matter in sewage. COD values obtained in our three experiments have been converted into corresponding organic matter values by using Porge's above factor. The results are summarised below:

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis		Anabaena cylindrica		Mixed algae	
	COD used	Organic matter	COD used	Organic matter	COD used	Organic matter
2 days	225	187.5	172	143.3	162	135.0
4 days	246	205.0	201	167.5	220	183.3
6 days	273	227.5	222	185.0	224	186.7

It is found that the bacteria had removed 187 to 227 mg. of organic matter within six days from sewage.

The Figs. 10, 11 and 12 show clearly how the organic matter is used up during bacterial oxidation on the different detention periods in our three different experiments; while the comparative values for organic matter and COD used up and remaining for

Fig. 13

BACTERIAL OXIDATION OF ORGANIC MATTER USING NOSTOC PYRIFORMIS

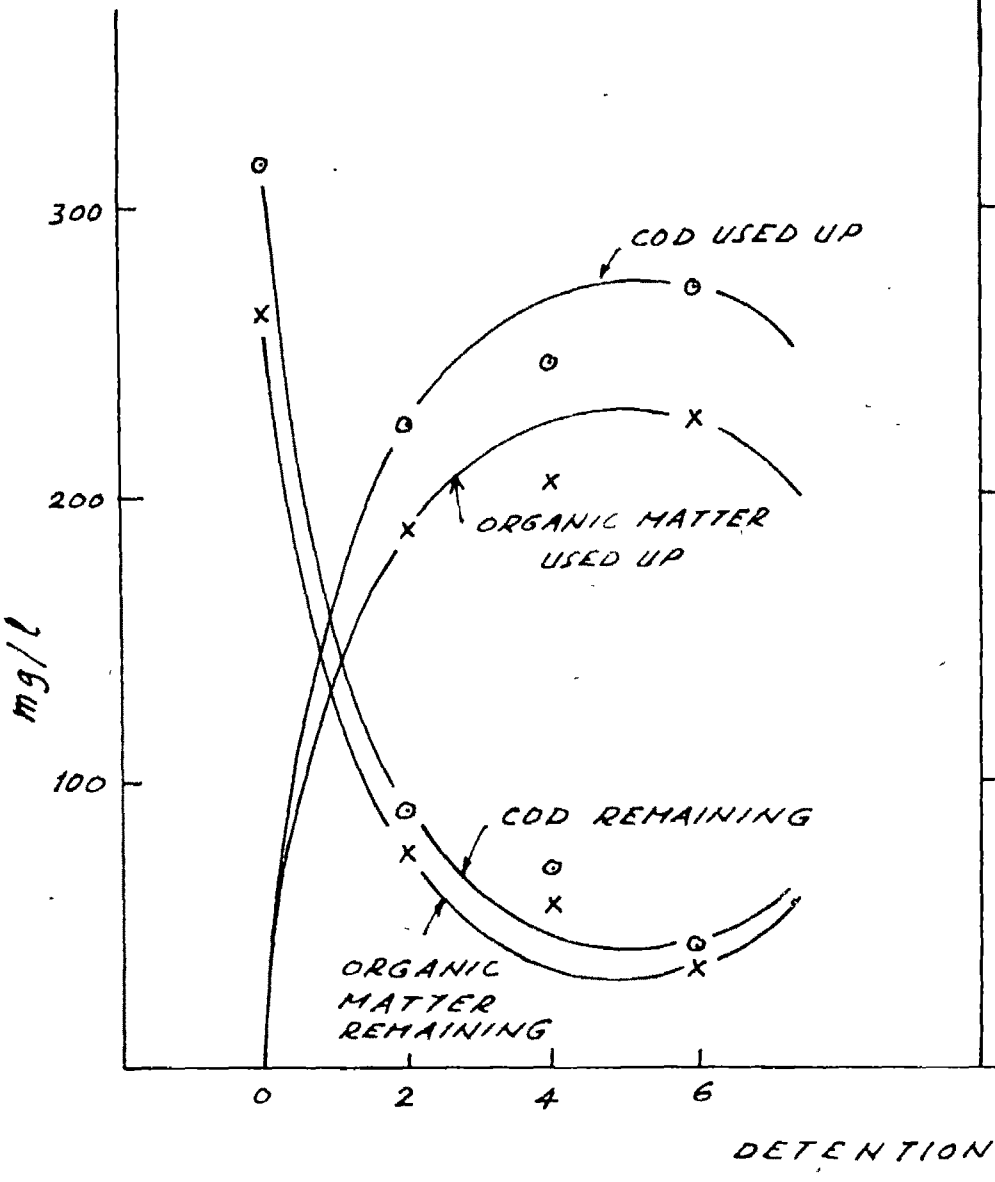
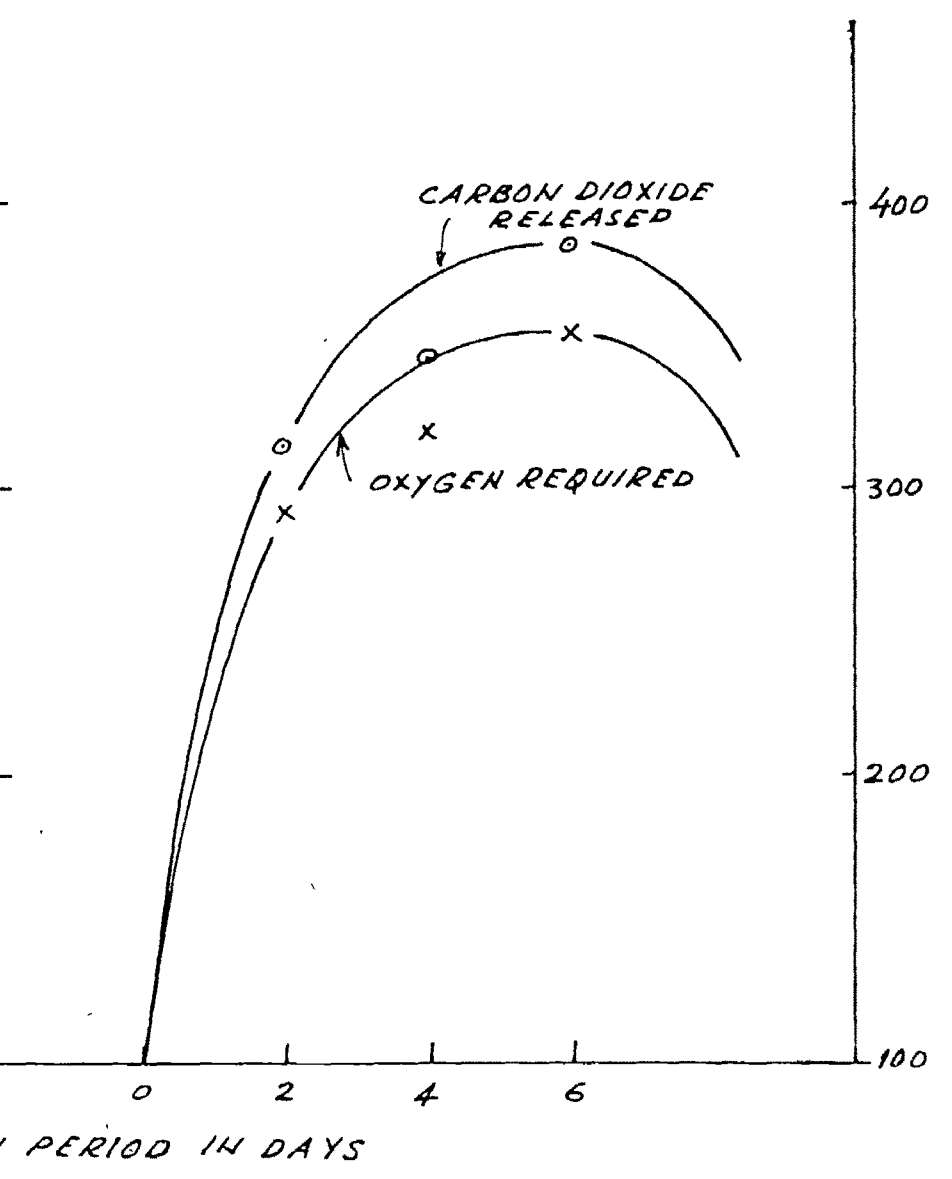


Fig. 14

TOTAL BIO-OXIDATION OF ORGANIC MATTER USING NOSTOC PYRIFORMIS



DETENTION PERIOD IN DAYS

Fig. 15

BACTERIAL OXIDATION OF ORGANIC MATTER USING ANABAENA CYLINDRICA

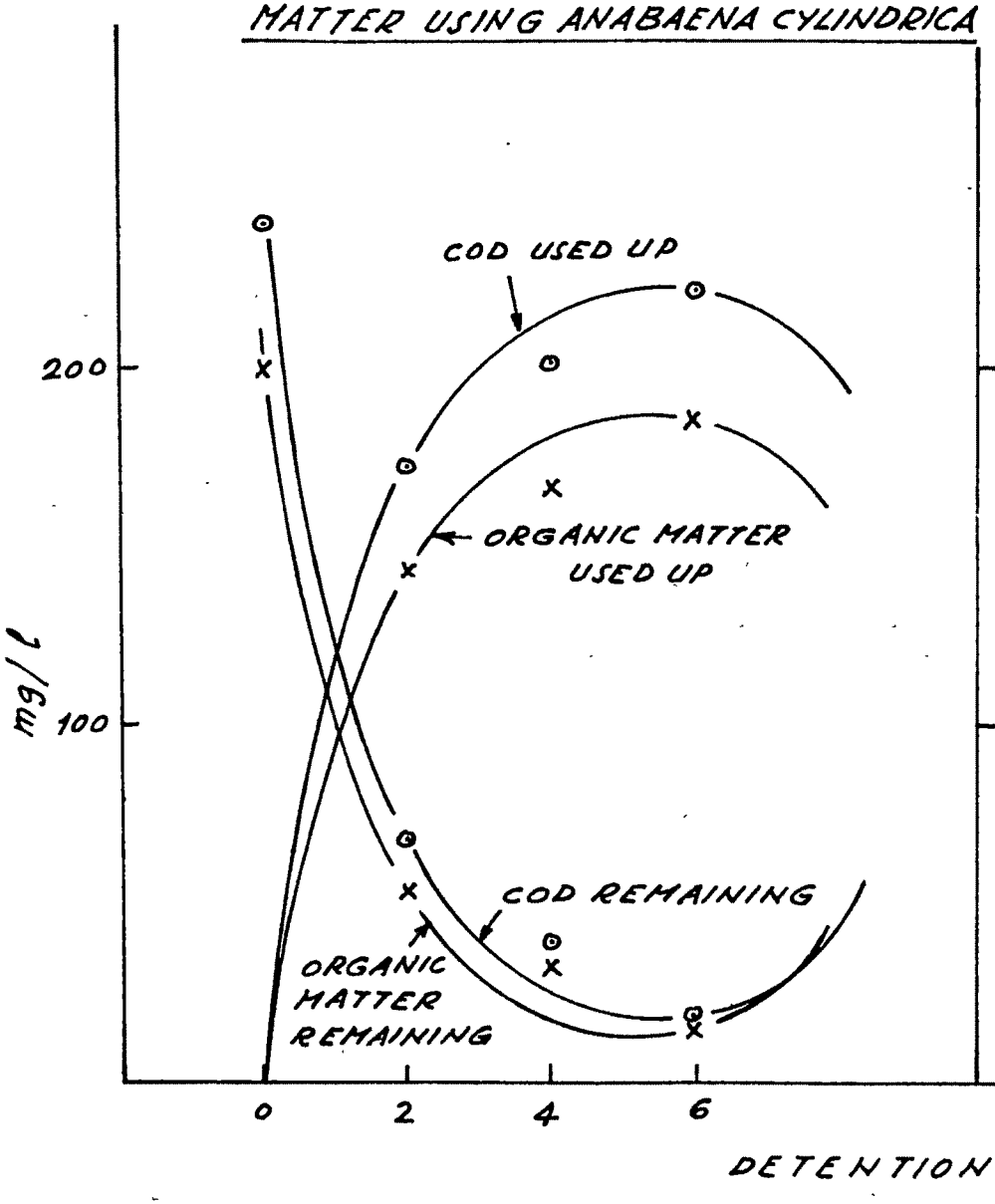
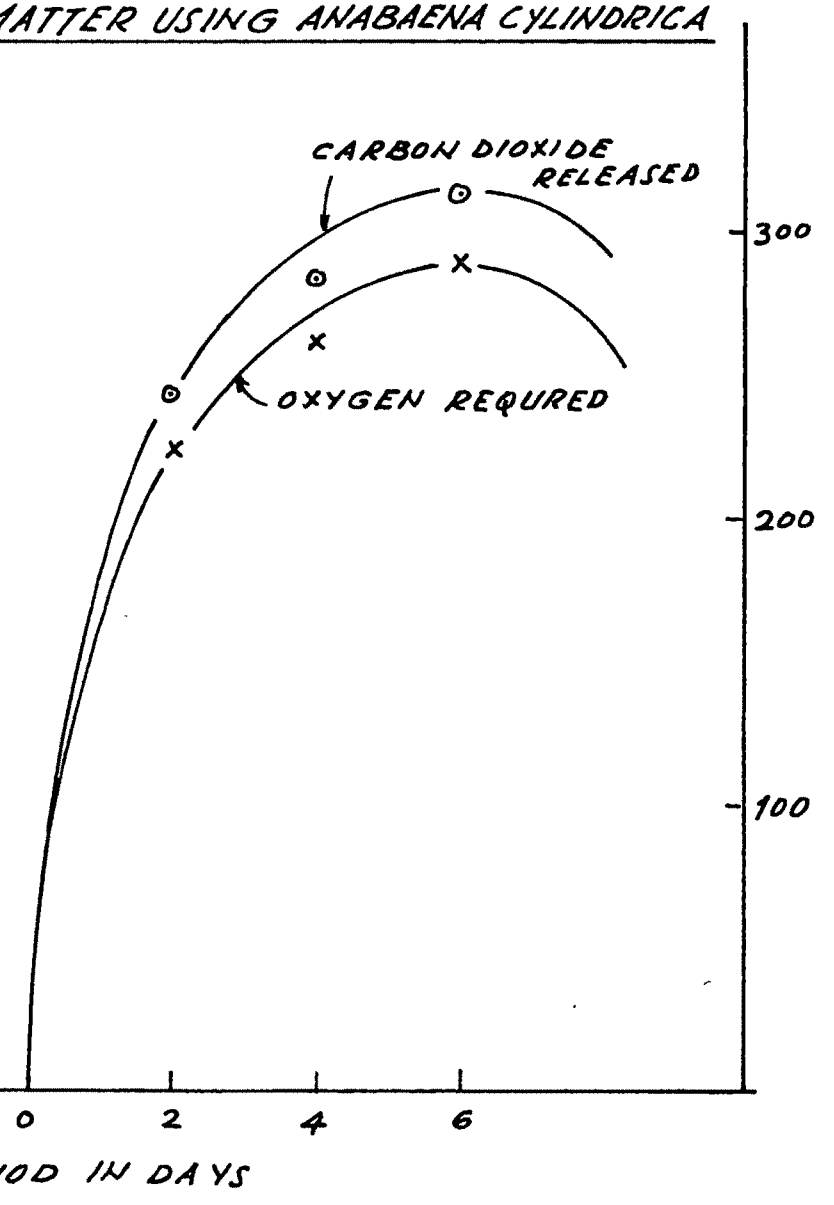


Fig. 16

TOTAL BIO-OXIDATION OF ORGANIC MATTER USING ANABAENA CYLINDRICA



DETENTION PERIOD IN DAYS

Fig. 17

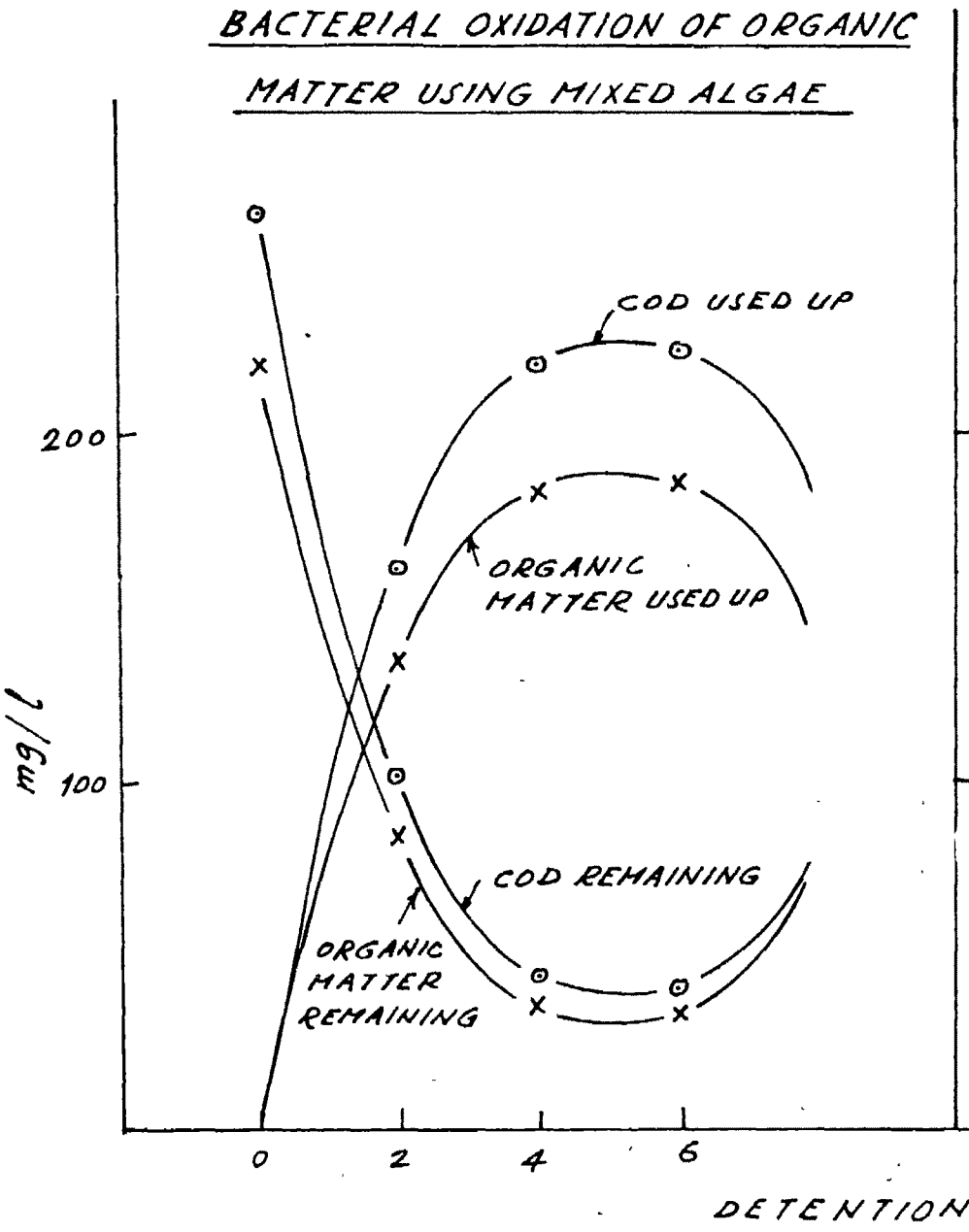
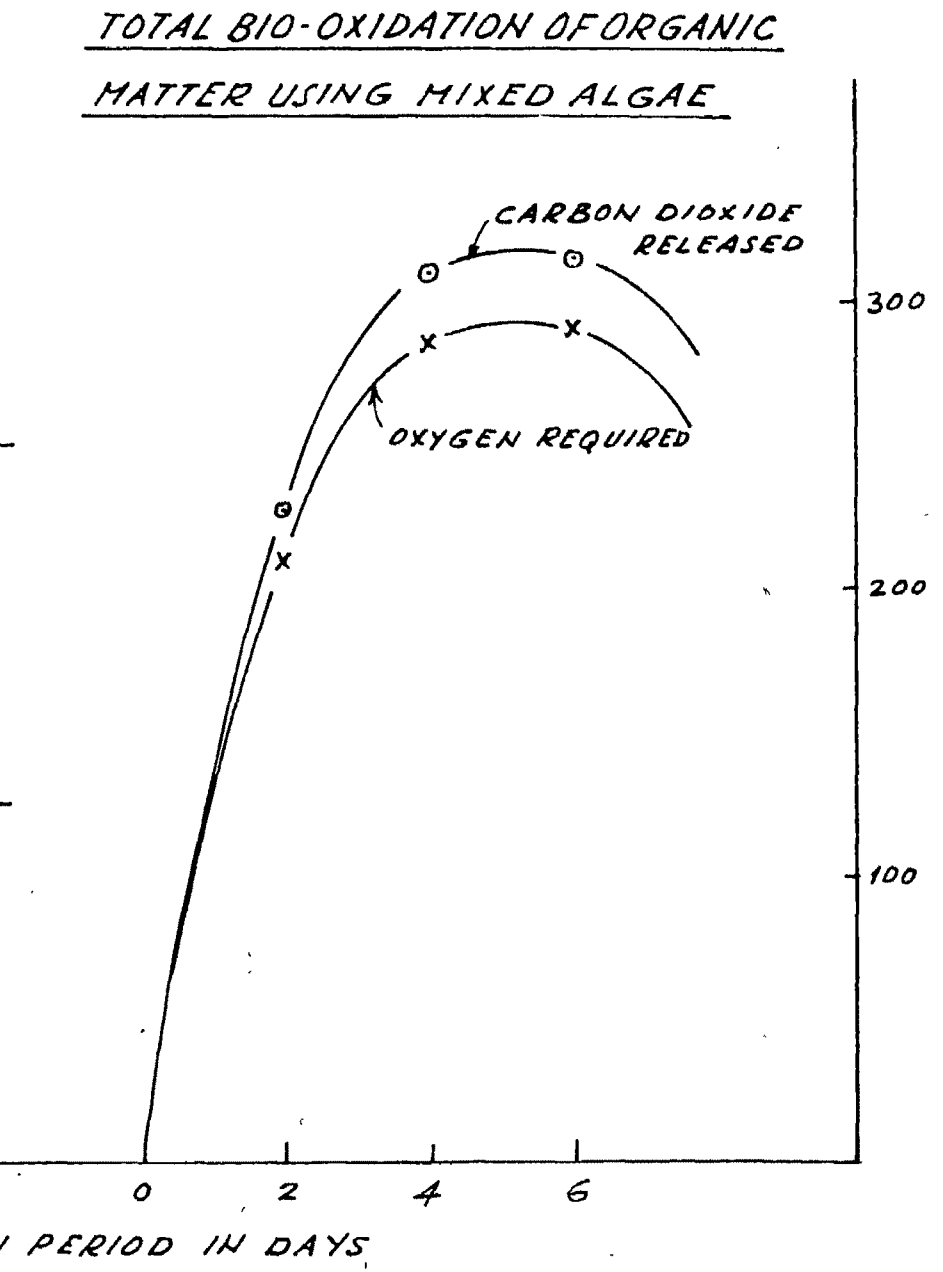

BACTERIAL OXIDATION OF ORGANIC MATTER USING MIXED ALGAE
COD USED UP
ORGANIC MATTER USED UP
COD REMAINING
ORGANIC MATTER REMAINING
200
100
mg/l
0
2
4
6
DETENTION PERIOD IN DAYS

Fig. 18


TOTAL BIO-OXIDATION OF ORGANIC MATTER USING MIXED ALGAE
CARBON DIOXIDE RELEASED
OXYGEN REQUIRED
300
200
100
0
2
4
6
DETENTION PERIOD IN DAYS

the different detention periods in our three experiments are clearly indicated in Figs. 13, 15 and 17.

Oxygen required and carbon dioxide released during bacterial oxidation:

Oswald, Hee and Gotaas (1958) found experimentally in high-rate aerobic ponds the oxidation of sewage to follow the reaction:

$$C_{11}H_{29}O_7N + 14\ O_2 + H^+ \longrightarrow 11\ CO_2 + 13\ H_2O + NH_4^+$$

Sewage organic matter which is represented by the formula $C_{11}H_{29}O_7N$ having a molecular weight of 287 gm. produces 484 gm. of CO_2 and requires 448 gm. of oxygen. Therefore 1 gm. of sewage organic matter will produce 484/287 or 1.69 gm. of carbon dioxide and will require 1.56 gm. of oxygen for bio-oxidation. The two factors are used in our calculation. So, oxygen required accordingly for bio-oxidation of organic matter in our experiments are shown below:

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis		Anabaena cylindrica		Mixed algae	
	organic matter	oxygen reqd.	organic matter	oxygen reqd.	organic matter	oxygen reqd.
2 days	187.5	292.5	143.3	223.5	135.0	210.6
4 days	205.0	319.8	167.5	261.3	183.3	285.9
6 days	227.5	354.9	185.0	288.6	186.7	291.2

It can be concluded from the table that 289 to 355 mg. of oxygen are required to degrade 185 to 227 mg. of organic matter within six days of detention period.

Next, carbon dioxide released during the total bio-oxidation of organic pollutants calculated accordingly in our experiments are shown below:

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis		Anabaena cylindrica		Mixed Algae	
	organic matter	carbon dioxide released	Organic matter	carbon dioxide released	organic matter	carbon dioxide released
2 days	187.5	316.9	143.3	242.2	135.0	228.1
4 days	205.0	346.4	167.5	283.1	183.3	309.8
6 days	227.5	384.5	185.0	312.6	186.7	315.5

It can be concluded also from the above table that 313 to 384 mg. of carbon dioxide are released during the degradation of 187 to 227 mg. of organic matter in six days.

In short, during bacterial oxidation phase 187 to 227 mg. of sewage organic matter were oxidised by bacteria in algae-treated samples in six days and this

was effected by 289 to 355 mg of oxygen and as a result 213 to 384 mg. of carbon dioxide were released for the use by algae for algal photosynthesis.

In brief, in the three algal treated samples:

187 to 227 mg of organic matter + 289 to 355 mg of oxygen $\xrightarrow{6 \text{ days}}$

313 to 384 mg. of carbon dioxide.

Carbon dioxide released and oxygen required during total bio-oxidation of organic matter for different detention periods in our three experiments are schematically represented in Figs. 14, 16 and 18.

B. Algal Photosynthesis

Photosynthesis is the biological starting point for synthesizing living substance out of non-living carbon dioxide, water and other inorganic nutrients. In this process radiant energy is absorbed from the sun by the algae and free molecular oxygen is released into the environment as long as sufficient light is available i.e. during the day time only. The end product is a basic organic material, a simple sugar or carbohydrate, a monosaccharide of which glucose is an

example. Photosynthetic oxygenation is responsible for the overall stabilization process in oxidation ponds.

Algae may release 20 times as much oxygen in photosynthesis as they utilise in metabolism (Palmer, 1956). The significance of this in the biological aerobic purification of organic wastes is obvious, since rapid decomposition of waste depends primarily on aerobic bacteria. So intensive cultivation of algae in a fresh sewage is a highly effective means both for supplying oxygen for aerobic decomposition of organic matter and for reclaiming nutrients from the wastes in the form of new algal cells.

During the first stage of oxidation of organic matter heterotrophic bacteria produce carbon dioxide and ammonia. Green algae utilising energy from the sun produce carbohydrates from carbon dioxide and water and assimilate the same together with the liberated ammonia and other inorganic products of biological significance for synthesising fresh algal cells each of which is capable of fixing solar energy.

One gram of algae produced is usually accompanied by 1.60 gm. of nascent oxygen. The heat

combustion of the algal cell is estimated at 5.5 to 6 K.cal. per gram, so that about 3.68 K.cal. of solar energy is fixed in the production of one gram of nascent oxygen. Since the amount of oxygen required by bacteria to oxidise the waste can be readily determined (i.e. BOD), the weight of the algae that must be grown and the quantity of solar energy that must be fixed in order to produce the required quantity of oxygen by photosynthesis may also be determined. The efficiency of light conversion energy by algae seldom exceeds 10% (Oswald, 1960).

Carbon dioxide used up and oxygen released during photosynthesis and photosynthetic energy conversion efficiency in our three experiments will be discussed below:

Carbon dioxide used up during algal synthesis:

Myers (1962) has found that 1.8 mg. of carbon dioxide are used up during the formation of 1.0 mg. of algal dry matter. This factor is used in calculating the carbon dioxide used up during algal photosynthesis in our three experiments. The results are shown below:

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis		Anabaena cylindrica		Mixed algae	
	algae formed	carbon dioxide used up	algae formed	carbon dioxide used up	algae formed	carbon dioxide used up
2 days	206	370.8	199	358.2	208	374.4
4 days	212	381.6	201	361.8	210	378.0
6 days	218	392.4	205	369.0	214	385.2

369 to 392 mg. of carbon dioxide are used up by algae in photosynthesis during 6 days for the formation of 205 to 218 mg. of algal biomass.

Production of photosynthetic oxygen:

The production of nascent photosynthetic oxygen was calculated according to Oswald and Gotaas (1957). They have found that 1.6 gm. of oxygen will be released during the formation of 1.0 gm. of algal biomass. This factor is used in calculating the amount of oxygen released during algal photosynthesis in our three experiments.

The results are shown in a tabular statement on the next page.

(The results are expressed in mg/l)

Detention period	Nostoc pyriformis		Anabaena Cylindrica		Mixed algae	
	algae formed	oxygen released	algae formed	oxygen released	algae formed	oxygen released
2 days	206	329.6	199	318.4	208	332.8
4 days	212	339.2	201	321.6	210	336.0
6 days	218	348.8	205	328.0	214	342.4

In short, during algal photosynthesis in our three experiments 369 to 392 mg. of carbon dioxide was fixed up in the formation of 205 to 218 mg. of algae releasing 328 to 349 mg. of nascent photosynthetic oxygen.

Algal biomass produced, carbon dioxide required and oxygen produced in our three experiments are shown diagramatically in Figs. 20, 22 and 24.

Overall photosynthetic energy conversion efficiency: (input-output energy balance).

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 continuously

stirred reactor with complete homogenity of the algal cells in suspension (Beck et al. 1969). "As in any continuous stirred tank reactor, there is a finite volume V in litres and flow rate F, in litres per day. The mean hydraulic residence time Q is then defined as

$$Q = V/F \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

For given mean residence time Q, in days, the total solar energy input per litre of pond volume is equal to

$$E_{in} = S.A.Q. \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

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 cm^2 per day and Q is the mean hydraulic residence time. For one litre of pond volume A = 1000 / d where d is the determined depth in cm.

$$\text{Therefore } E_{in} = \frac{1000 \times S \times Q}{d} \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

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

$$E_{out} = h. Cc \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

Where E_{out} is the total energy tied up in synthetised algae in calories per litre; h, is the heat of combustion of algae in calories per milligram and Cc, is the concentration of algae in mg/l. Equations (2) and (4) can be equated by assuming that only a portion of the energy input is converted to algal biomass, so that

$$E_{in}\, e = E_{out} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

Where e is an efficiency factor. The equation (5) can be expanded thus :

$$\frac{1000.\ S.\ Q.\ 1}{d} = h.Cc$$

and therefore,

$$\text{Photosynthetic efficiency, } e = \frac{h.Cc.\ d}{1000.S.Q.}$$

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

The above equation is used in calculating the overall photosynthetic energy conversion efficiency of the three algae used in our experiments.

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

Depth of the liquid volume (d) = 3.5 cm.

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

10.5 lux = 1 gram calorie.

∴ 375 lux (S) = 35.7 gram - calories

1 mg. of algae contains (h) = 5.5 calories (heat of combustion).

Mean hydraulic residence time (Q) = 6 days

C_c = maximum quantity of algal biomass produced in 6 days in 1.5 litres of sewage.

$e = C_c \times 5.5 \times 3.5 / 35.7 \times 1500 \times 6$

$= C_c \times 5.99 \times 10^{-5}$; Percentage efficiency = e x 100

Percentage photosynthetic energy conversion efficiencies in our three experiments are shown below in a tabular statement:

Name of the Algae	Algae formed in 1500 ml in 6 days (mg)	% efficiency
Nostoc pyriformis	327.0	1.96
Anabaena cylindrica	307.5	1.85
Mixed algae	321.0	1.92

From the above table it will be seen that the photosynthetic energy conversion efficiency in our three experiments varied between 1.85 to 1.96% with an average of 1.91%.

Wassink et al. (1953) have reported 12 to 20% efficiency in both small and large scale cultures of Chlorella. Gloyna (1971) has stated that the usual efficiencies range from 2 to 9% with 5% a common figure. Ganapati and Sreenivasan (1970) have reported a maximum efficiency of 10% in a sewage polluted fish pond in Madras. Pasche (1960) has reported 7% and Talling (1961) 6%. Myers (1955) has stated that 20% is taken as a reasonable maximum value of efficiency for use of white light. Oswald and Gotaas (1957) found that the overall efficiency under a wide variety of environmental conditions seldom exceeds 10 or 12% of the available light energy. Oswald et al. (1957) found the efficiencies of outdoor ponds to range between 1 to 10% with most values in the narrow range of 3 to 7%. Efficiencies of 5 to 8% may be attained with algal cell concentrations dense to utilize the nutrients and permit harvesting of cells (Gotaas and Oswald, 1955). Miss Heffert (Rabinowitch, 1955) obtained an average of 8% utilization of total incident

light for several months in algal ponds fertilized with sewage. Ganapati (1971) has reported values ranging from 6.6 to 13.8% in oxidation ponds in India.

Compared to above efficiency values, the values we have obtained in our three experiments are low, on account of our using low intensity of illumination.

C. "Algal-bacterial Symbiosis"

The mode of nutrition of algae is autotrophic and that of bacteria is heterotrophic and the two can work together, one helping the other in commensal relationship. In other words, bacteria metabolise the organic components of the waste water and release some substances like carbon dioxide utilisable by algae. During synthesis of fresh algal cells, algae release oxygen, which is utilisable by the bacteria for stabilization of organic matter.

Stoichiometry of "algal-bacterial symbiosis":

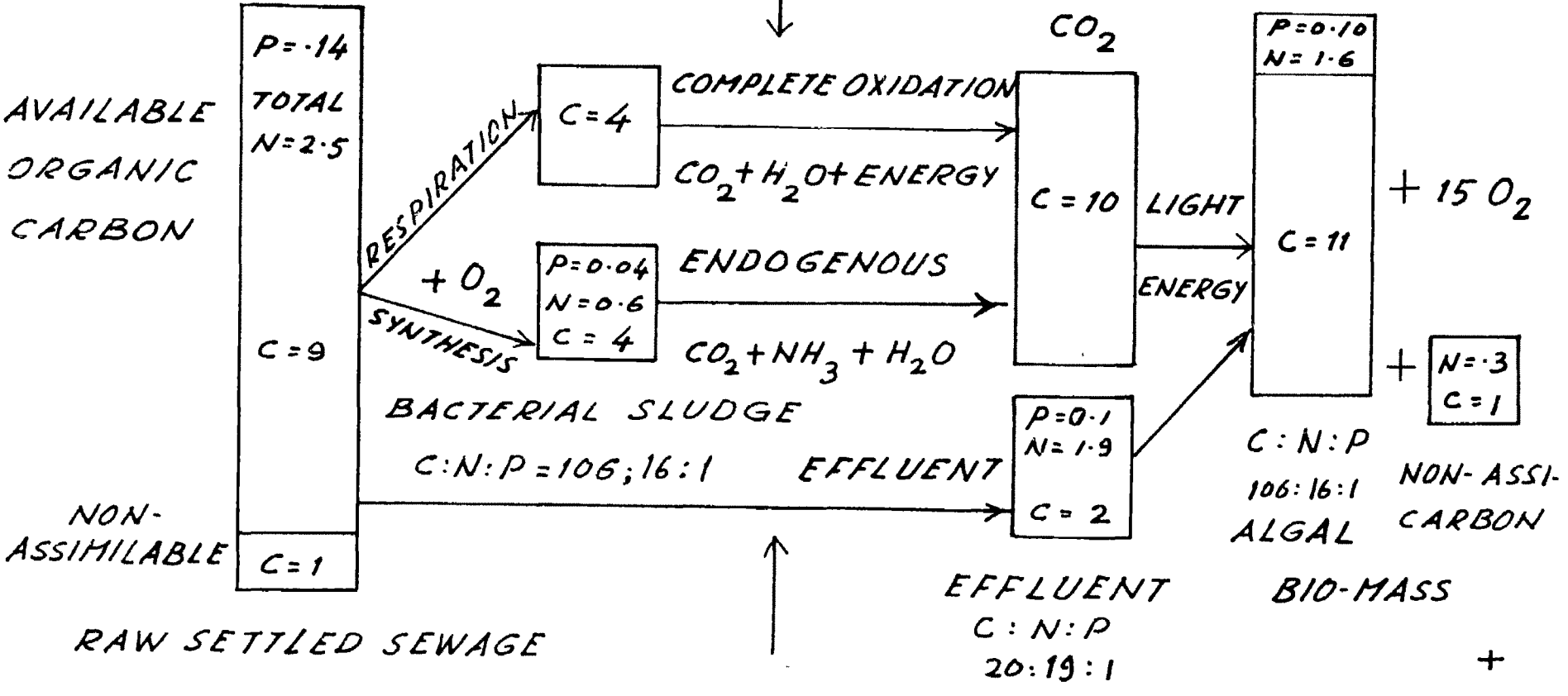
The basic principles of the "symbiotic system" in oxidation pond are: (i) the production of organic matter (algae) is accompanied by the absorption of

Fig. 19

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

A BALANCED SYSTEM OF

COMPLETE BACTERIAL OXIDATION AND | ALGAL SYNTHESIS AND PHOTO-SYNTHETIC OXYGEN PRODUCTION



radient energy from the sun and the concomitant release of nascent oxygen, (ii) the destruction of old organic matter involves the utilization of an equivalent amount of oxygen and release of energy. The final degradation products of aerobic bacterial oxidation of organic matter are carbon dioxide ammonia and water which are identical to the chief needs of algal photosynthesis plus radient energy. A schematic representation of the metabolic processes taking place during " algal-bacterial symbiosis " is shown in Fig. 19 (modified after Stumm, 1968).

An attempt is made to compare the quantity of carbon dioxide released during total bacterial oxidation of organic matter with that required for algal biomass production of the three algal types during "algal-bacterial symbiosis." Comparison of the quantities of oxygen produced during algal photosynthesis with that required for total bacterial oxidation of sewage organic matter during "algal bacterial symbiosis" of the three types of algae investigated is also made.

The quantity of oxygen released during algal photosynthesis is compared below with the quantity of oxygen required for bacterial oxidation of organic matter in our three experiments:

(The results are expressed in mg/l)

Detention period	Oxygen released during algal photosynthesis	Oxygen required for bacterial oxidation	Difference (Excess %)
(a) Nostoc pyriformis:			
2 days	329.6	292.5	37.1 (12.7%)
4 days	339.2	319.8	19.4 (6.1%)
6 days	348.8	354.9	-6.1 (?)
(b) Anabaena cylindrica:			
2 days	318.4	223.5	94.9 (42.5%)
4 days	321.6	261.3	60.3 (23.1%)
6 days	328.0	288.6	39.4 (13.7%)
(c) Mixed algae:			
2 days	332.8	210.6	122.2 (58.0%)
4 days	336.0	285.9	50.1 (17.5%)
6 days	342.4	291.2	51.2 (17.6%)

There is always an excess of photosynthetic oxygen produced over that required for total bacterial oxidation of sewage organic matter excepting on one occasion, so that the eco-system is always kept under aerobic conditions.

Further, the quantity of carbon dioxide released during bacterial oxidation of organic pollutants is compared below with the amount of carbon dioxide required for algal photosynthesis in our three experiments:

(The results are expressed in mg/l)

Detention period	Carbon dioxide used in algal photosynthesis	Carbon dioxide released during bacterial oxidation	Difference (Excess %)
(a) Nostoc pyriformis:			
2 days	370.8	316.9	53.9 (17.0%)
4 days	381.6	346.4	35.2 (10.2%)
6 days	392.4	384.5	7.9 (2.1%)
(b) Anabaena cylindrica:			
2 days	358.2	242.2	116.0 (48.0%)
4 days	361.8	283.1	78.7 (27.8%)
6 days	369.0	312.6	56.4 (18.0%)
(c) Mixed algae:			
2 days	374.4	228.1	146.3 (64.1%)
4 days	378.0	309.8	68.2 (22.0%)
6 days	385.2	315.5	69.7 (22.1%)

It will be seen from the above three tabular statements that the quantities of carbon dioxide required for algal photosynthesis in respect of all

the three types of algae investigated are greater than the corresponding amounts released during bacterial oxidation of sewage organic matter. The increase varies from 2 to 64 percent over that released during bacterial oxidation or the deficiency ranges from 2 to 64 percent of the quantity released during total bacterial oxidation, and the excess required for the algal biomass formed should have come from two sources:(a) from the atmosphere and the bicarbonate - carbonate equilibrium system. The availability of the bicarbonate ion *per se* for photosynthesis is an important factor. The importance of the relationship between free carbon dioxide and alkalinity with respect to pH has been described by King (1970); (b) from the extra-cellular product glycolate released by algae in the system. According to Fogg (1970): "Possibly glycolate may be reabsorbed, so that it might be looked on as an extra-cellular reserve product, but as yet there is no evidence for this and it is more likely that the glycolate released is utilized by bacteria " (for carbon dioxide production).

Carbon dioxide used and oxygen released during algal photosynthesis and oxygen used and carbon

Fig. 20

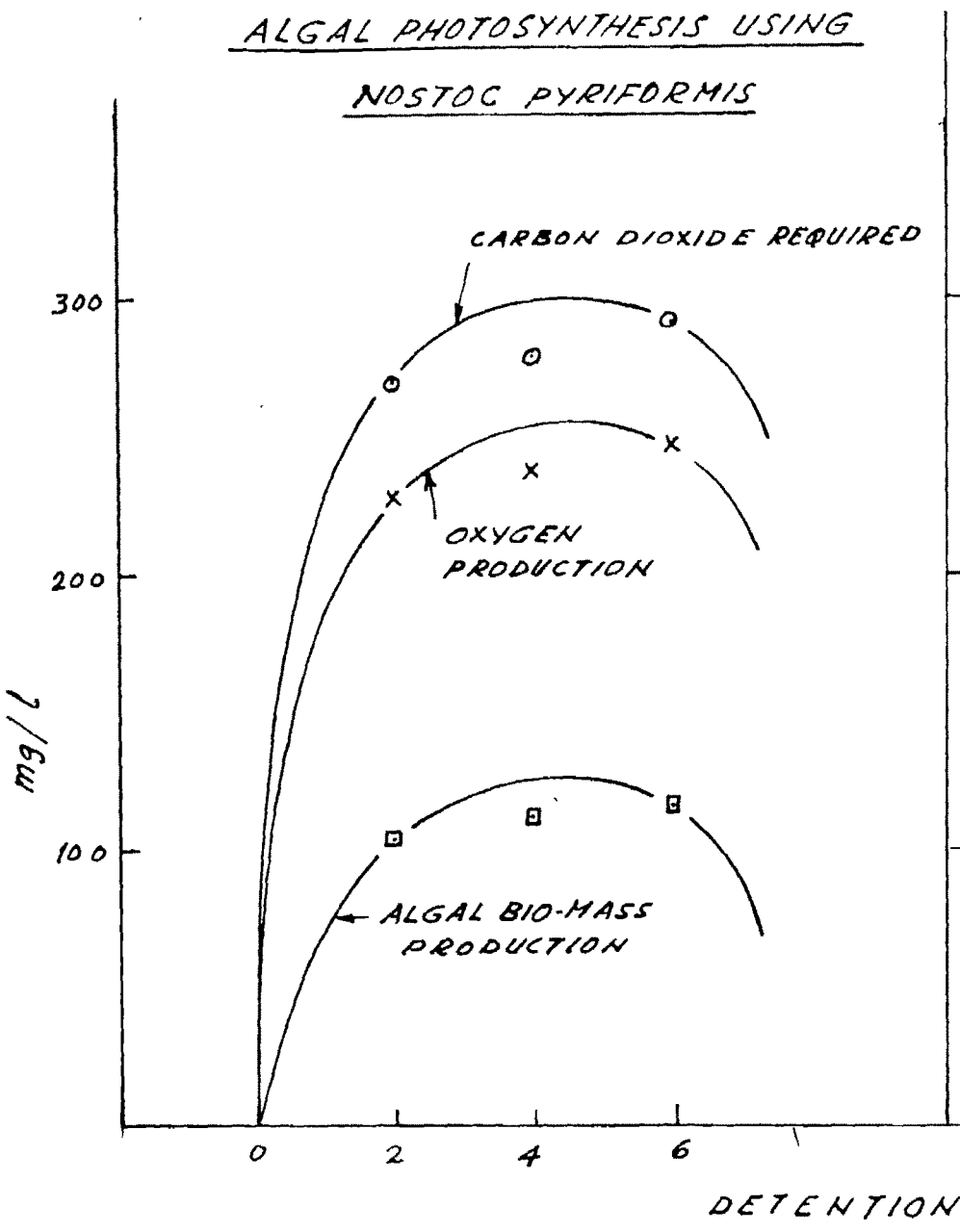

ALGAL PHOTOSYNTHESIS USING
NOSTOC PYRIFORMIS
CARBON DIOXIDE REQUIRED
OXYGEN PRODUCTION
ALGAL BIO-MASS PRODUCTION
300
200
100
mg/l
0
2
4
6
DETENTION PERIOD IN DAYS

Fig. 21

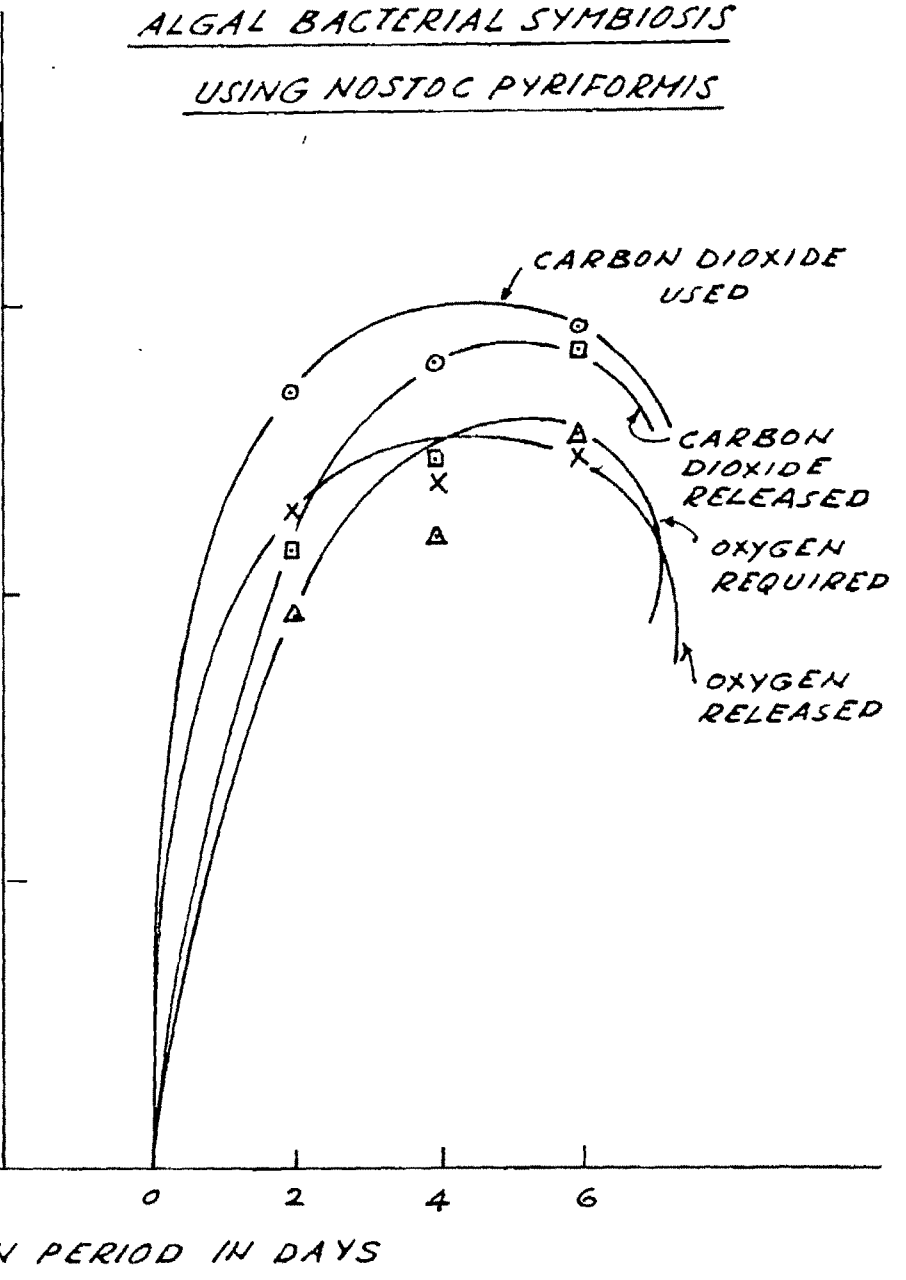

ALGAL BACTERIAL SYMBIOSIS
USING NOSTOC PYRIFORMIS
CARBON DIOXIDE USED
CARBON DIOXIDE RELEASED
OXYGEN REQUIRED
OXYGEN RELEASED
0
2
4
6
DETENTION PERIOD IN DAYS

Fig. 22

ALGAL PHOTOSYNTHESIS USING

ANABAENA CYLINDRICA

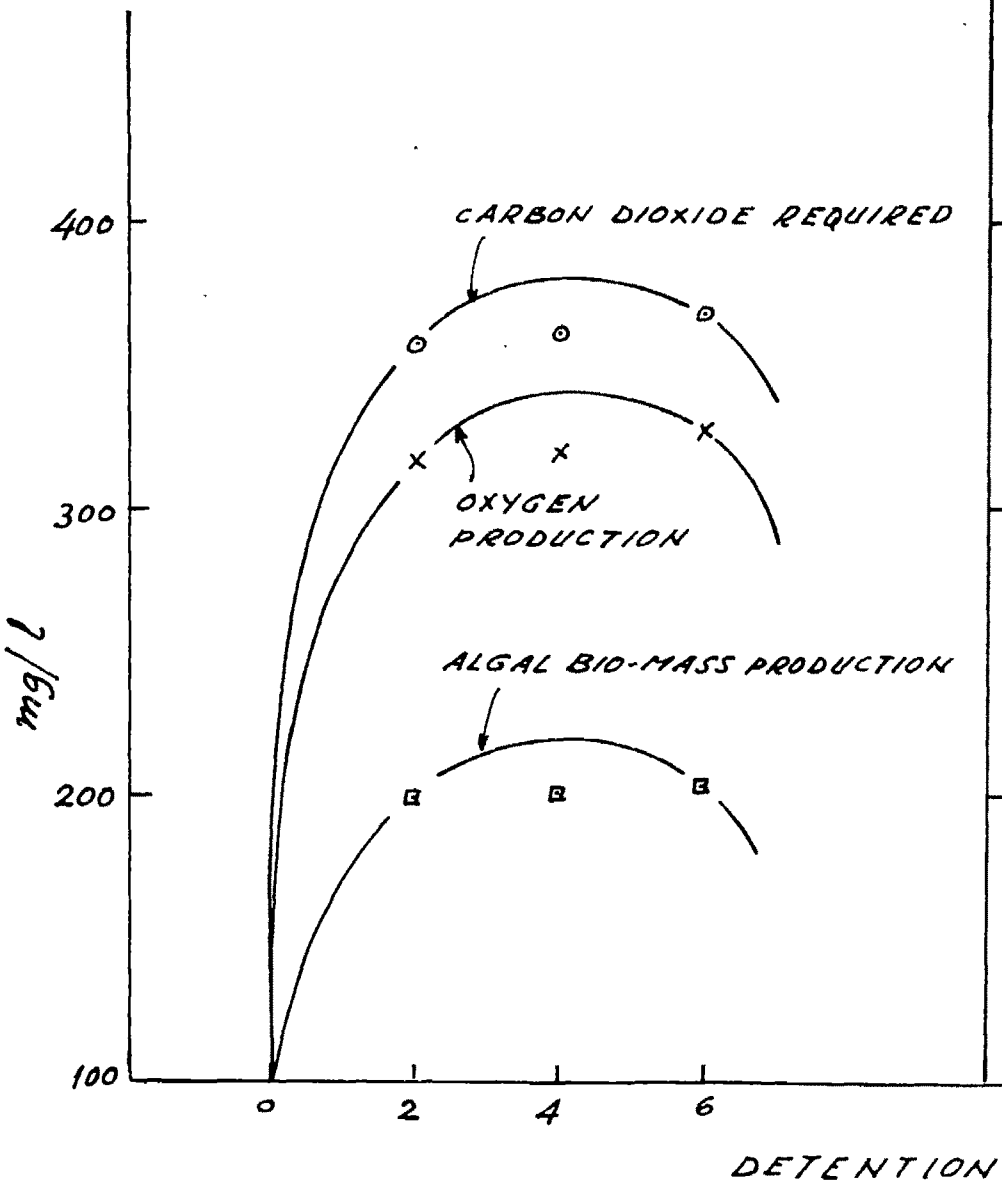


Fig. 23

ALGAL - BACTERIAL SYMBIOSIS

ANABAENA CYLINDRICA

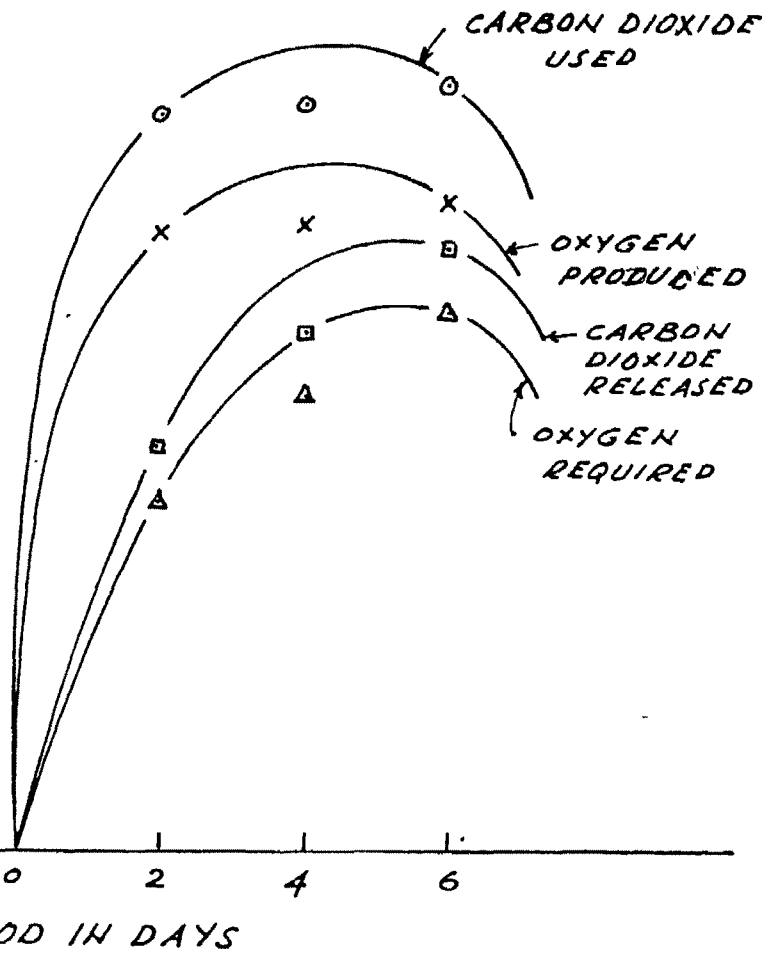


Fig. 24

ALGAL PHOTOSYNTHESIS USING MIXED ALGAE

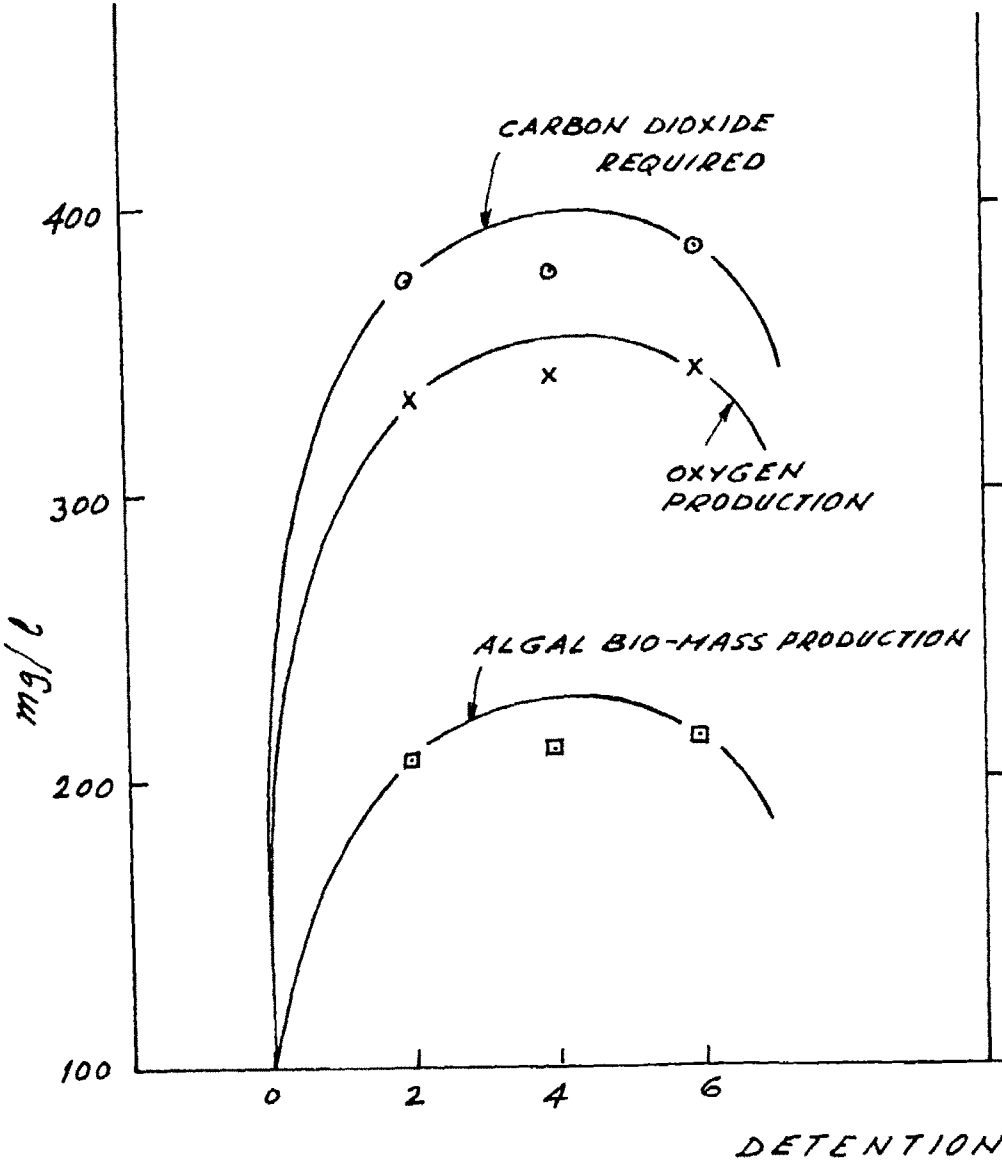
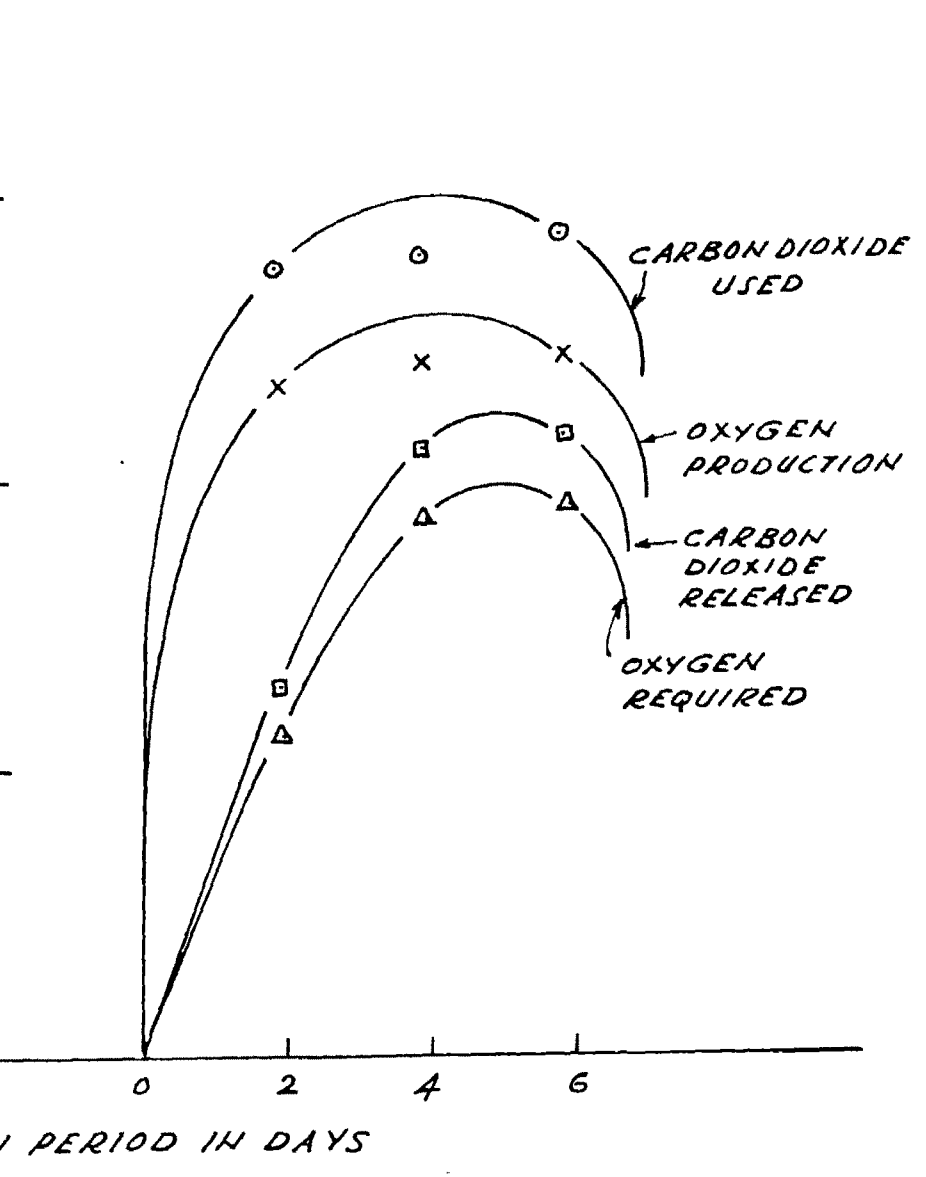


Fig. 25

ALGAL-BACTERIAL SYMBIOSIS USING MIXED ALGAE



dioxide released during bacterial oxidation in our laboratory model high-rate oxidation ponds are shown diagramatically in Figs. 21, 23 and 25.

Regression analysis of nutrient strength expressed as COD and algal biomass and correlation coefficients between the two:

The nutrient strength of a waste is ordinarily expressed in terms of 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 (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 remains in suspension and thus decrease

the energy available for algal growth.

Regression analysis relating algal growths in each case with the corresponding "used-up COD" have been worked out for the individual alga.(Standard Methods, 1971). The correlation coefficients "r" in each case are also calculated (Fischer, 1970) and are indicated in the tabular statement below, along with the corresponding values of the two constants "m" and "b" of the regression equation:

Algae	Regression analysis constants		Correlation coefficient "r"
	"m"	"b"	
Nostoc pyriformis	+ 0.249	+ 150.3	+ 0.997
Anabaena cylindrica	+ 0.117	+ 178.5	+ 0.96
Mixed algae	+ 0.070	+ 196.6	+ 0.79

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 sewage we find a high degree of direct correlation between algal growthsand their corresponding " used-up COD values".

D. Removal of Biostimulatory Nutrients

Two groups of nutrients are involved in algal-bacterial symbiosis; major and micronutrients. The former consist of carbon, nitrogen and phosphorus and the latter of trace elements like copper, manganese, zinc, iron etc. The major nutrients carbon, nitrogen and phosphorus are used up by heterotrophic bacteria and autotrophic algae, the two main partners involved in "algal-bacterial symbiosis".

Stumm (1968) has shown that the main constitutional elements in both bacteria and algae are the same (carbon, nitrogen and phosphorus) and that they are present 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 and bacteria synthesis.

The carbon value is usually expressed in terms of BOD_5 by Public Health Engineers, as the biological oxygen demand is a measure of the carbon oxidised and as one molecule of carbon combines with one molecule of oxygen.

Nitrogen enters synthesis reactions of bacteria and algae at the oxidation level of ammonia. Therefore, if any other nitrogen source except ammonia is to be utilised, it must first be converted into ammonia before it becomes available. Nitrate nitrogen and nitrite nitrogen are available for use in the production of bacterial protoplasm (Symons and McKinney, 1958).

Nutrients in raw settled sewage:-

The proportions in which the major nutrients present in the sewage samples used for our three experiments are shown below in a tabular form:

	Raw sewage by using					
	Nostoc pyriformis		Anabaena cylindrica		Mixed Algae	
	Actual (mg/l)	Reduced	Actual (mg/l)	Reduced	Actual (mg/l)	Reduced
C:N (Am-N)	197:364	5.4:1	153:34	4.5:1	160:36	4.4:1
C:P	197:19.5	10.1:1	153:16	9.6:1	160:16.8	9.5:1
C:COD :BOD_5	12:135 :197	12:26.3 :16.4	12:240 :153	12:20: :12.8	12:264 :160	12:22 :13.3
N:P	36.4:19.5	1.9:1	34:16	2.1:1	36:16.8	2.1:1

Porges (1960) has found the C:COD:BOD_5 ratio as 12:32:21.9. Our corresponding values are lower.

Sawyer (1971) has recently recorded the N:P ratios of waste waters. He has stated that predetergent domestic sewage was slightly rich in phosphorus with a N:P ratio of 10 and that the modern domestic sewage having a N:P ratio of 3, is excessively rich in phosphorus. In our case we get the N:P ratio as 2.

Stumm (1968) has observed that carbon is deficient in domestic sewage for utilizing all nitrogen and phosphorus into bacterial solids in the above proportions.

Nutrients in algal bacterial symbiosis:

Sawyer (1971) has stated: carbon, nitrogen and phosphorus form the three important constituents of algal tissues, where carbon alone accounts for 35 to 50 percent. Kuentzel (1969, 1970, 1971) therefore considers that the complete removal of organic matter from waste waters is necessary for preventing eutrophication in receiving waters. So algal growth can never be controlled solely by the removal of phosphorus alone. Nitrogen and phosphorus containing

substances are furnished by domestic waste waters.

Amounts of organic carbon, ammonia nitrogen and phosphate phosphorus utilized in "algal-bacterial symbiosis" and C:N, C:P and N:P ratios in view of their utilization are discussed below.

(i) Organic carbon utilised in " algal-bacterial symbiosis".

Photosynthetic oxygen values in turn are used for calculating the organic carbon used up according to Vollenweider (1969). "Assuming that the gas exchanges are the results of photosynthetic production of the conventional hexose, the photosynthetic quotient,

(PQ = oxygen output/carbon dioxide uptake by volume)

should be unity and hence :

g. carbon = g. oxygen x 0.375 [99]

Carbon values calculated from photosynthetic oxygen are compared below with the values for organic matter calculated according to Porges (1960):

Detention period	Organic matter used up from COD values(Porges) (mg/l)	Organic carbon used up from photosynthetic oxygen values (mg/l)
(a) Nostoc pyriformis		
2 days	187.5	123.6
4 days	205.0	127.2
6 days	227.5	130.8
(b) Anabaena cylindrica		
2 days	143.3	119.4
4 days	167.5	120.6
6 days	185.0	123.0
(c) Mixed algae		
2 days	135.0	124.8
4 days	183.3	126.0
6 days	186.7	128.4

From the above table, it will be seen that the values for organic carbon, calculated from photosynthetic oxygen are less than the organic matter calculated from COD values according to Porges.

(ii) Nitrogen utilised in "algal-bacterial symbiosis"

Nitrogen assimilation is calculated on the basis of the amount of ammonia nitrogen used up from the eco-system as a result of algal growth. This an over-

simplification of the real system found in the growth cultures where decomposition of organic matter, bacterial cellular synthesis, endogenous respiration and algal growth are taking place almost simultaneously and therefore a constant flux of nitrogen forms takes place.

The total ammonia nitrogen utilised in all the three algal cultures as a whole is calculated as well as the ammonia nitrogen used up by the algae alone for their cellular synthesis has been calculated according to the actually determined nitrogen content of the algae, which are not pure but are admixed with a small amount of organic matter. The actually determined nitrogen content in the algae is reported below on the basis of their algal dry weight:

Nostoc pyriformis	=	7,78%
Anabaena cylindrica	=	8.20%
Mixed algae	=	8.40%

Next, the results of total nitrogen utilization and nitrogen utilization by the three algal specimens are shown in a tabular from below:

Detention period (in days)	Total utilization		Utilization in algal synthesis		
	Am-N (mg/l)	% of Am-N used	Algal dry Wt. (mg/l)	N used (mg/l)	% of Am-N used over influent
Nostoc pyriformis					
0	36.4	-	-	-	-
2	9.9	72.8	206	16.0	44.0
4	5.4	85.2	212	16.5	45.3
6	3.0	91.8	218	17.0	46.7
Anabaena cylindrica					
0	34.0	-	-	-	-
2	8.2	75.9	199	16.3	47.9
4	6.4	81.2	201	16.5	48.5
6	2.7	92.0	205	16.8	49.4
Mixed algae					
0	36.0	-	-	-	-
2	8.0	77.8	208	17.5	48.6
4	5.9	83.6	210	17.6	48.9
6	2.2	93.9	214	18.0	50.0

From the table, it will be seen that nearly 92 to 94% of ammonia nitrogen have been utilised within six days in the three algal cultures. But only about 45 to 50% of the influent nitrogen have been utilised within six days by algae for their growth. The difference

in the ammonia nitrogen content must have been utilised for other biochemical reactions and/or lost to the atmosphere.

Algae seem to prefer ammonia nitrogen when both ammonia nitrogen and nitrate nitrogen are present. It is also reported that nitrate assimilation results in the production of OH^- ions which causes a rise in pH while ammonia nitrogen assimilation lowers the pH by the formation of H^+ ions.

(iii) Phosphorus used in "algal-bacterial symbiosis".

Phosphorus is used as orthophosphate in "algal-bacterial symbiosis". So, phosphorus assimilation is calculated on the basis of the amount of phosphorus used up in the ecosystem and as a result of algal growth. In the ecosystem the following reactions are taking place almost simultaneously:

a) decomposition of organic matter
b) bacterial cellular synthesis,
c) endogenous respiration and
d) algal growth

The organic matter from the ecosystem is removed as bacterial solids, while the inorganic nutrients are removed as algal bio-mass.

The total phosphorus utilized in all the three algal cultures has been calculated, and that used by the algae alone for their cellular synthesis has also been calculated according to the estimated phosphorus content of the algae. The actually determined phosphorus content of the algal cells is reported below in terms of % of algal dry weight:

Nostoc pyriformis	=	0.98
Anabaena cylindrica	=	1.14
Mixed algae	=	0.99

The results of total phosphorus utilization and phosphorus utilization in algal synthesis are shown in a tabular form below:

Detention period in days	Total utilization			Utilization in algal synthesis		
	PO_4 (mg/l)	P used (mg/l)	% of P used	Algal dry Wt. (mg/l)	P used (mg/l)	% of P used over influent (mg/l)
Nostoc pyriformis						
0	19.5	6.5	-	-	-	-
2	7.4	2.5	61.5	206	2.02	31.1
4	6.0	2.0	69.2	212	2.08	32.0
6	5.7	1.9	70.8	218	2.14	32.9
Anabaena cylindrica						
0	16.0	5.3	-	-	-	-
2	5.3	1.8	66.0	199	2.27	42.8
4	1.4	0.5	90.6	201	2.29	43.2
6	1.0	0.3	94.3	205	2.34	44.2
Mixed algae						
0	16.8	5.6	-	-	-	-
2	5.7	1.9	66.1	208	2.06	36.8
4	3.6	1.2	78.6	210	2.08	37.1
6	2.7	0.9	83.9	214	2.12	37.9

It will be seen that nearly 71 to 94% of the phosphate phosphorus have been used up within six days in the three algal cultures but only about 33 to 44% of phosphorus has been utilized with six days by the algae alone for their growth. The rest of the phosphate must have been precipitated on account of higher pH reached in six days detention period.

(iv) The ratios of C:N and C:P:

Various studies on waste water purification show the optimum rates of carbon to nitrogen used up in stabilization. In terms of COD, the COD to nitrogen ratio is found to be 25:1 (Porges, 1960). Sawyer (1956) has reported a wide BOD to N ratio of 32:1, equal to carbon to nitrogen ratio of 17.5:1 with longer periods of detention.

It has been stated that the critical levels are much lower than nitrogen with the BOD_5 to phosphorus varying from 90:1 upto 150:1 indicating one unit of phosphorus is required for 49 to 82 units of carbon (Porges, 1960).

In the case of the three algal growth cultures studied, the following ratios have been found in respect of BOD_5 used up to ammonia nitrogen used up

Stabilisation ratios	C : N Ratio		Stabilisation ratios	C : P ratio	
	Actual (mg/l)	Reduced		Actual (mg/l)	Reduced
Nostoc pyriformis					
BOD : N	171:33.4	5.1:1	BOD : P	171:4.6	37.2:1
COD : N	273:33.4	8.2:1	COD : P	273:4.6	59.3:1
Org.C:N	130.8:33.4	3.9:1	Org.C:N	130.8:4.6	28.4:1
Organic matter:N	227.5:33.4	6.8:1	Organic matter	227.5:4.6	49.5:1
Anabaena cylindrica					
BOD : N	141:31.3	4.5:1	BOD : P	141 : 5.0	28.2:1
COD : N	222:31.3	7.1:1	COD : P	222 : 5.0	44.4:1
Org.C:N	123:31.3	3.9:1	Org.: P	123 : 5.0	24.6:1
Organic matter:N	185:31.3	5.9:1	Organic matter	185 : 5.0	37.0:1
Mixed algae					
BOD : N	139:33.8	4.1:1	BOD : P	139 : 5.1	27.3:1
COD : N	224:33.8	6.6:1	COD : P	224 : 5.1	43.9:1
Org.C:N	128.4:33.8	3.8:1	Org.C:P	128.4: 5.1	25.2:1
Organic matter:N	186.7:33.8	5.5:1	Organic matter	186.7: 5.1	36.6:1

BOD_5 used up to phosphorus used up, COD used up to ammonia nitrogen used up, COD used up to phosphorus used up and organic carbon used up to ammonia nitrogen used up, organic carbon used up to phosphorus used up during "algal bacterial symbiosis":

From the sub-joined table, it will be seen that the ratios of C:N and C:P are found to be lower in the "algal bacterial symbiosis" taking place in the high-rate aerobic ponds than the reported ones.

The uptake of phosphorus is influenced by light (Gest and Kaman, 1948) and its availability is influenced by the pH of the medium.

(v) <u>The ratio of N:P in "algal bacterial symbiosis":</u>

Sawyer (1971) has recently recorded that the primary producers use nitrogen and phosphorus in a ratio of 15 : 1 and that natural bicarbonate activity serves as an adequate source of carbon dioxide for algal blooms in all wastes excepting acidic wastes.

The N:P ratio in the algae studied has been calculated as follows:

Algae	The ratio of N : P (utilised) in 6 days			
	Total utilisation		Algal synthesis utilisation	
	Actual	Reduced	Actual	Reduced
Nostoc pyriformis	33.4:4.6	7.3:1	17.0:2.14	7.9:1
Anabaena cylindrica	31.3:5.0	6.3:1	16.8:2.34	7.2:1
Mixed algae	33.8:4.7	7.2:1	18.0:2.12	8.5:1

It will be seen from the above table that our values under Indian conditions are nearly one half of the values stated by Sawyer (1971).