DISCUSSION OF RESULTS

C H A P T E R - V :

DISCUSSION OF RESULTS

PAGE NO:

Main series of experiments with the two algae. Chlorella vulgaris and Euglena gracilis:

(a) Bacterial oxidation of polluting organic matter

PAGE NO:

CHAPTER- V

Discussion of Results

(a) Bacterial Oxidation of pollution organic matter:

(1) Theory of bio-oxidation:

There are 4 phases of bacterial oxidation of polluting organic matter. They are as follows:

(i) Application of the four cardinal principles to waste treatment system:

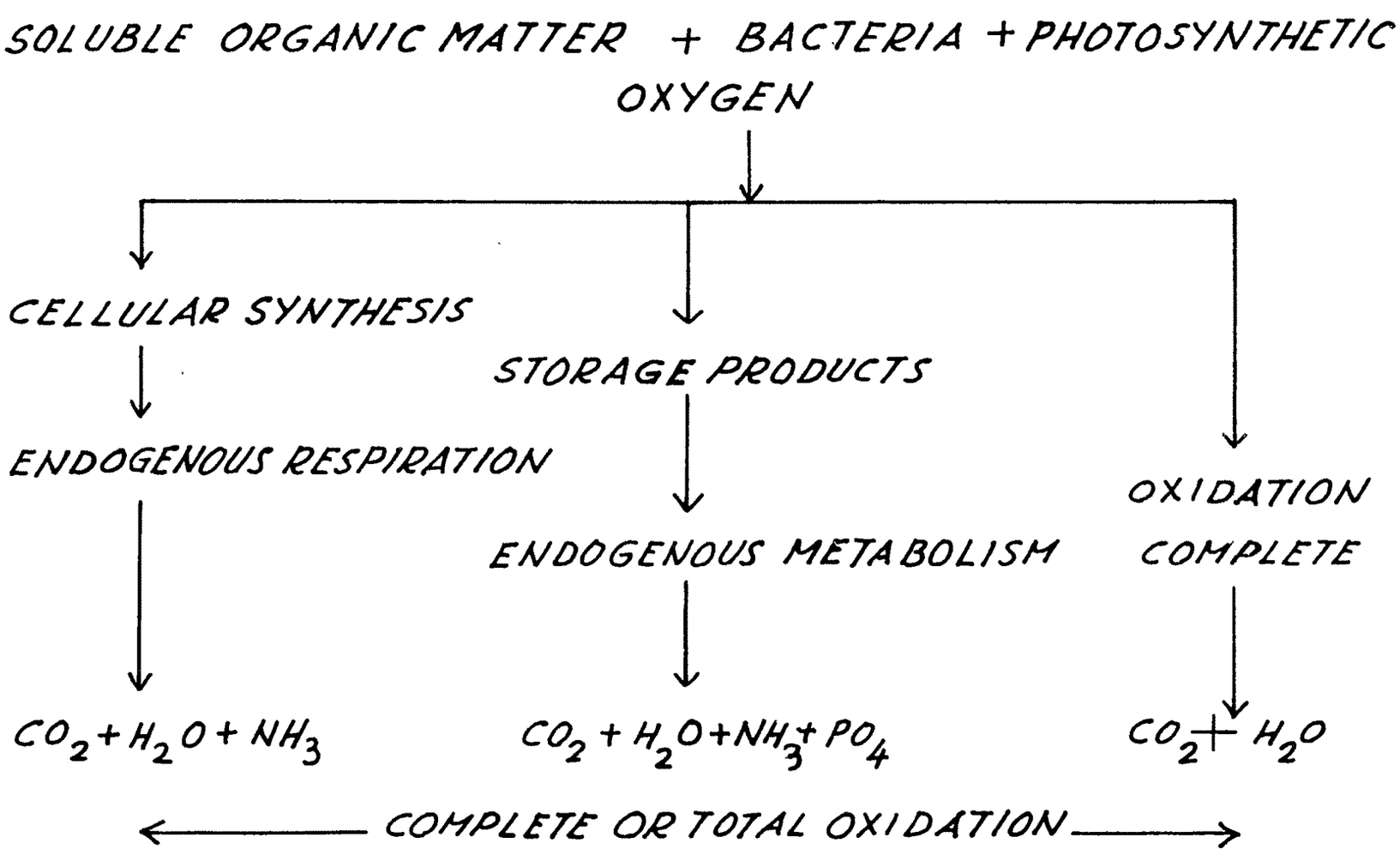
The main purpose of aerobic biological treatment for stabili--zation of organic matter is the removal of organic carbon in the organic substances of the waste water. This removal is brought about by reactions emboided in principles like the complete oxidation of organic compounds and biosynthesis of the bacterial cells. It is on account of this biosynthesis, the activated sludge process maintains and even increases itself (Symons and Mckinney 1958)

The phenomenon of mechanical flocculation, bio-flocculation, bioprecipitation and surface feaeration:

The phenomena of mechanical flocculation bio-flocculation and bio-precipitation are of common occurence in nature and act as partial and intermediary processes of sewage purification (Heukelekian 1941). The process of flocculation is coalescence of finely divided suspended matter in sewage acting in the absence of biologically active slime, primarily under the influence of physical forces. Bio-floceula--tion is also coalescence of finely divided suspended matter but acting under the influence of biological agencies. Bio-precipitation is distinguished from bio-floceulation by the conversion of soluble substrates into cellular protoplasm by micro organisms.

The design criteria and the methods of operation in high-rate aerobic ponds are conductive to foster these phenomena which seem to act as partial and intermediate process of sewage purification.

COMPLETE OR TOTAL BACTERIAL OXIDATION OF ORGANIC MATTER (MODIFIED AFTER PORGES 1960]



(ii) Complete oxidation:

In this phase of bacterial oxidation, the soluble organic matter is completely oxidised to carbon dioxide and water and energy as the final degradation product of metabolism.

(iii) Incomplete oxidation:

In this phase of oxidation, due to the lack of specific bacterial enzymes, the soluble polluting matter is not completely converted to carbon-dioxide but to some other intermediate compounds which accumulate inthe medium as end products of respiratory metabolism.

(iv) Endogenous metabolism:

This phase of oxidation starts when the nutrients in the medium is very low after aerating for a longer period. To get enough energy for the maintenance at this stage, bacteria oxidise their own body rdserved products like Glycogen,fat, volutin, etc.

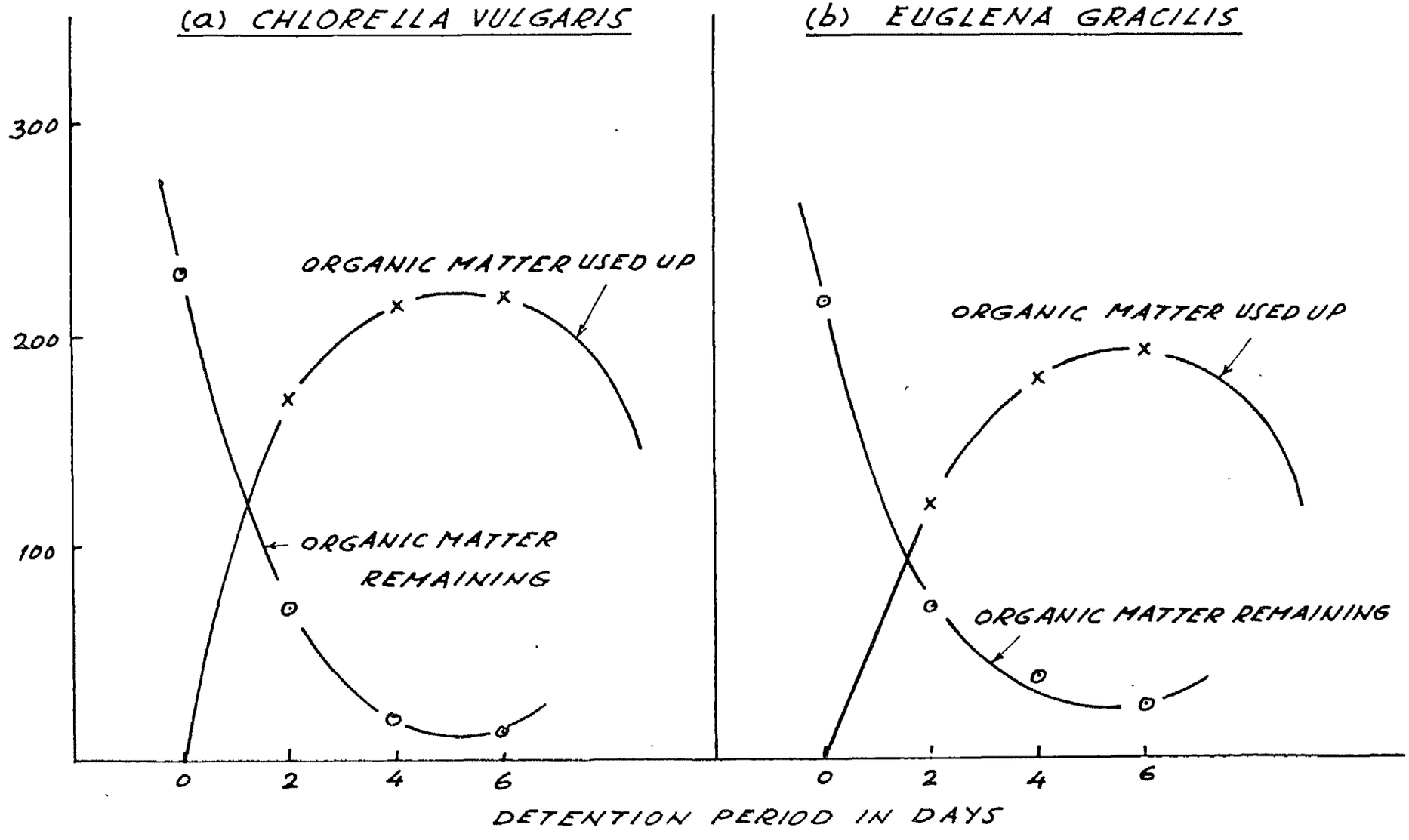
2. Application of the theory to the results

1. Removal of polluting organic matter as COD.

Detention time	Rawsewage	+Chlorella	Rawsewage	+ Euglena
	COD (mg/l)	CODused up (mg/l)	COD (mg/1)	COD used up (mg/1)
0 Day	275	-	257	-
2 days	72	+203	86	+171
4 days	18	+257	45	+212
6 days	12	+263	30	+227

It is clear from the above results that by using an alga Chlorella vulgaris with raw sewage, after 6 days, about 96% of COD is removed and by using Euglena gracilis about 88% of COD is removed on account of the three metabolic processes mentioned above.

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



Quantitative evaluation of algal-bacterial symbioses in the case of the two algal types experimented with Baroda raw setted and strained sewage:

In order to evaluate algal-bacterial symbioses (or how one kind of organism helps the other kind), it is necessary to know the quantity of CO_2 liberated during total bio-oxidation of sewage organi matter for the production of each of the two algal bio-masses, and in turn also how much of the photosynthetic oxygen is liberated by each of the algal bio-masses during the photosynthesis for total bacterial oxidation of sewage organic matter. But it is not possible to estimate directly either of the two gases in the two ecosystems during algal-bacterial symbiosis for, they are not phased metabolic processes (i.e; processes taking place one after the other) but are considered to be not only almost simultaneous, but are also stated to be utilized as soon as they are liberated in the eco system (Oswald 1960) The two metabolic processes are illustrated in Fig.

So, attempts were made to estimate indirectly the quantities of the two gases by methods which are based upon certain well-esta-blished factors and equations connecting CO_2 production from and oxygen requirements for total oxidation of sewage organic matter; and photosynthetic oxygen production from 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 rela-tions of facts from the two most indispensable and important parameter - COD and algal biomass which have been actually determined in our laboratory experiments.

"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 organic matter itself. But we have to know the quantity of or-

ganic matter oxidized by bacteria and porges (1960) has furnished a method of estimating approximately the same from COD values by using the conversion factor 1.2.COD values when divided by 1.2 give the corresponding "organic matter" equivalent in the wastewater. The rest of the calculations are shown under:-

ii. Conversion of COD values into organic matter values according to Porges (1960)

According to Porges, the values of organic matter obtained from COD values, if we divide COD values by the figure 1.2 Applying this factor to our results, we get the following results.

Detention time	Rawsewage + Chlorella		Rawsewage + Euglena	
	COD used up (mg/l)	Organic matter (mg/1)used up	COD used up (mg/l)	Organic matt--er (mg/1) used up
2 days	203	169.1	171	142.5
4 days	257	214.2	212	176.6
6 days	263	219.2	227	189.1

iii. Oxygen required for total bio-oxidation according to Oswald et al (1958) 's equation:

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

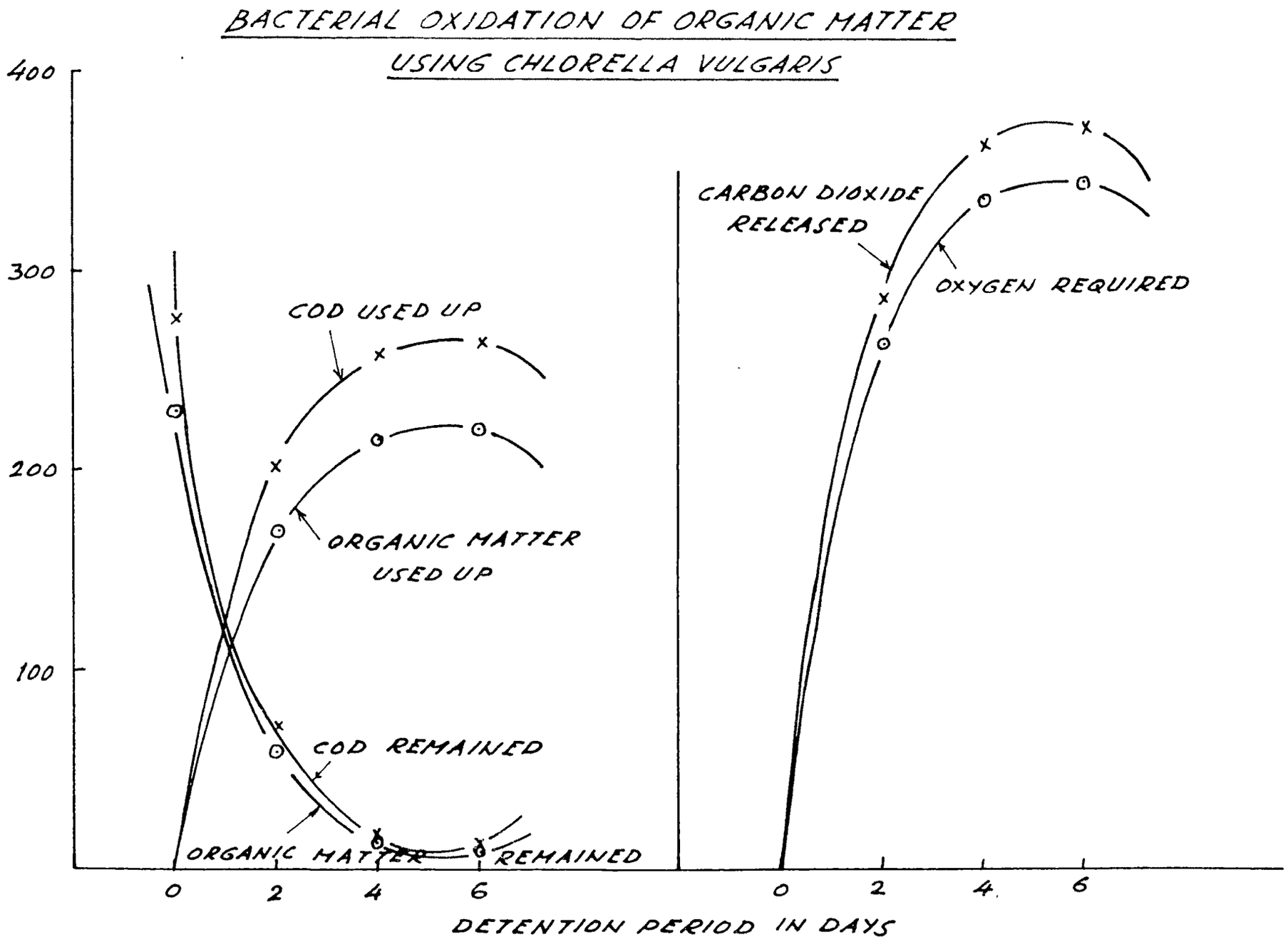
$$C_{11} H_{29} O_7 N + 14O_2 H \text{ -- } 11 CO_2 + 13H2O + NH_4$$

(Organic matter = 287+448 gm O_2) -- 484 gm CO_2 + $13H_2O$ + NH_4

So, one gm of sewage organic matter will produce 1.69 gm CO_2 and require 1.56 gm of O_2.

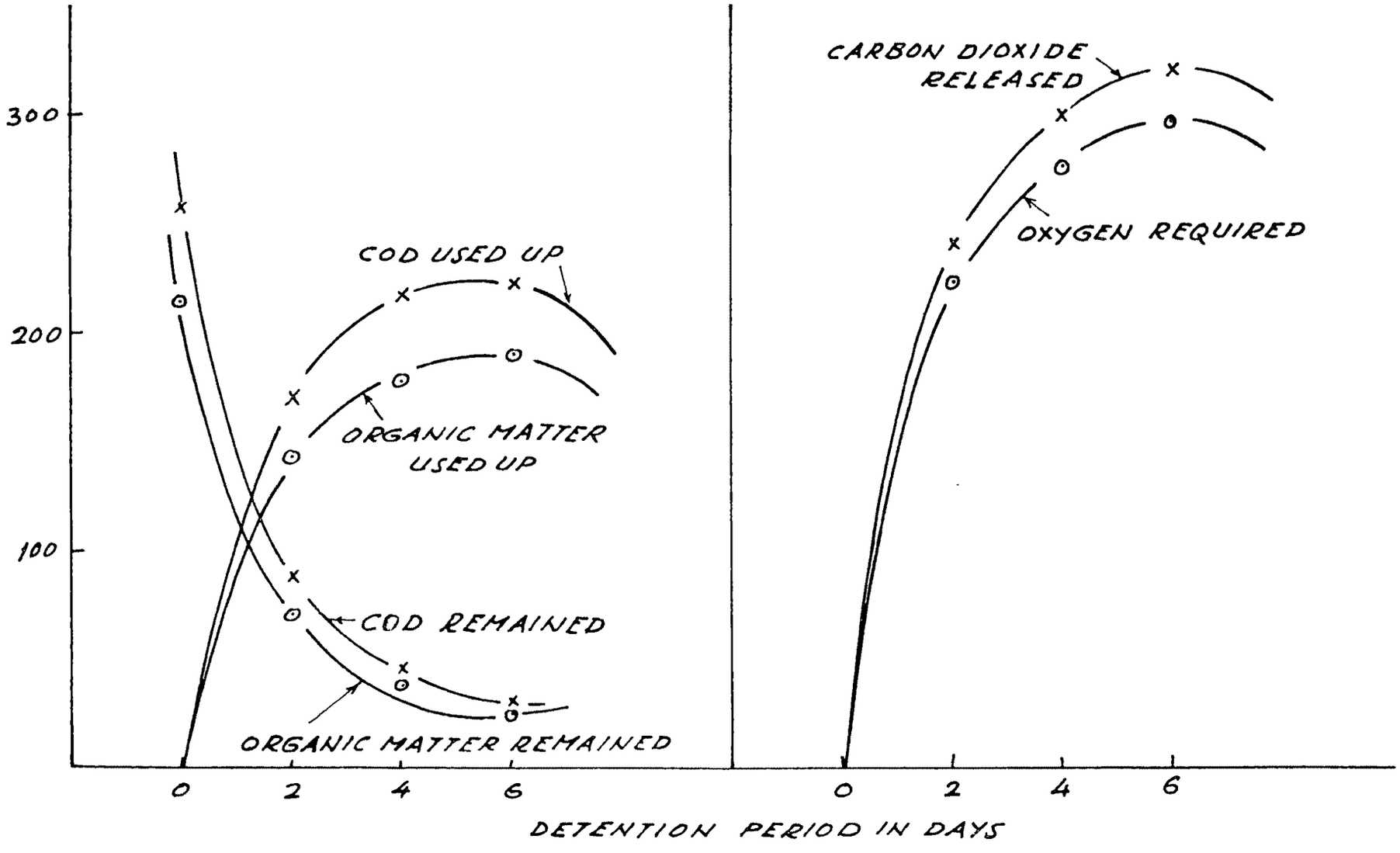
IV. Carbon dioxide released by bacterial oxidation:

According to Oswald et al (1958), in high-rate aerobic ponds, during total bacterial oxidation of one gm. of organic matter, 1.69


BACTERIAL OXIDATION OF ORGANIC MATTER
USING CHLORELLA VULGARIS
400
300
200
100
COD USED UP
ORGANIC MATTER
USED UP
COD REMAINED
ORGANIC MATTER REMAINED
CARBON DIOXIDE
RELEASED
OXYGEN REQUIRED
0
2
4
6
0
2
4
6
DETENTION PERIOD IN DAYS

BACTERIAL OXIDATION OF ORGANIC MATTER

USING EUGLENA GRACILIS



gm of carbon dioxide are released. Applying these factors to our results, the following results are obtained.

Detention time	Rawsewage + Chlorella		Rawsewage + Euglena	
	Organic matter used up (mg/l)	CO_2 released (mg/l)	Organic matter used up (mg/l)	CO_2 released (mg/l)
2 days	169.1	285.7	142.5	240.8
4 days	214.2	362.0	176.6	298.4
6 days	219.2	370.4	189.1	319.5

V. Oxygen required and carbon-dioxide released during bio-oxidation of sewage organic matter according to Oswald et al (1958)

According to Oswald et al (1958) one gm.of organic matter will require, 1.56 gm.of oxygen for its complete oxidation and as a result will produce 1.69 gm. of CO_2. Applying this to our results.

Detention time.	Rawsewage +Chlorella			Rawsewage + Euglena		
	Organic matter used up (mg/l)	CO_2 released (mg/l)	O_2 required. (mg/l)	Organic matter used up (mg/l)	CO_2 released (mg/l)	O_2 required (mg/l)
2 days	169.1	285.7	263.8	142.5	240.8	222.3
4 days	214.2	362.0	334.1	176.6	298.4	275.4
6 days	219.2	370.4	341.9	189.1	319.5	295.0

VI. Bacterial growth and synthesis according to Sawyer (1956):

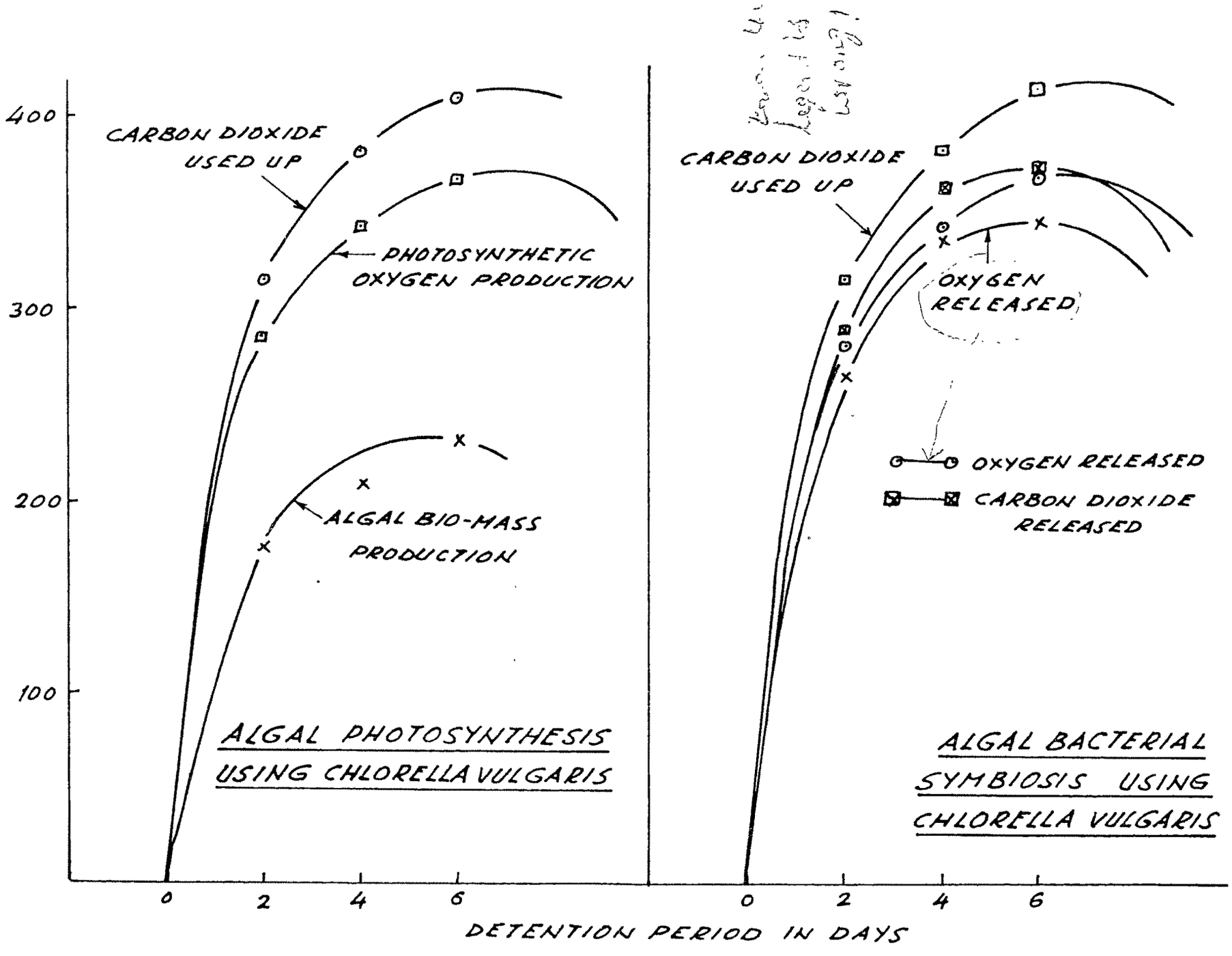
Sawyer (1956) has found a formulae to calculate the total bacterial mass in treated flasks from its BOD_5 values. According to him:

Total bacterial growth = 0.5 x BOD_5 used up (mg/l)

applying this to our results, we get:

Should slight

Detention time	Raw sewage + Chlorella		Raw sewage + Euglena		
	BOD_5 used up (mg/l)	Total bacterial mass (mg/l)	BOD_5 used up (mg/l)	Total bacterial mass(mg/l)	


400
300
200
100
CARBON DIOXIDE USED UP
PHOTOSYNTHETIC OXYGEN PRODUCTION
ALGAL BIO-MASS PRODUCTION
ALGAL PHOTOSYNTHESIS USING CHLORELLA VULGARIS
0
2
4
6
CARBON DIOXIDE USED UP
OXYGEN RELEASED
OXYGEN RELEASED
CARBON DIOXIDE RELEASED
ALGAL BACTERIAL SYMBIOSIS USING CHLORELLA VULGARIS
0
2
4
6
DETENTION PERIOD IN DAYS

1	2	3	4	5
2 days	100	50.0	95	47.5
4 days	123	61.5	139	69.5
6 days	125	62.5	142	71.0

VII Total active and decreasing bacterial bio-mass according to Mckinney (1962)

Mckinney (1962) has found a formula and to find out, bacterial active and total mass, the difference between the two will give the decreasing mass for activated sludge process. Applying the same to our case, we get,

The active bacterial mass = $M_a = S/(1+K_3t)$ where,

S = total bacterial mass; K_3 = constant = 0.006: T=time in hours

And the total bacterial mass = COD used up (mg/l)/2.13

The difference between the total bacterial mass and active mass will give the decreasing mass:

Applying these formulae to our results we get:-

Detenti-on time.	Rawsewage + Chlorella Vulgaris			Rawsewage+Euglena Gracilis		
	Total mass (mg/l)	Active mass (mg/l)	Decreasing mass (mg/l)	Total mass (mg/l)	Active mass (mg/l)	Decreasing mass (mg/l)
2 days	95.3	73.99	21.31	80.3	62.34	17.96
4 days	120.6	76.52	44.08	101.8	64.59	37.21
6 days	123.5	66.25	57.25	104.7	56.22	48.58

From the above results, it is evident that on 4th day, the active mass of bacteria in the case of chlorella and Euglena is maximum which declines on 6th day and this may be due to endogenous metabolism.

(VIII) Different phases of Microbiat metabolism in high-rate aerobic ponds:

High-rate oxidation pond process is a complete process involving a variety of microorganisms and a multitude of bio-chemical

reactions. But in simplest terms it may be depicted by the following equation.

$$\text{Food} + \text{Microbes} + O_2 \rightarrow \text{Newcells} + CO_2 + H_2O + NH_3 + \text{energy}.$$

The fundamental reason for oxidation pond to purify sewage is that micro-organisms want to proliferate for which they need food and energy. The energy required for the multiplication is obtained by oxidizing a part of the food available in the organic waste. The microorganisms (primarily bacteria) oxidise the organic compounds liberating the end products such as CO_2, H_2O and NH_3. Oxygen required for the oxidation is derived from algae is as photosynthetic oxygen.

Initially when the F/M (food to microorganisms) ratio is high, multiplication of cells is limited only by the cells ability to utilise the substrate and the generation time of the cells. At this stage the organisms are in a "log phase" or in "assimilatory phase". In our experiment this period lasts for 2 days (0-2 days) during this interval the maximum nutrients COD or BOD and organic matter are utilised. After some time, the food availbale can not support in bacterial growth at that former rate and hence limits the growth. In other words, F/M ratio decreases. This phase is known as declining phase, or as endogenous phase. In this phase, the bacteria live on the dead bacteria, and on themselves utilising the reserve food in the form of glycogen, volution and fat which are present in their bodies. In our case the endogenous phase period is between 2-6 days. And in this period the values of COD, BOD reduction are less organic matter utilised is less than they are utilised in the assimlatory phase. The bio-chemical nutrients like sugar, protein, aminoacid and organic acids are used up more in assimilatory phase than in the endogenous phase/vide Table, below.

COMPLETE BACTERIAL OXIDATION IN HIGH RATE AEROBIC OXIDATION POND

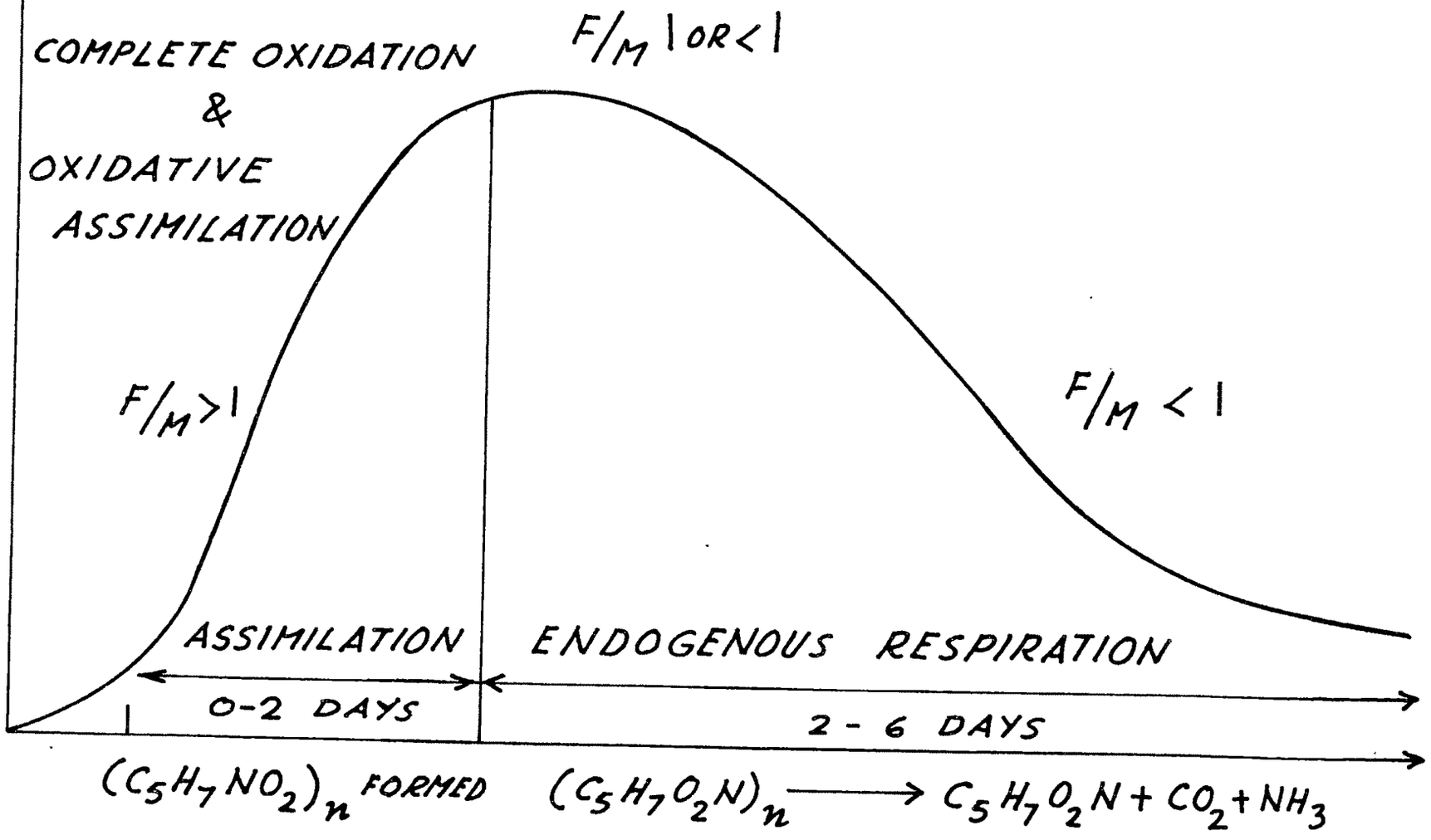
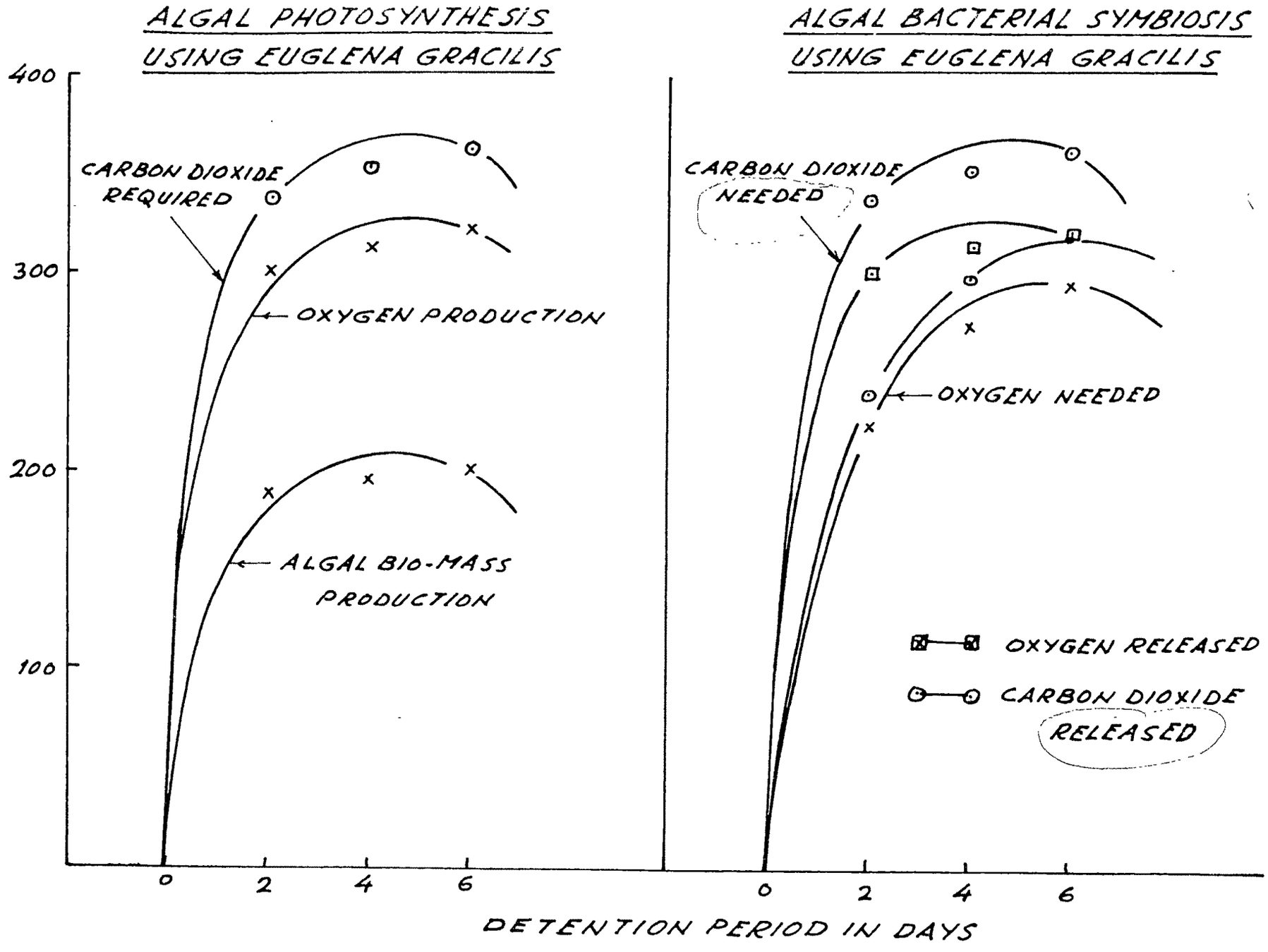
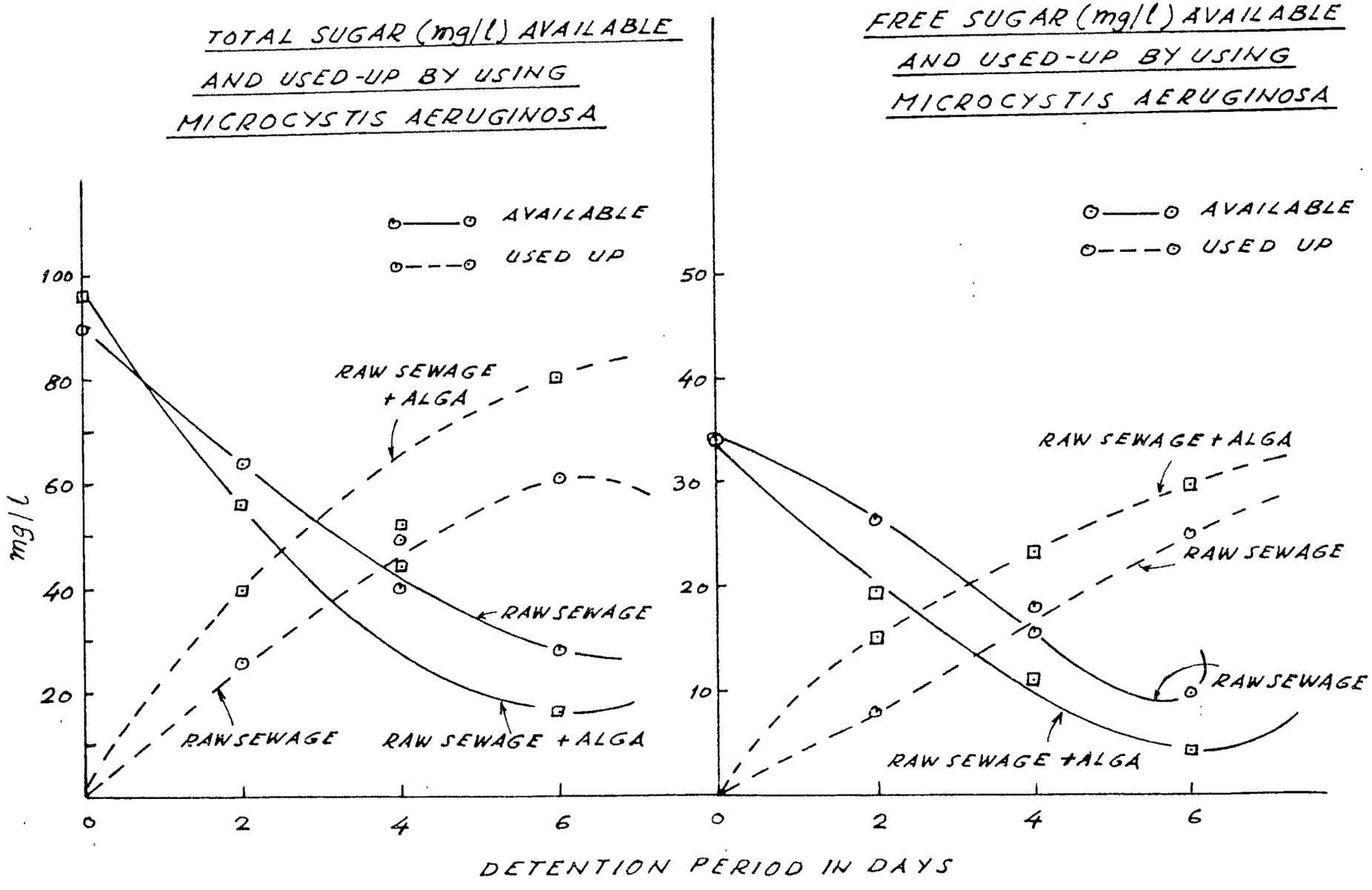


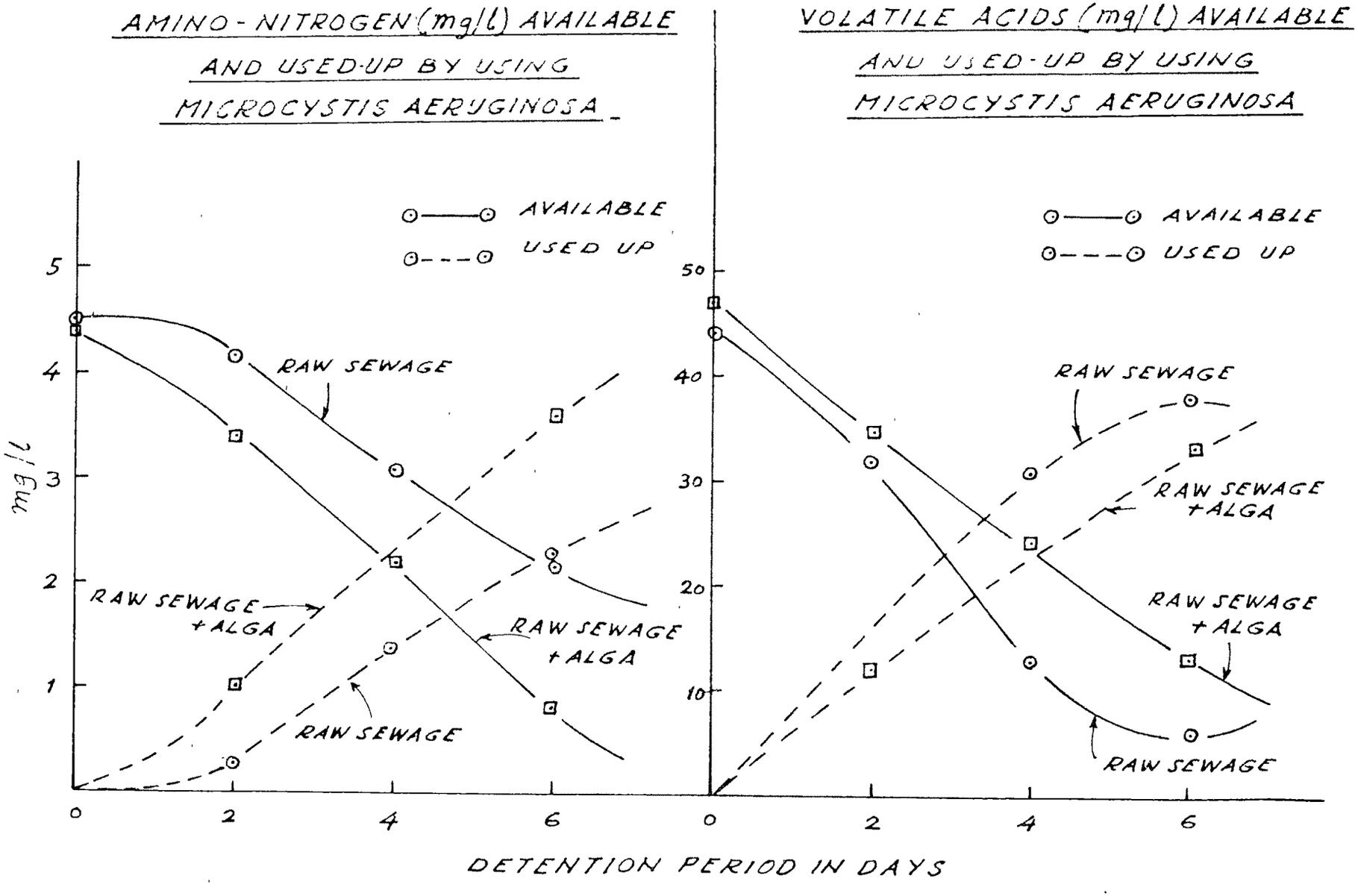
TABLE - XI

Removal of Nutrients during the Assimilatory and Endogenous phases of Algal-Bacterial symbiosis using the three algae and Baroda Raw Sewage.

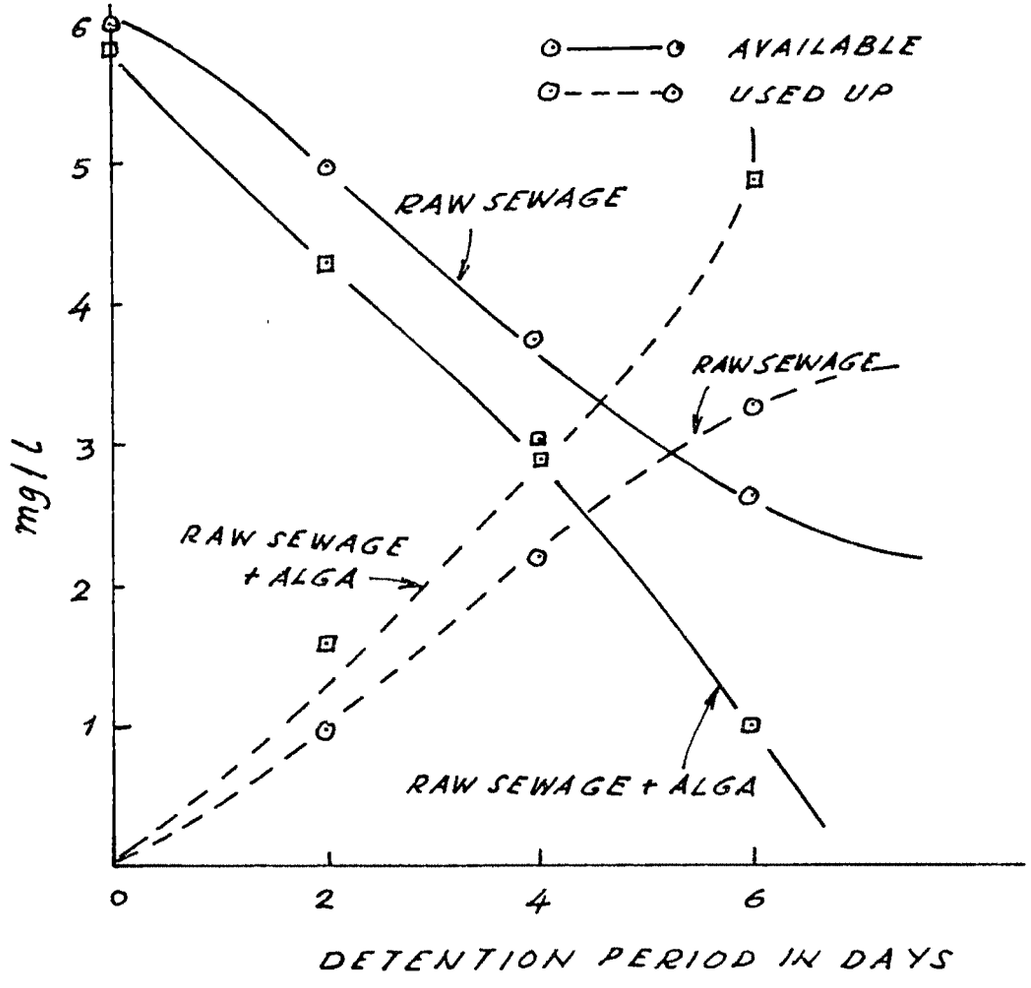
Algae	Chlorella vulgaris			Euglena gracilis		
	Detention periods			Detention periods		
phase	Assimila tory.	Endogenous		Assimila tory	Endogenous	
Days	0-2	2-4	4-6	0-2	2-4	4-6
Geo-chemical Tests (mg/l)						
1. Am-N	14.8	7.6	1.9	16.2	3.6	4.2
2. PO_4	5.7	1.5	0.3	12.0	0.8	1.2
Bio-chemical Tests(mg/l)						
3. BOD	203	54	6.0	171	41	15
4. Organic matter	169.1	45.1	5.0	142.5	34.1	12.5
5. Algae formed	175	213	230	188	196	201
BIOCHEMICAL TESTS USING Microcystis aeruginosa (mg/l)						
1. Free sugar	14.9	8.4	6.5			
2. Total sugar	40.0	12.5	27.2			
3. Protein	1.6	1.4	1.9			
4. Amino-N	1.0	1.2	1.4			
5. Organic acids	12.0	19.2	7.2			


ALGAL PHOTOSYNTHESIS
USING EUGLENA GRACILIS
ALGAL BACTERIAL SYMBIOSIS
USING EUGLENA GRACILIS
400
300
200
100
CARBON DIOXIDE
REQUIRED
OXYGEN PRODUCTION
ALGAL BIO-MASS
PRODUCTION
CARBON DIOXIDE
NEEDED
OXYGEN NEEDED
OXYGEN RELEASED
CARBON DIOXIDE
RELEASED
0
2
4
6
0
2
4
6
DETENTION PERIOD IN DAYS


TOTAL SUGAR (mg/l) AVAILABLE AND USED-UP BY USING MICROCYSTIS AERUGINOSA
AVAILABLE
USED UP
RAW SEWAGE + ALGA
RAW SEWAGE
RAW SEWAGE
RAW SEWAGE + ALGA
mg/l
100
80
60
40
20
0
2
4
6
FREE SUGAR (mg/l) AVAILABLE AND USED-UP BY USING MICROCYSTIS AERUGINOSA
AVAILABLE
USED UP
RAW SEWAGE + ALGA
RAW SEWAGE
RAW SEWAGE
RAW SEWAGE + ALGA
50
40
30
20
10
0
2
4
6
DETENTION PERIOD IN DAYS


AMINO - NITROGEN (mg/l) AVAILABLE AND USED-UP BY USING MICROCYSTIS AERUGINOSA
AVAILABLE
USED UP
RAW SEWAGE
RAW SEWAGE +ALGA
RAW SEWAGE +ALGA
RAW SEWAGE
mg/l
5
4
3
2
1
0
2
4
6
VOLATILE ACIDS (mg/l) AVAILABLE AND USED-UP BY USING MICROCYSTIS AERUGINOSA
AVAILABLE
USED UP
RAW SEWAGE
RAW SEWAGE +ALGA
RAW SEWAGE +ALGA
RAW SEWAGE
50
40
30
20
10
0
2
4
6
DETENTION PERIOD IN DAYS

PROTAIN (mg/l) AVAILABLE AND USED-UP BY USING MICROCYSTIS AERUGINOSA



(b) Algal photosynthesis with Chlorella and Euglena:

1. THEORITICAL CONSIDERATIONS:

a. Concepts of algal growth:

Jewell and McCarty (1968) have summarised:-
The photosynthetic process of algal development as detailed below: —

$$a\,CO_2 + CNO_3 + e\,PO_4^{-3} + (C+3e)H^+ + \tfrac{1}{2}(b-C-3e) + H_2O + \text{Sunlight}$$

$$\rightarrow C_aH_bN_c\,O_d\,P_e + (a+b/4+5c/4-d/2+5e/4)O_2$$

In other words, the rate N (or P) assimilation by algal cells is a function of the rate at which organic material is synthe--sized.

In most waste treatment systems utilizing algae, algal nutrients flow continuously through the system and thus are constan--tly renewed.

(b) Light intensity:

Light penetration is directly affected by incident light and inversely affected by depth and culture intensity. Optimum light intensities for maximum algal growth range from 200 to 400 foot candles (ft-C) and the lower limit may be 100 ft. Oswald (1963) reported that in laboratory studies with settled sewage, an average of 4% of the incident light energy was fixed by the algal cultures, and that conversion efficiency varied inversely with intensity, duration of light and detention time and directly with temperature and carbon-dioxide concentration.

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 ponds.

(c) Efficiency of light energy conversion:

In both small and large scale cultures of Chlorella, it is

reported to be 12 to 20%, provided the intensity of illumination is not too high (Wassink et al 1953). Gloyna (1971) has stated that the usual efficiencies range from 2% to 9% with 5% a common figure. Ganapati and Srinivasan (1970) have reported a maximum efficiency of 10% in a sewage polluted fish pond in Madras. 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 exceeded 10 or 12% of the available light energy. Oswald et al (1957) found the efficiencies of outdoor ponds to range between 1 and 10% with most values in the narrow range of 3 to 7%. Effiei--cencies of 5 to 8% may be attainable with algal cell concentrations dense to utilise the nutrients and permit harvesting of cells (Gotaas and Oswald 1955). Ganapati (1971, p 33) has reported a value of 6.6 to 13.8% in Indian oxidation ponds.

(d) Temperature:

As in the case of all organisms, temperature affects the growth rate of algae, normally following the Van't Hoff rule accor--ding to which growth rate doubles for each 10°C rise in temperature, within the range of temperature tolerance.

(e) Carbon Source:

It is usually the limiting element when algae are cultured in sewage. Algae normally use free CO_2 as an inorganic carbon source, though some algae are reported to use the bicarbonate ion. Use of artificially introduced carbon dioxide 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% carbon-dioxide normally and this amount is adequate to sustain maximum photosynthetic efficiency if a sufficient volume of the mixture is brought into contact with cell surface (Davis, 1953).

Active photosynthesis causes the pH to increase to 10 or more according to the absorption of atomospheric carbon-dioxide by the culture. Under such conditions, this carbon-dioxide appears as a bicarbonate ion and becomes available to algae immediately. So, algae may compensate for a storage of carbon-dioxide by increasing the carbon-dioxide 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 carbon-dioxide is used up, the half bound carbon dioxide (bicarbonates) from 4/5 to 5/8 is then availed and used up (Birge and Juday, 1911). When the first two sources are exhausted, the fully bound CO_3 or monocarbonates may also have to be used up (Schutouz, 1926, Maucha 1929; Neresheimer and Ruttner 1929, Juday, Birge and Melocke, 1935).

(f) 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 Wolf (1954), but they prefer Ammonia-nitrogen to nitrate nitrogen when both sources are provided in the same culture, (Harvey, 1940, Schuler, et al 1953)

Many blue green algae can fix atmospheric nitrogen (Fogg, 1947) when an extremely low concentration of dissolved nitrogen is available in the medium. So for nitrogen removal nitrogen-fixing algae should not be used in a treatment plant.

Algae use also phosphorus as orthophosphate (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.

2. Application to the connected results:

i. Carbon-dioxide used up during algal-bio-mass production:

Myers (1962) has shown that for production of 1 mg. of dry algal matter, 1.8 mg. of CO_2 are required. Applying this factory to our results, we get as follows:

Detention time	Rawsewage + Chlorella vulgaris		Rawsewage+Euglena Gracilis	
	Algal bio-mass (mg/l)	CO_2 used up (mg/l)	Algal bio-mass (mg/l)	CO_2 used up (mg/l)
2 days	175	315	188	338.4
4 days	213	383	196	352.8
6 days	230	414	201	361.8

ii. Quantity of photosynthetic oxygen released into the eco system:

According to Oswald (1960, 1963) the weight of oxygen produced during algal photo synthesis in the culture medium is calculated using his formulae.

Weight of Oxygen produced = Weight of algae produced x 1.60

Applying this to our results, we get the following data:

Detention time	Rawsewage+Chlorella vulgaris		Rawsewage+ Euglena gracilis	
	Algal bio.mass (mg/l)	Oxygen released (mg/l)	Algal bio-mass(mg/l)	Oxygen released (mg/l)
2 days	175	280	188	300.8
4 days	213	340.8	196	313.6
6 days	230	368	201	321.6

(C) In-Put, Out-Put energy balance:

1. Theoritical considerations:

Gotaas and Oswald (1953) and Oswald and Gotaas(1957) have

developed an in-put -out-put energy balance system for estimating the over-all photosynthetic energy conversion efficiency, in which a basic assumption is made that the system under study is a continu--ously, stirred reactor with complete homogenity of the algal cells in suspension (Beck et al, 1969). As in any continuosuly stirred tank reactor, there is a finite volume V, in liters and flow rate F, in liters per day. The mean hydraulic residence time Q is then defined as :

$$Q = V/F \qquad \ldots\ldots\ldots \quad 1$$

For a given mean residence time Q, in days, the total solar energy input per liter of pond volume is equal to Ein = S.A.Q...2 Where Ein is the total energy input in calories per liter, S, the daily solar energy input in calories per square Cm. per day; A, the surface area of the one liter of pond volume receiving sunlight in Cm^2 per day and Q is the mean hydraulic residence time. For one liter of pond volume A = 1000/d where d is the determined depth in cm.

$$\text{Therefore Ein} = \frac{1000\ S.Q.}{d} \qquad \ldots\ldots\ldots \quad 3$$

The energy out-put in the form of synthetised algae is defined as:

$$E_{out} = h\, C_c \qquad \ldots\ldots\ldots \quad 4$$

Where E_{out} is the total energy tied up in synthetised algae in calories per liter, 'h' is the heat of combination of algae in calories per milligram, and C_c, is the concentration of algae in mg/l Equations 2 and 4 can be equated by assuming that only a portion of the energy in-put is converted to algal bio-mass, so that,

$$E_{in}\, e = E \text{ out} \qquad \ldots\ldots\ldots \quad 5$$

Where e is an efficiency factor.

The equation 5 can be expended thus;

$$\frac{1000\ S.Q.e.}{d} = h\ C_c \qquad \ldots\ldots 6$$

$$e = \frac{h\ C_c\ d}{1000\ S.Q.} \qquad \ldots\ldots 7$$

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

2. Application to the results:

The above equation $e = \frac{h\ C_c.d}{1000.S.Q.}$ 7

is used in calculating the overall photosynthetic energy conversion efficiency of the 2 algae used up in our experiments. The pertinent data used in our calculations are shown for the algae Chlorella vulgaris and Euglena gracilis.

Chlorella vulgaris:

(a) Volume of the culture fluid i.e; raw sewage used = 1.5 liters

(b) Depth of the fluid volume = 3.5 cm.

(c) Maximum quality of algal bio-mass produced in 6 days in 1.5 liters of sewage = 230+115=345 mg.

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

(e) 10.5 lux = 1 gm-calorie

Therefore 375 lux = 35.7 gram-calories.

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

Applying this value in equation (7)

$$e = \frac{345 \times 5.5 \times 3.5}{35.7 \times 1500 \times 6} = 0.0207 = 2.07\%$$

Similarly

h for Euglena gracilis

$$e = \frac{301.5 \times 5.5 \times 3.5}{35.7 \times 1500 \times 6} = 0.018 = 1.80\%$$

(d) Algal-bacterial symbiosis in high-rate aerobic ponds:

1. Theoritical considerations:

Algal-bacterial symbiosis is the result of the combined

activity of heterotrophs and autotrophs. Since algae obtain their energy for synthesis from sunlight, there is no necessity for them to metabolize organic compounds like the bacteria and the fungi.

The mode of nutrition of algae is autotrophic. Bacteria and algae can work together. One helping the other in commensal relation-ship. In other words, the bacteria metabolize the organic compounds of the waste and release some substances like CO_2 and NH_3 utiliza-ble by algae. During synthesis of fresh algal cells, algae release oxygen, which is utilized by the bacteria for stabilization of organic matter.

In the absence of sunlight, some algae are reported to obtain the energy to remain alive from metabolism of organic matter just like bacteria and fungi. This organic matter normally comes from stored food within their cells but in some cases it can come from the organic matter in the wastes.

But in the treatment affected by an oxidation pond results from a complete symbiosis between bacteria and algae. (Ludwig, Oswald, Gotaas and Lynch 1951).

2. Application to related results:

(i) Quantity of oxygen released during algal photosynthesis is compared with the quantity of oxygen required for total bacterial oxidation of polluting organic matter of Baroda sewage.

The results for the two algae are shown under

(a) Chlorella vulgaris:

Detention time	Oxygen released during algal photosynthesis	Oxygen required during bacterial oxidation(mg/l)	Difference	Excess %
2 days	280.0	264.4	15.6	6
4 days	340.8	334.1	6.7	2
6 days	368.0	341.9	26.1	8

(b) Euglena gracilis

Detention time	Oxygen released during algal photosynthesis (mg/l)	Oxygen required during bacterial oxidation(mg/l)	Difference	Excess %
2 days	300.8	222.3	78.5	35.3
4 days	313.6	275.4	38.2	13.8
6 days	321.6	295.0	26.6	9.0

So, it is found, in both the experiments using Chlorella vulgaris and Euglena gracilis, that photosynthetic oxygen production is greater than the quantity needed for total bacterial oxidation of sewage organic matter.

ii. Carbon dioxide released during total bio-oxidation of polluting organic matter is compared with the quantity used up for algal bio-mass production in algal-bacterial symbiosis:

(a) Chlorella vulgaris:

Detention time	Carbon dioxide used in algal photosynthesis (mg/l)	Carbon-dioxide released during the bacterial oxidation(mg/l)	Difference	Excess %
2 days	315.0	285.7	29.3	10.2
4 days	383.4	362.0	21.4	5.9
6 days	414.0	370.4	43.6	11.2

(b) Euglena gracilis:

Detention time	Carbon-dioxide used in algal photosynthesis (mg/l)	Carbon dioxide released during the bacterial oxidation(mg/l)	Difference	Excess %
2 days	338.4	240.8	97.6	40.5
4 days	352.8	298.4	54.4	18.2
6 days	361.8	319.5	42.3	13.2

From the above results it is found that the amount of carbon-dioxide released by bacterial oxidation is less than the amount of carbon-dioxide needed for algal photosynthesis.

iii. Explanation for the excess of carbon dioxide used up during algal photosynthesis in algal bacterial symbiosis:

It is seen that sewage organic matter alone is not sufficient for the formation of the required amount of algal bio-mass. As the organic matter alone can not supply the required amount of carbon-dioxide to the algae. Other sourcesof carbon dioxide also must have helped in photosynthesis and they are (a) atmospheric carbon dioxide and (b) bicarbonate-carbonate equilibrium system. The two latter sources vary from 8 to 41% of the organic carbon-dioxide released from sewage organic matter in the two experiments.

(e) Regression analyses of nutrient strength expressed as COD and algal bio-mass and their correlation coefficients:

1. 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 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 linearly with increased BOD and their decreases probably because strong wastes contain excess colloidal material and bacteria which remain in suspension and thus decrease the energy available for algal growth.

2. Application to the connected results:

Our studies confirm the observation 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 highdegree of direct correlation between algal growths of two different kinds and their corresponding used up COD. Regression analysis relating algal growths in each case with the corresponding used up COD have been worked out for the indivi-dual as well as for all the algae together. (Standard Methods, 1971) The correlation coefficient "r" in each case is also indicated in the tabular statement along with the corresponding values for the two constants 'm' and 'b' in the regression equation.

$$m = \frac{n\sum x \cdot y - \sum y \cdot \sum x}{n\sum y^2 - (\sum y)^2} \qquad b = \frac{\sum y^2 \sum x - \sum y \cdot \sum x \cdot y}{n\sum y^2 - (\sum y)^2}$$

$$r = \frac{n\sum x\ y - \sum y \cdot \sum x}{\left(n\sum x^2 - (\sum x)^2\right)\left(n\sum y^2 - (\sum y)^2\right)}$$

Where n = number of observations

x = algal bio-mass expressed as mg/l.

y = COD used up expressed as mg/l

Applying the above formula to our results we get:-

Name of the algae	Regression analyses	Constant	Correlation constant
	'm'	'b'	'r'
i) Chlorella vulgaris	+0.861	-0.341	+0.990
ii) Euglena gracilis	+0.224	+149.4	+0.990

(f) Bio-stimulatory nutrients and their removal during algal photosynthesis:

1. Theoritical considerations (N.P.)

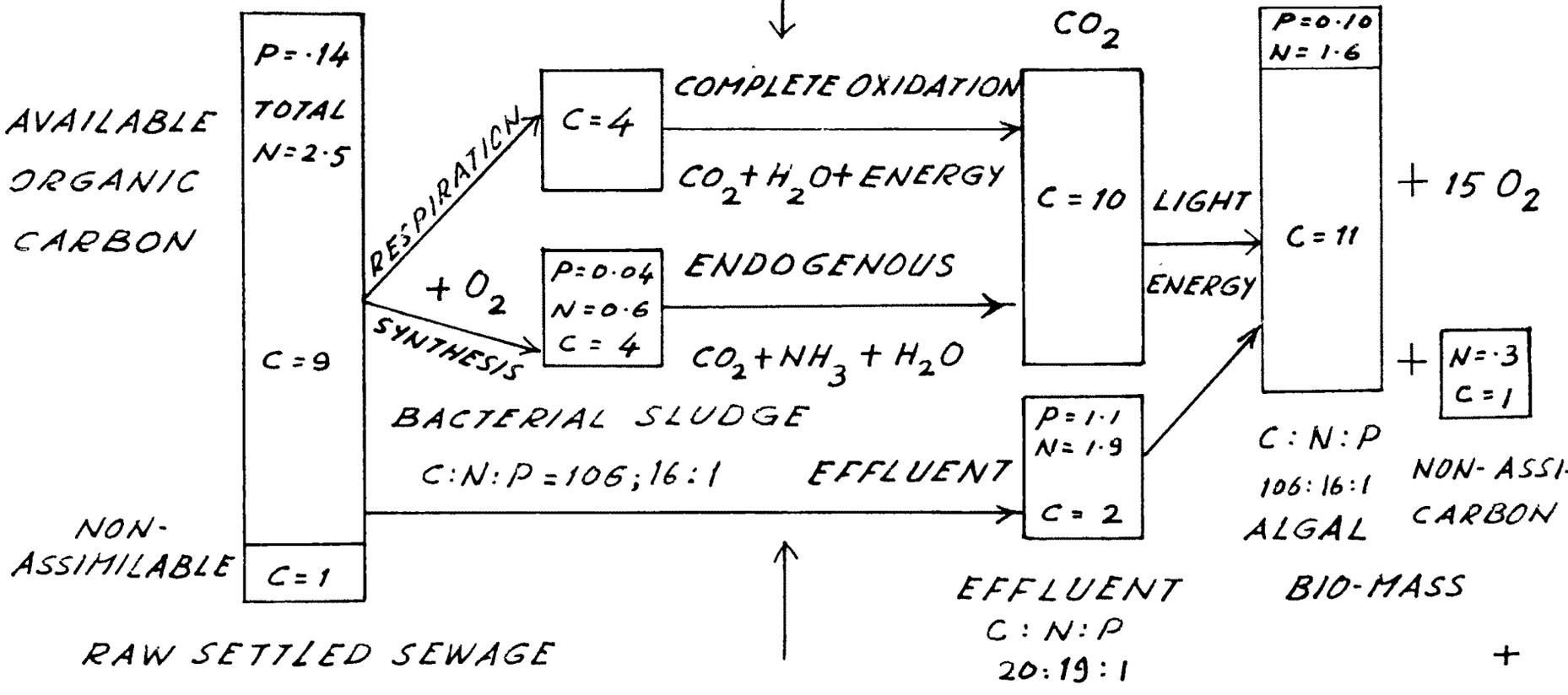
Two groups of nutrients are involved in algal-bacterial symbiosis; major and micro-nutrients. The former consist of carbon,

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



nitrogen, phosphorus and the latter of trace elements like copper, manganese, arsenic etc. The major nutrients are used up by hetero trophic bacteria and autotrophic algae, the two main partners in the game of algal bacterial symbiosis.

Stumm (1968) has laboured to show that only the main consti--tutional elements in both bacteria and algae are the same (C,N,P) and they are also found in the same constant properties of 105:16:1 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 bacterial synthesis.

Nutrients in raw settled sewage:

The major nutrients (C,N and P) and the micro-nutrients are always present. The proportions in which the major nutrients are present are: (a) C:N (Am-N) = 280:29 N 20:1 (b) C:P 280:12:5 or 67:1 The relation between C, COD and BOD_5 is 12:32:21.9 for ordinary wastes (Porges, 1960, 81) and we get a ratio of 12:31:23.3 Stumm (1968) has observed that C is deficient in domestic for utilizing all N and P into bacterial solids and algae in the above proportions.

2. Application to the connected results:

(i) Phosphorus:

Phosohorus is used as orthophosphate in algal-bacterial symbiosis. It is used in algal growth reactions, at 0.94% of the dry algal weight for chlorella vulgaris and at 1.15% of the algal dry weight for Euglena ~~(Krenapyti and Kudpy)~~. Based on this factor, the following calculations are made. The amount of phosphorus and phos--phates used up during algal-bacterial symbiosis are shown as under:-

(a) Chlorella Vulgaris:

Detentiontime	Algal bio-mass (mg/l)	PO_4 P (PO_4 mg/l) found	P	PO_4 P (PO_4 mg/l) used up	P	%of PO_4 used up	PO_4 P content of algal bio-mass at 9.94% of algal dry weight	P	PO_4 % utilization by algal bio-mass.
0 Day	-	9.0	3.0	-	-	-	-	-	-
2 Days	175	3.3	1.1.	5.7	1.9	53%	1.64	0.55	18.2
4 Days	213	1.75	0.58	7.25	2.42	80%	2.00	0.66	22.2
6 Days	230	1.40	0.46	7.60	2.53	84%	2.16	0.72	24.0

From the above results, it is found that by using Chlorella vulgaris, on 6th day about 84% of PO_4 o/p is utilized in the process of algal bacterial symbiosis.

Euglena gracilis:

Detention time	Algal bio mass	PO_4 P (mg/l) found		PO_4 P (mg/l) used up		PO_4 P content of algal bio mass at 1.15% of algal dry weight.		% of PO_4 used up	PO_4 % utilization by algal bio-mass.
0 Day	-	18.4	6.1	-	-	-	-	-	-
2 Days	188	6.4	2.1	12.0	4.0	2.16	0.72	65%	11.7
4 Days	196	5.6	1.9	12.8	4.3	2.25	0.75	70%	12.2
6 Days	201	4.4	1.5	14.0	4.7	2.31	0.77	76%	12.6

The above table shows that 76% of the phosphate or phosphorus have been used up by alga Euglena gracilis in the process of algal-bacterial symbiosis. The wide difference in % utilization by the algae Chlorella vulgaris and Euglena gracilis may be due to different types of algae. Analysis of pure algae shows the concentrations of phosphates of about 1% of the algal dry weight (Oswald, Golueke and Gee 1961, P.37) ~~&~~ The phosphate content of Chlorella is 2.16 mg/l on 6th day and for Euglena 2.31 mg/l. Considering 1.15 % of dry algal wt The utilization of phosphate phosphorus in the case of Chlorella works out to be 24.0

and for Euglena 12.6% on 6th day. The rest of the phosphates must have been precipitated as calcium phosphates and magnesium phosphates on account of higher pH reached in six days detention time.

ii. Nitrogen:

Nitrogen assimilation is calculated on the basis of the amount of ammonia nitrogen used up from the ecosystem as a result of algal bacterial symbiosis. This is 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 a constant flux of nitrogen forms take place. The ammonia nitrogen consumed in the two algal growth cultures are shown below:

(a) Chlorella vulgaris:

Detention time	Algal bio mass	Am-N Found	Used up (mg/l)	% of Am-N used up	Nitrogen content of algal bio-mass (mg/l) at 7.85% of dry algal wt.	% utilization of Am-N by algal biomass.
0 Day	-	27.1	-	-	13.73	50.6
2 Days	175	12.3	14.8	54.6%	13.73	50.6
4 Days	213	4.7	22.4	82.6%	16.72	61.7
6 Days	230	2.8	24.3	90.0%	18.05	66.6

(b) Euglena gracilis

Detention time	Algal bio-mass	Am-N found (mg/l)	Used up	% of Am-N used up	Nitrogen content of algal biomass (mg/l) at 8.14% of the dry algal weight.	% utilization of Am-N by algal bio-mass.
0 Day	-	26.0	-	-	-	-
2 Days	188	9.8	16.2	62.3%	15.30	58.8
4 Days	196	6.2	19.8	76.1%	15.95	61.3
6 Days	201	2.0	24.0	92.3%	16.36	62.9

It is clear from the above data that 66.6 to 62.9% on the total ammonia nitrogen used up has been utilized by the algas Chlorella vulgaris and Euglena gracilis respectively in 6 days. ~~The nitrogen content of the algal cells has been found to vary between 8 and 10% in this laboratory.(Senapati and Asthana)~~ Assuming it as 7.85% the nitrogen content of Chlorella vulgaris is about 18.05 mg/l and that of Euglena gracilis[15] about 16.36 mg/l on 6th day, assuming it as ~~8.14%~~ 7.85% of the algal dry wt. So, the utilization of ammonia nitrogen in the first case works out to be 90% and that in the second case about 92.3% the average being 91%. So the rest of ammonia nitrogen must have been utilized for the other biochemical reactions.

Algae require nitrogen either as ammonia nitrogen or as nitrate nitrogen but they seem to prefer ammonia-nitrogen when both are provided together (Harvey 1940, Schuler et al, 1953) It is also reported that nitrate assimilation results in the production of hydroxyl ions which cause a rise in pH while ammonia-nitrogen assimilation lowers the pH by the formation of hydrogen ions. Conventional sewage treatment processes like activated sludge and trickling filter only removes factor of N and P in sewage effluents. These nutrowaste cause entroplication in receiving waters. The effluent on the high-rate oxidation ponds, the only device capable of considerable removal of N and P.

(g) Biological conditions:

It is seen from tables IV and V that rotifers are absent in the control Rawsewage flasks, but are present in ~~high-rate aerobic oxidation ponds~~ algae treated flasks which are an indirect proof of the photosynthetic oxygen being present in the algae treated rawsewage and as there is no free oxygen present in control flask, the rotifers seem to be absent.

(h) Bio-Chemical conditions:

The bio-chemical changes e.g. the reduction in free sugar, total sugar, protein, amino-N and organic acids in the two experiments ponds are attributed to the metabolic activities of the different types of bacteria present in the system.

(i) Bacteriological Results:

Microbes in action in sewage purification:

1. The activated sludge process was developed by Arden and lockett in 1914 and since then it has attained such a popularity and use that no other process had attained. For more than half a century that it has been in use; much empirical knowledge has been gained about the technical operation of the process, but unfortunately little is known still as to what effects the stabilizing action that takes place when air is diffused into activated sludge. It is no doubt, the metabolic processes which are taking place then, that form the basis of purification. But the available literature reveal very little about the nature of the purification processes. Only a few basic facts have been found regarding the ecology and metabolism of activated sludge process upto the present day.

About the middle of the centruy Oswald, Gotaas and Golueke of the Sanitary Engineering School of the university of California developed their high-rate aerobic oxidation pond, a new low cost method of sewage purification, which is based on the primordeal process of photosynthesis. Much less is known about the mechanism of purifica-tion of this new process than in the case of the activated sludge process for, the discoverers of the new process themselves state as follows:

(i) "Although reports which list the specific organisms involved in aerobic oxidation in atabilization ponds are not available, it is

extremely likely that the aerobic bacteria of ponds which are mainly contained in a yelbw-brown flocculent sludge (the substance created during bio-flocculation) differ but little from those found in activated sludge or in trickling filter slimes (22)" (Oswald 1960). (1) Russel and Barrow (1916) isolated 13 varieties of non-nitrifying bacteria from activated sludge. Nine of them belonged to the Bacillus group of aerobic spore formers, formed acid but no gas from glucose and hydrolysed starch and casein.

(ii) Kamm (1917) got almost the same results like Russel and Bartow.

(iii) Buswell and Long (1923) stated that activated sludge consis--ted of zoogleal masses mixed with filamentous bacteria.

(iv) Harris et al (1927) found that 61% of the organisms in acti--vated sludge were of the Aerobacter aerogenes type andthe rest of the Proteus type. The results of the earlier workers seemed to show that coliforms and spore-formers predominated in activated sludge and thus played an important role in purification.

(v) Butterfield (1935) and Winogradsky (1937) isolated Zooglea forming organisms from activated sludge and the latter classified her strain as a Nitrocystis sp. But the former described his strain as Zooglea ramigera. After the isolation of this organism by Butterfield in 1935, this organism was considered as the most dominating organism in activated sludge until the middle of this century by several investigators (Heukelekian, Littman,Wattie, etc.) who described it as rod-shaped, motile with one polar flagellum, aerobic, non-spore forming, Gram negative, capsule forming producing ammonia from gelatin, scant growth on agar or gelatin but producing well organised flocs when aerated in sterile sewage.

From 1937 to 1943, Butterfield, Heukelekian, Wattie and coworkers studied the purification of sterile sewage using pure

cultures of Zooglea ramigera strains. Butterfield, Ruchhoft and Mcnamee (1937) found 50% BOD reduction after 5 hours aeration and 80% after 24 hours.

According to Heukelekian and Littman (1939), activated sludge consisted of flocculent masses of Zooglea ramigera.

(vi) Butterfield and Wattie (1941) suggested that the active organism concerned in purifying sewage in trickling filters and in activated sludge process was formed by Zooglea ramigera.

(vii) Allen (1944) showed that most of the organisms in activated sludge were proteolytic, Gram-negative, rod-shaped organisms belonging to the genera Achromobacter, Flavobacterium and Pseudomonas. Coliforms were found only in smaller numbers.

(viii) After the middle of this century, Mckinney and Horwood (1952), Mckinney and Weichlein (1953) isolated several organisms other than Zooglea ramigera and which had the ability to form cultures. They found Escherichia coli, E-intermedia, Paracolobacterium Aerogeno--ids, Nocardia actinomorpha, Bacillus cereus and a number of strains of the genera Pseudomonas, Alcaligenes and Flavobacterium to possess this capacity. These floc-forming bacteria reduced the BOD in waste water to 66-68% after 24 hours aeration.

Calaway et al (1952) showed that the distribution of predomi--nent species diffused with filter depth in the case of the sewage filtrate through sand, The upper 12" had the greatest number and widest distribution of species. 14 species were isolated from various levels. Flavobacterium and Bacillus were predominant throughout the filter and zoogleal bacteria were found in high numbers in the upper 12" of sand.

(ix) Fieldman (1955) stated that Zooglea-ramigera was the main bacterial species responsible for purification in trickling filters.

On account of the ability of the organism to flocculate and to stabilise polluting organic matter, it was universally considered asthe organisms primarily responsible for purification in activated sludge process.

(x) Jasewicz and Porges (1956) found 26% Alcaligenes, 34% flavobacterium, 14% micrococceus and 16% Pseudomonas in a dairy-waste activated sludge.

(xi) Duganand Lundgren (1960) isolated a Gram-negative rod with floc forming properties from activated sludge. This bacterium did not affect carbohydrates.

These investigators did not attempt to elucidate the mechanism of floc formation by the bacteria; and also about the role of the floc-forming bacteria in activated sludge formation under natural conditions and in stabilizing waste-water (van gil 1964)

(xii) Van gils (1964, p 38) found Pseudomonas to be a minor part of the predominant bacteria in sewage grown activated sludge. Members of the genera Achromobacter, Alcaligenes, flavobacterium were found to be the main constituents of the bacterial flora of such sludges. Most of the Gram-negative rod shaped strains isolated from the domes--tic types of activated sludge did not produce acid from glucose. A large part of these strains was not able to affect glucose at all, thus presumably belonged to the genera Alcaligenes and Lophomonas. The strains attacking glucose without production of acid apparently belonged to the genus Achromobacter or if they had yellow colonies to the genus flavobacterium.

A smaller number of Gram-negative rod shaped strains utilised glucose aerobically with production of acid; many of these strains had yellow colonies and probably were representatives of the genus Flavobacterium, while of the remainder a few had a positive

oxidase reaction test (Pseudomonas) and the others were considered to belong to the genus Achromobacter.

(j) Bacteria in action in high-rate aerobic ponds:

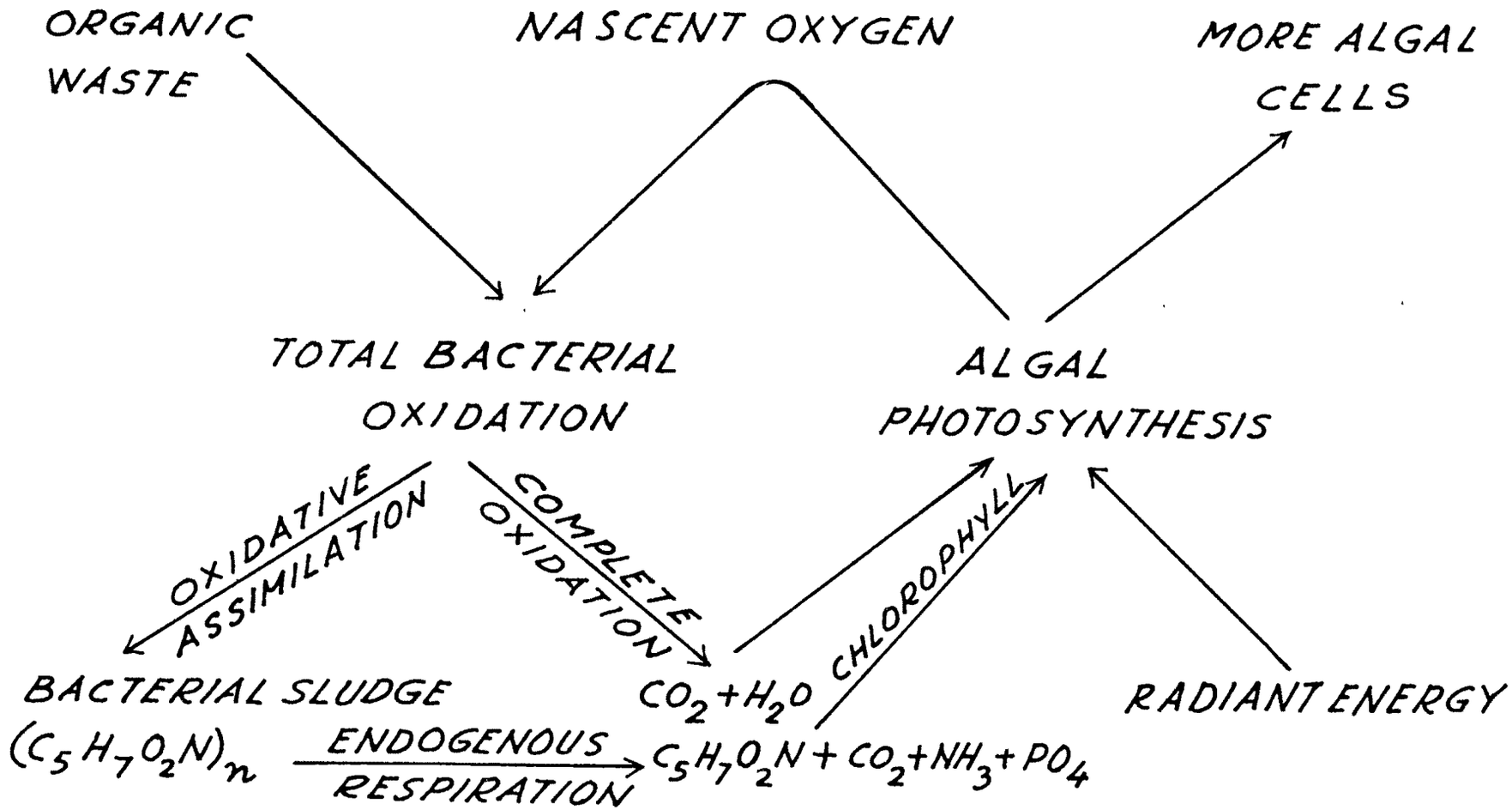
The microorganisms isolated and identified are listed in tables 7 to 10 and on pages 43 to 44. They are almost the same as those found in an activated sludge process.

(k) Basic facts about the metabolic reactions taking place in an activated sludge process:

The main object of aerobic biological waste treatment is the removal of organic substances from the waste water. This is achieved by two important metabolic processes taking place in the ecosystem. They are: (a) complete oxidation of a part of the organic substrates resulting in the formation of CO_2, H_2O and energy, and (b) biosynthesis and growth accompaning the decomposition of the remaining organic substrates, and newly formed cells are a major end-product of this intermediate metabolism. It is on account of the latter process that the activated sludge, process maintains and even increases itself (Symons and Mckinney, 1958). So a very striking feature of microbial metabolism in waste treatment systems generally is the relatively enormous amount of new bacterial cells which are normally produced during the oxidation of organic substrates.

One should therefore expect to find a heavy accumulation of bacterial sludge in the high-rate aerobic pond system also. But Oswald (1960, p. 384) has stated that a healthy sludge comparable to activated sludge is maintained in the pond and that following an initial accumulation, the volume of aerobic sludge does not increases but rather remains constant indicating that "total oxidation" is taking place. In our three laboratory batch culture experiments

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



with the Chlorella ~~three different types~~ of algae, there was no accumulation of sludge as in the classified activated sludge process but comparatively less brownish deposits were seen intermixed with algae when viewed under a microscope. Also, the formation of a constant volume of bacterial sludge as in Oswald's field ponds and very little sludge in our laboratory experiments is possible only if the system is operated on endogenous metabolism resulting in "total oxidation" of bacterial sludge.

Hoover et al (1952) found that activated sludge rapidly removed organic matter from solution and converted much of it into protoplasm which was degraded when all organic matter was removed. This fundamental concept was applied to dairy waste treatment by Porges (1960) who concluded from his exhaustive studies that it should be theoretically possible to arrange conditions so as to maintain a balanced system in which sludge or bacterial cells did not accumulate. All that would be required are sufficient nutrients to produce enough cells to replace those being oxidized by endogenous respiration. He has added that this ideal state has been approached but not attained. But it would appear that this ideal state has been attained in Oswald's high-rate aerobic pond system by operati--onal details.

Very soon however, it was found that it was impossible to "burn up" activated sludge completely by aeration. In the ideal case the reduction in mass of activated sludge by aerobic digestion balances the growth of new sludge so that no surplus sludge is left for disposel. From the experience in U.S.A. it is known that "total oxidation" of sludge cannot be achieved since there is always a fraction which is inert and which cannot be broken down further by aeration. Kountz and Forney (1959) and Washington and Symons (1962) found the non-degradable portion remaining to be about 20% of the maximum mass of microorganisms found or 11 to 15% of the ultimate

BOD_5 removed. Mowhorter and Heukelekian (1964) reported the inert matter to be 12% of the initial, COD and Washington and Hetling (1965) to be about 10% of the COD consumed. So the "constant volume" of sludge reported by Oswald (1960 p 384) in the high-rate aerobic pond may consist essentially of inert matter and active cells. Further work is necessary to determine the nature of its biochemical constituents.

It would therefore, seem that the high-rate aerobic oxidation pond system is operated on endogenous metabolism resulting in total oxidation and therefore one would also expect to find entirely different types of organisms during its assimila-tion and endogenous phases. In fact Jasewicz and Porges (1956) and Porges (1960) have made a complete survey of the bacteria in action in a dairy waste activated sludge. They found the presence of Pseudomonas and Achromobacteriaceal when the sludge was in endogenous phase and the presence of Bacillus and Bacterium in its assimilation phase. However, these results were not confirmed by Admse (1968) in his systematic and equally through investigati-on of the bacterial flora of a similar dairy waste activated sludge. He found no significant difference in composition of the activated sludge bacterial flora before and after feeding. But in our own case also, no significant difference in composition of the bacterial flora in the two phases are found, and conditions favouring endogenous metabolism also exist in the high-rate aerobic ponds.

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" WHY TOTAL OXIDATION IS IMPOSSIBLE IN HIGH-RATE OXIDATION PONDS"?