CHAPTER - IV

R E S U L T S

Performance characteristics of the three laboratory experiments:

Three series of experiments were carried out. In the first series, the alga used was Nostoc pyriformis; in the second Anabaena cylindrica and in the third, mixed algae consisting of Chlorella vulgaris, Scenedesmus obliqus, Oscillatoria chalybea and Oscillatoria obscura in almost equal proportions. In the first two series of experiments, the samples were tested only for physico-chemical conditions on zero, second, fourth and sixth day; while in the third series of experiments dealing with the mixed algae, the samples were tested for physico-chemical, biochemical and biological conditions on zero, second, fourth and sixth day. The analyses were done in duplicate and the average of the results are given in the tabular statements (Appendix).

Physical variables

Temperature, colour and pH were determined in all the three series of experiments. The results are shown in the Tables 1, 2 and 3 (Appendix).

Temperature:

In the first series of experiment, the temperature of the liquid varied between 29.7 to 29.9°C, in the second series, between 28.9 to 29.1°C; and in the third series, between 28.4 to 28.6°C.

Colour:

The colour remained brownish in all the control flasks. For the algae-treated flasks the brown colour of the sewage changed to bluish-green from second day onwards in case of Nostoc and Anabaena, but it changed to predominantly greenish in case of mixed algae. On the sixth day the green and bluish-green colour became intense due to maximum algal growth.

pH :

The pH changes in all the flasks have been summarised in a tabular form below:

Detention periods	Control (raw sewage) pH	Control (raw sewage) difference over zero day	Algae-treated pH	Algae-treated difference over zero day
Nostoc pyriformis (first series)				
0 day	7.3	-	7.3	-
2 days	7.4	+ 0.1	8.5	+ 1.2
4 days	7.2	- 0.1	8.9	+ 1.6
6 days	7.4	+ 0.1	9.8	+ 2.5

Detention periods	Control (raw sewage) pH	difference over zero day	Algae-treated pH	difference over zero day
Anabaena cylindrica (second series)				
0 day	7.2	-	7.2	-
2 days	7.4	+ 0.2	8.4	+ 1.2
4 days	7.4	+ 0.2	8.9	+ 1.7
6 days	7.3	+ 0.1	9.8	+ 2.6
Mixed algae (third series)				
0 day	7.5	-	7.5	-
2 days	7.6	+ 0.1	8.8	+ 1.3
4 days	7.6	+ 0.1	9.4	+ 1.9
6 days	7.8	+ 0.3	10.0	+ 2.5

From a study of the above table it will be seen that there was a slight change of pH in the control flasks. But it was found to increase in the algae treated samples from the second day onwards to about 10 on the sixth day for all the three algal samples. The increase has to be attributed mainly to phenolphthalein alkalinity resulting from photosynthetic activities of the algae.

Chemical variables

Phenolphthalein alkalinity, ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, orthophosphate, BOD_5 and COD were determined in all the three series of experiments. The results are shown in the Tables 1,2 and 3 (Appendix).

Phenolphthalein alkalinity:

Phenolphthalein alkalinity in all the flasks have been summarised below:

Detention period	Control		Algae-treated	
	Phenol-phthalein alkalinity (mg/l)	increase over zero day	Phenol-phthalein alkalinity (mg/l)	increase over zero day
Nostoc pyriformis				
0 day	28	-	28	-
2 days	36	8	65	37
4 days	40	12	78	50
6 days	42	14	90	62
Anabaena cylindrica				
0 day	36	-	36	-
2 days	52	16	72	36
4 days	61	25	84	48
6 days	65	29	98	62
Mixed algae				
0 day	26	-	26	-
2 days	35	9	81	55
4 days	40	14	92	66
6 days	45	19	106	80

It will be seen from the above table that comparatively less increase in the phenolphthalein alkalinity was found in the control flasks. But in case of the algal flasks it

shot up on the second day and gradually increased upto the sixth day. This shows that the carbon dioxide from the bicarbonates was used up for algal photosynthesis resulting in increase in phenolphthalein alkalinity and in pH.

Ammonia nitrogen:

The amounts of ammonia nitrogen present in all the flasks have been summarised below:

Detention period	Control		Algae-treated	
	Am-N (mg/l)	% of reduction	Am-N (mg/l)	% of reduction
Nostoc pyriformis				
0 day	36.4	-	36.4	-
2 days	35.0	3.9	9.9	72.8
4 days	34.0	6.6	5.4	85.2
6 days	32.9	9.6	3.0	91.8
Anabaena cylindrica				
0 day	34.0	-	34.0	-
2 days	36.2	-6.5	8.2	75.9
4 days	31.8	6.5	6.4	81.2
6 days	30.4	10.6	2.7	92.1
Mixed algae				
0 day	36.0	-	36.0	-
2 days	26.8	25.6	8.0	77.8
4 days	26.4	26.7	5.9	83.6
6 days	21.4	40.6	2.2	93.9

There was comparatively less reduction in the control flasks. But there was a sharp fall in the case of all the algal samples. 73 to 78% of reduction in algal flasks have been found within 2 days and 90 to 94% reduction within 6 days.

The data indicate that ammonia nitrogen was used as a nutrient for the algal growth and no specificity was found for different algae.

Nitrite and nitrate nitrogen:

A trace of nitrite and nitrate was found in the sewage and no significant changes were found for the control and algal flasks.

Orthophosphate:

The amounts of phosphate present in the control and in the algal flasks have been summarised below:

Detention period	Control		Algae-treated	
	PO_4 (mg/l)	% of reduction	PO_4 (mg/l)	% of reduction
Nostoc pyriformis				
0 day	19.5	-	19.5	-
2 days	17.8	8.7	7.4	62.1
4 days	18.4	5.6	6.0	69.2
6 days	20.0	-2.6	5.7	70.8

Detention period	Control		Algae-treated	
	PO_4 (mg/l)	% of reduction	PO_4 (mg/l)	% of reduction
Anabaena cylindrica				
0 day	16.0	-	16.0	-
2 days	14.3	10.6	5.3	66.9
4 days	12.8	20.0	1.4	91.3
6 days	14.0	12.5	1.0	93.8
Mixed algae				
0 day	16.8	-	16.8	-
2 days	16.1	4.2	5.7	66.1
4 days	12.8	23.8	3.6	78.6
6 days	14.0	16.7	2.7	83.9

No appreciable change was found in the control flasks. But algal flasks showed 62 to 67% decrease within 2 days 71 to 94% within 6 days, indicating that orthophosphates from sewage were utilized as a nutrient for algal growth.

BOD_5 at 20°C:

The results of control and algal flasks have been summarised below:

Detention period	Control		Algae-treated	
	BOD_5 (mg/l)	% of reduction	BOD_5 (mg/l)	% of reduction
Nostoc pyriformis				
0 day	197	-	197	-
2 days	114	42.1	56	71.6
4 days	102	48.2	44	77.7
6 days	61	69.0	26	86.8

Detention period	Control		Algae-treated	
	BOD_5 (mg/l)	% of reduction	BOD_5 (mg/l)	% of reduction
Anabaena cylindrica				
0 day	153	-	153	-
2 days	108	29.4	43	71.9
4 days	85	44.4	25	83.7
6 days	64	58.2	12	92.2
Mixed algae				
0 day	160	-	160	-
2 days	104	35.0	68	57.5
4 days	78	51.3	26	83.8
6 days	65	59.4	21	86.9

60 to 70% of reduction in BOD_5 was found in the control flasks within 6 days. But the same amount of reduction was found in the algal flasks within 2 days and 87 to 92% within six days.

This shows that organic matter from sewage influents has been more rapidly removed in algae treated flasks to a greater extent than in the control flasks.

COD:

The results of the control and algal flasks have been summarised below:

Detention period	Control		Algae-treated	
	COD (mg/l)	% of reduction	COD (mg/l)	% of reduction
Nostoc pyriformis				
0 day	315	-	315	-
2 days	230	27.0	90	71.4
4 days	164	47.9	69	78.1
6 days	97	69.2	42	86.7
Anabaena cylindrica				
0 day	240	-	240	-
2 days	180	25.0	68	71.7
4 days	125	47.9	39	83.8
6 days	100	58.3	18	92.5
Mixed algae				
0 day	264	-	264	-
2 days	156	40.9	102	61.4
4 days	110	58.3	44	83.3
6 days	104	60.6	40	84.8

60 to 70% reduction of COD was found in the control flasks within 6 days and the same reduction was found in algal flasks within 2 days and 85 to 93% within 6 days.

The results confirm that degradable organic matter is rapidly removed but to a greater extent in algal-bacterial symbiosis.

60 to 70% reduction of COD and BOD_5 in the control flasks has to be attributed to the phenomena of mechanical

flocculation, bioflocculation and bioprecipitation which are of common occurence in nature (Huekelekian, 1941) and to comparatively slower surface reaeration phenomena. In the case of the algal samples the greater percentage reduction has to be ascribed to photosynthetic oxygen furnished quickly to bacteria as a result of algal photosynthesis in the growth cultures.

Biochemical variables

The estimations of protein, amino acid nitrogen, sugars and volatile acids were done only in one experiment dealing with the mixed algae. The results are shown in the Table -4 (Appendix).

Protein:

The amounts of protein present in the control and algal flasks have been summarised below:

Detention period	Control		Algae-treated	
	Protein (mg/l)	% of reduction	Protein (mg/l)	% of reduction
0 day	6.8	-	6.6	-
2 days	5.4	20.6	4.5	31.8
4 days	3.6	47.1	2.8	57.6
6 days	2.3	66.2	1.5	77.3

The values for protein decreased in both the control and algal flasks. But the degree of decrease varied. In the control flask about 66% decrease was found within 6 days and in the algae-treated flasks nearly 77% decrease. Thus the latter showed a higher percentage of decrease. The amounts of protein used up and remaining in the ecosystem for different detention periods are diagramatically represented in Fig. 2. The two curves run almost opposite to each other.

Amino-acid nitrogen:

The amounts of amino-acid nitrogen present in the control and in the algal flasks have been summarised below:

Detention period	Control		Algae-treated	
	Amino-N (mg/l)	% of reduction	Amino-N (mg/l)	% of reduction
0 day	5.7	-	5.6	-
2 days	4.7	17.5	4.5	19.6
4 days	3.5	38.6	2.3	58.9
6 days	1.1	80.7	0.7	87.5

The data show a gradual decrease and a higher percentage of decrease in algae treated flasks. The control flasks showed 81% decrease while the algae-treated flasks

Fig. 2

PROTEIN USED UP AND REMAINING IN THE ECOSYSTEM WITH THE MIXED ALGAE

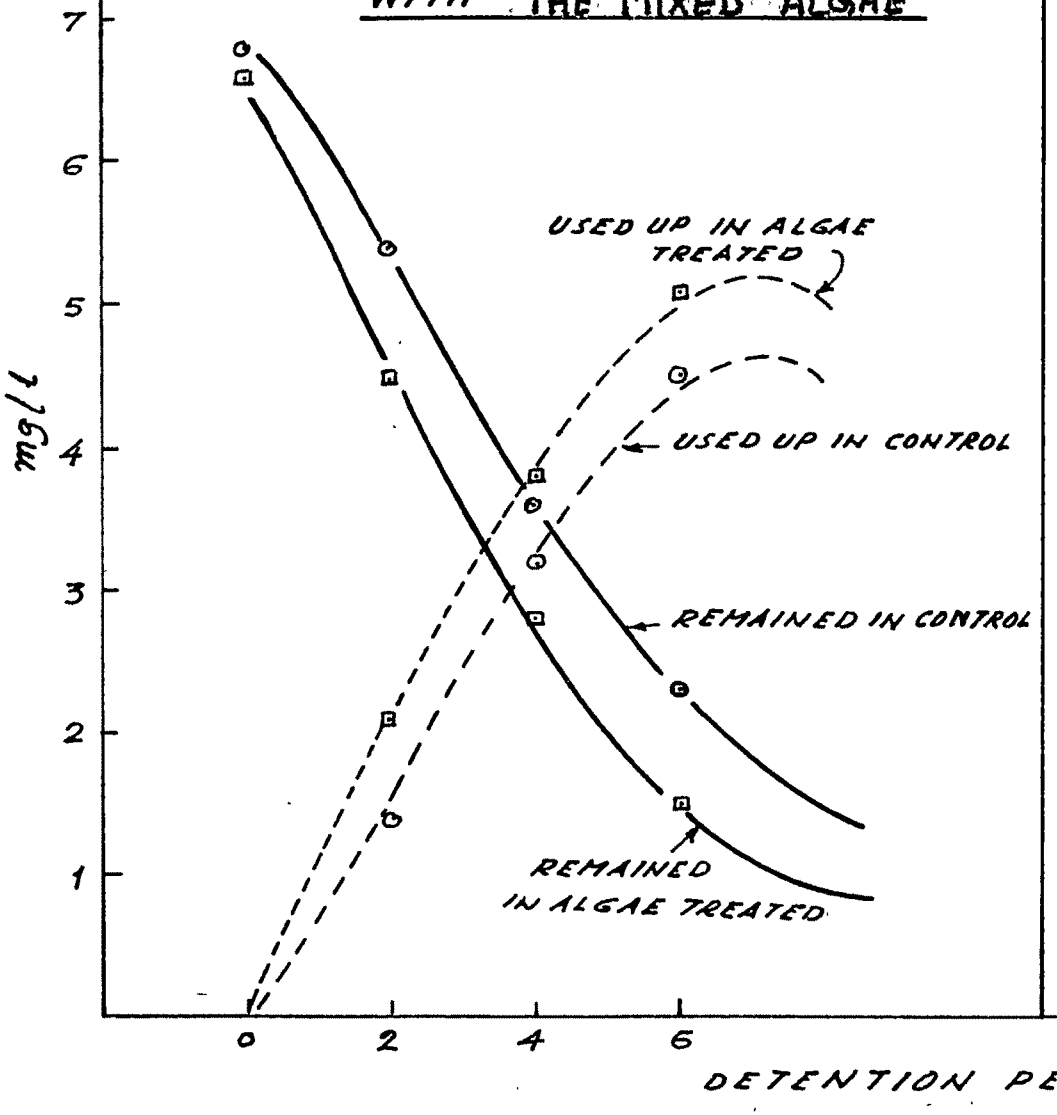
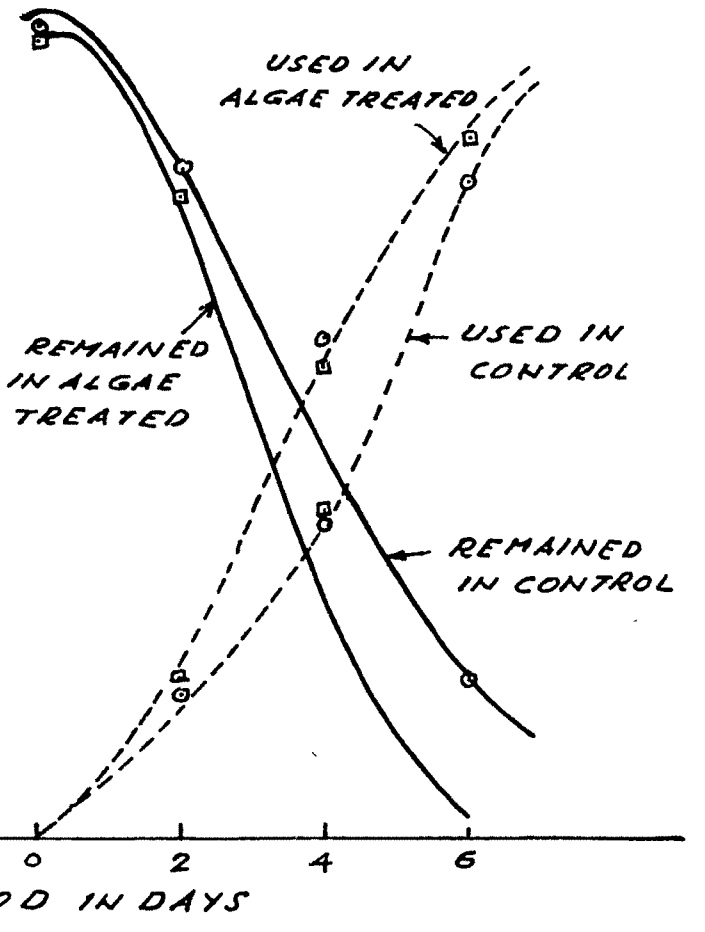


Fig. 3

AMINO NITROGEN USED UP AND REMAINING IN THE ECOSYSTEM WITH THE MIXED ALGAE



DETENTION PERIOD IN DAYS

88% decrease within 6 days. These results are shown in Fig. 3.

Total sugar:

The amounts of total sugar present in the control and in the algal flasks have been sumarrised below in a tabular form.

Detention period	Control		Algae-treated	
	Total sugar (mg/l)	% of reduction	Total sugar (mg/l)	% of reduction
0 day	80.0	-	83.9	-
2 days	61.8	22.8	56.0	33.3
4 days	41.2	48.5	30.0	64.2
6 days	29.2	63.5	16.3	80.6

In this case also both the cultures showed a gradual decrease but a higher percentage of decrease in the algae treated flasks. The control flasks showed 64% decrease and the algae-treated 81% decrease within 6 days. The amounts of total sugar used up and remaining in the ecosystem for the different detention periods are shown in Fig. 4.

Free sugar:

The amounts of free sugar present in the control and

Fig. 4

TOTAL SUGAR USED UP AND REMAINING IN THE ECOSYSTEM WITH THE MIXED ALGAE

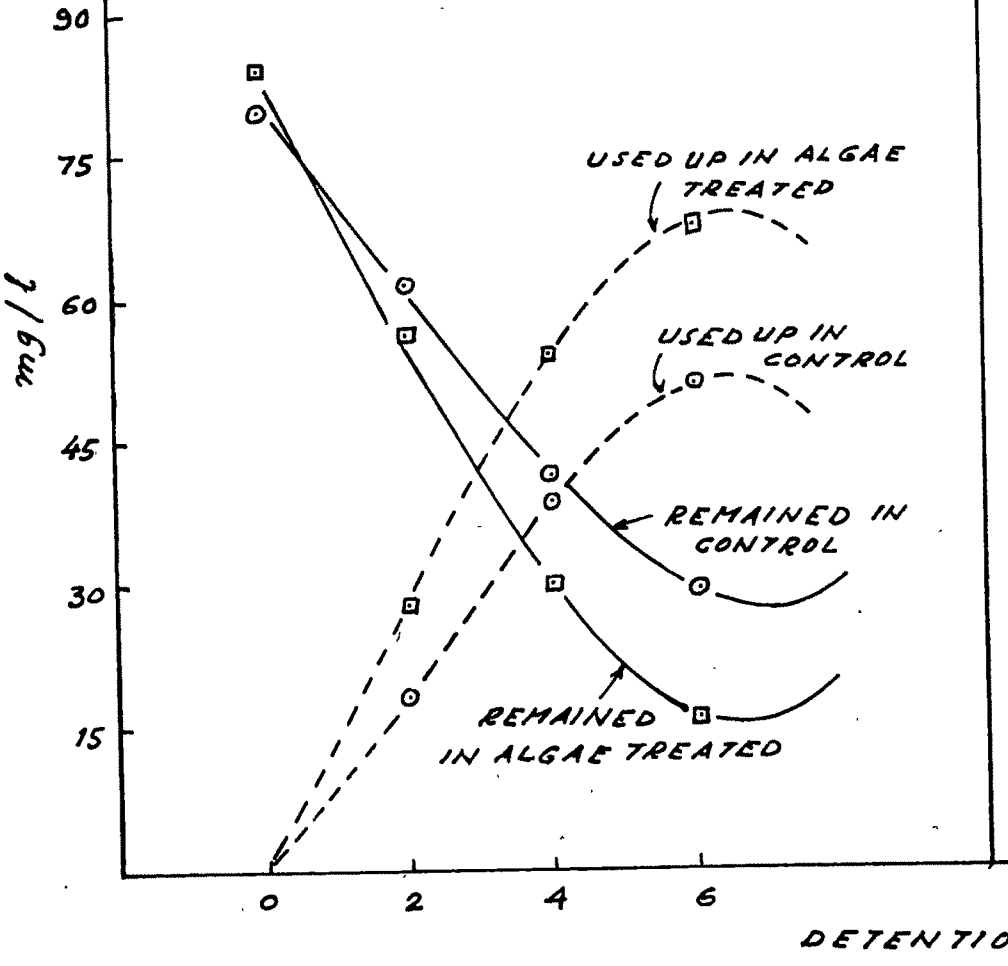
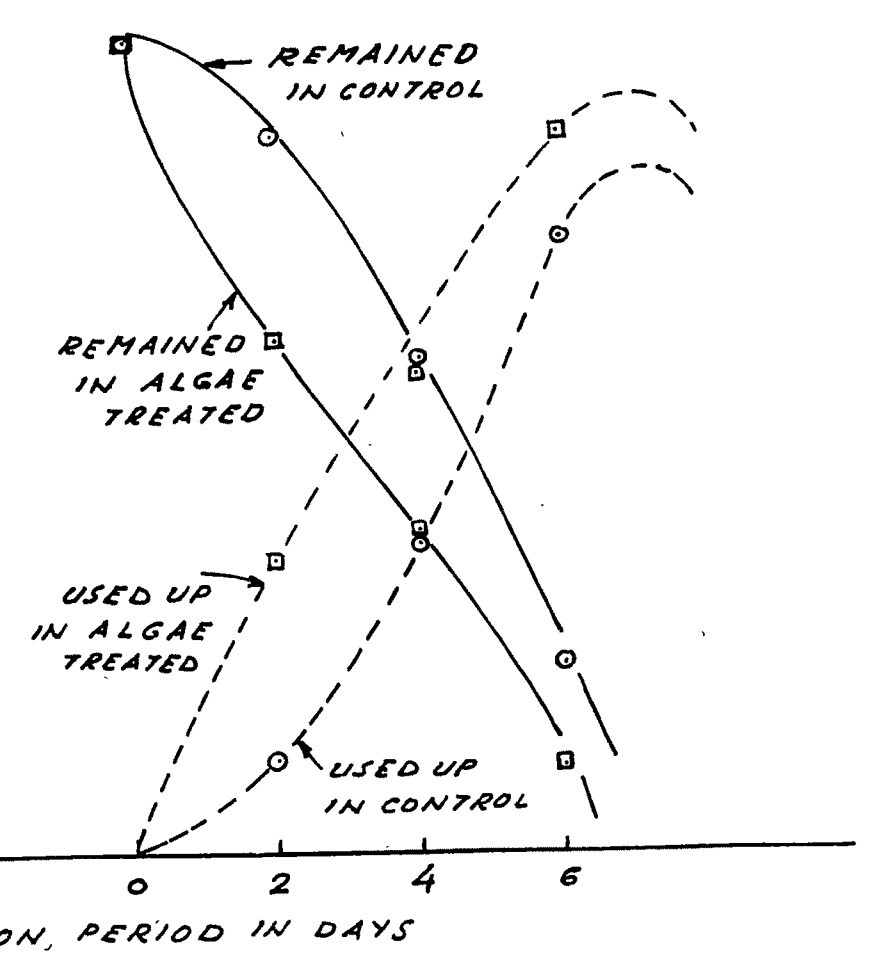


Fig. 5

FREE SUGAR USED UP AND REMAINING IN THE ECOSYSTEM WITH THE MIXED ALGAE



in the algal flasks have been summarised below:

Detention period	Control		Algae-treated	
	Free sugar (mg/l)	% of reduction	Free sugar (mg/l)	% of reduction
0 day	28.2	-	28.2	-
2 days	25.0	11.3	18.0	36.2
4 days	17.2	39.0	11.2	60.3
6 days	6.6	76.6	2.9	89.7

A gradual decrease and a higher percentage of decrease in the algal flasks were indicated in this case also. The control showed 77%, while the algae treated 90% decrease within 6 days. This has been clearly demonstrated in Fig. 5 with respect to the amount used up and remaining in the ecosystem.

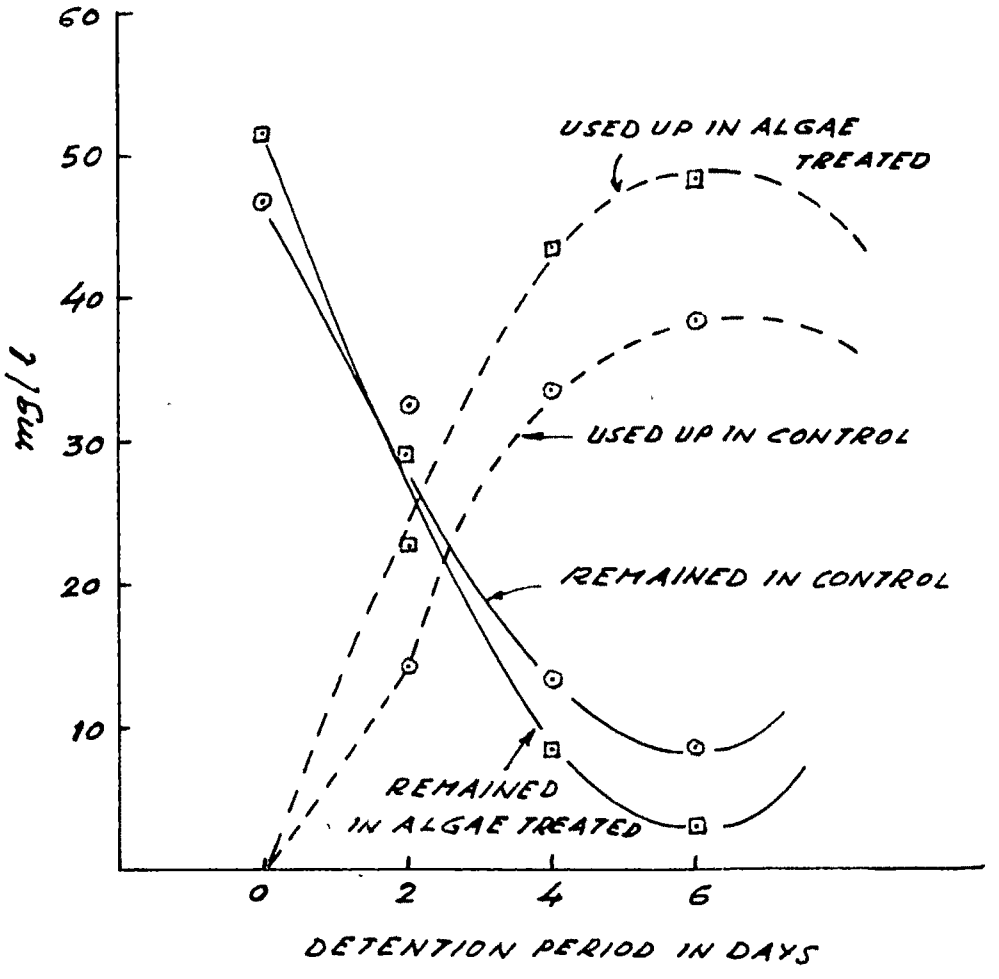
Volatile acids (lower fatty acids):

The amounts of volatile acids present in the control and in the algae treated flasks have been summarised below:

Detention period	Control		Algae-treated	
	Volatile acids (mg/l)	% of reduction	Volatile acids (mg/l)	% of reduction
0 day	46.8	-	51.6	-
2 days	32.5	30.6	29.0	43.8
4 days	13.2	71.8	8.2	84.1
6 days	8.4	82.1	3.1	94.0

Fig. 6

VOLATILE ACIDS USED UP AND REMAINING IN THE ECOSYSTEM WITH THE MIXED ALGAE



Again the reactions were similar. The control showed 82% decreased and the algae-treated 94% decrease within 6 days. The amounts used up and remaining in the ecosystem for different detention periods are shown in Fig. 6.

In short, the reduction of biochemical variables like protein, amino nitrogen, sugars and volatile acids in the control has to be attributed to metabolic activities of bacteria. Surface reaeration a comparatively slow process has helped in providing oxygen to bacteria for degradation of organic matter. But the higher percentage of decrease in the algal-treated samples has to be attributed to the greater and more intimate availability of photosynthetic oxygen than reaeration.

Biological changes

Algal dry weights were estimated in all the three experiments for each detention period. The results are shown in the Tables 1, 2, 3 and 5 (Appendix). It will be seen from the tables that algal dry weight on second day ranged between 199 to 208 ppm. Most of the algae developed within two days. After two days the increase in the production of algae was not much. On the sixth day algal dry weight ranged between 205 to 218 ppm.

Other biological changes were observed in only one experiment dealing with mixed algae. The results are shown in the Table - 5 (Appendix).

From a study of the table it will be seen that:

i. Zoogloeal growth was very rarely seen throughout the study period

ii. Dark brown to light brown filaments resembling Leptothrix ochracea (?) were always seen in both the cultures but in varying numbers on all days. (Photograph No.1).

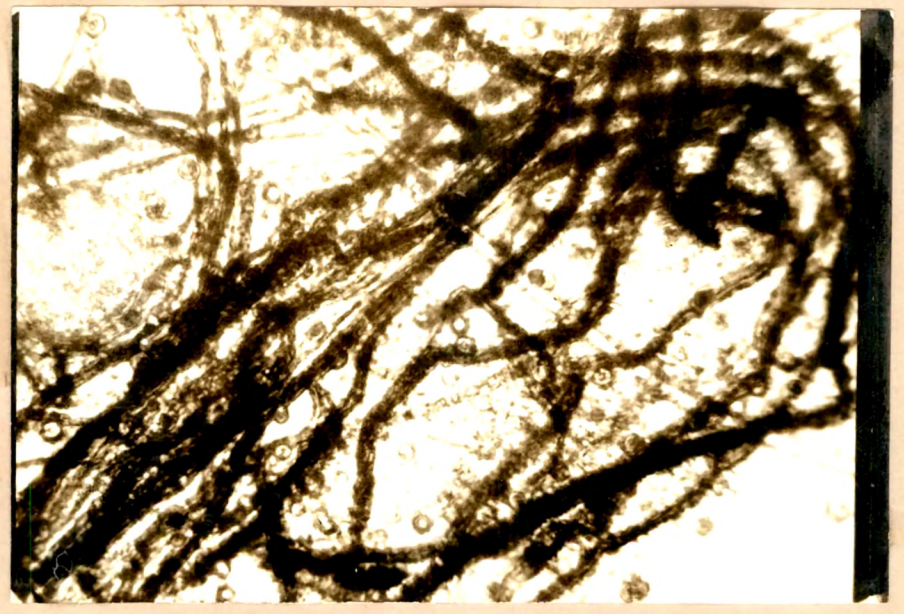
iii. Brownish flocculent precipitates were seen either at the bottom or suspended in the control flasks only and not in the algae-treated flasks where they were seen intermixed with the algal filaments but in considerably smaller numbers as organic debris.

iv. Paramoecium caudatum was seen only in the control flask on fourth day but it disappeared on the sixth day.

v. Spathidium spathula was seen in both the control and algae-treated flasks but in smaller numbers.

vi. Aspidisca costata was observed in both the culture flasks but in smaller numbers.

Photograph No. 1



Dark brown filaments resembling
Leptothrix ochracea

Photograph No. 2

Rotifer Lecane sp. in the algal Sample

vii. Vorticella globosa was seen only in the control flask in large numbers on fourth day but it disappeared on sixth day.

viii. Vorticella spp. were most common only in the control flask.

ix. The rotifer, 'Lecane sp' was abundantly found only in the algal flask. (Photograph No.2).

x. Both in control and the algal flasks were practically free from Annelid worms excepting for stray (rrr) occurence in the latter case.

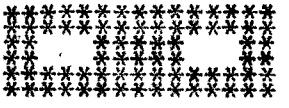
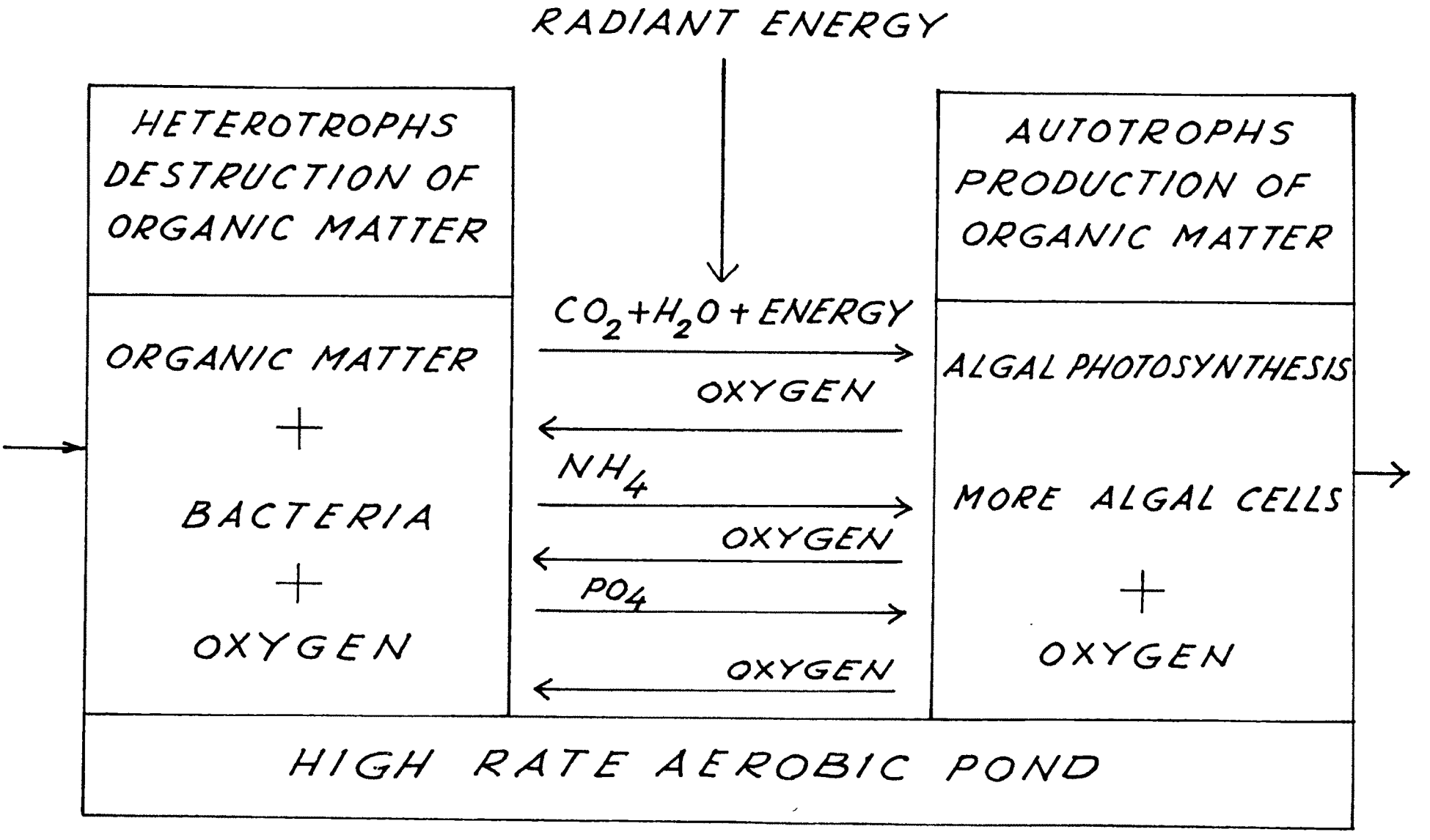


Fig. 7


RADIANT ENERGY
HETEROTROPHS DESTRUCTION OF ORGANIC MATTER
AUTOTROPHS PRODUCTION OF ORGANIC MATTER
ORGANIC MATTER
+
BACTERIA
+
OXYGEN
$CO_2 + H_2O$ + ENERGY
OXYGEN
NH_4
OXYGEN
PO_4
OXYGEN
ALGAL PHOTOSYNTHESIS
MORE ALGAL CELLS
+
OXYGEN
HIGH RATE AEROBIC POND