CHAPTER - IV

EXPERIMENTAL RESULTS

Performance characteristics of the three laboratory experiments:

Three series of experiments were carried out. In the first series, the alga used was Scenedesmus obliqus; in the second Microcystis aeruginosa and in the third, mixed algae consisting of thick and thin varieties of Oscillatoria, Spirulina and Microcystis. In the first series of experiment, the samples were tested for physico-chemical, biological and bacteriological conditions on zero, second, fourth and sixth day of detention period. In the second series of experiment, the samples were tested for physico-chemical conditions and algal biomass while in the third series of experiment dealing with the mixed algae, the samples were tested only for biochemical tests on zero, second, fourth and sixth day detention period. The analyses were done in duplicate and the average of the results are given in the tabular statements (Appendix).

a) Physical conditions : (vide Tables 1 and 2, Appendix)

i) Colour :

The colour remained brownish in all the control flasks containing only raw sewage, while in the algae treated flasks the brown colour of the sewage changed to green from second day onwards in case of Scenedesmus obliqus and blue green in the case of Microcystis aeruginosa. On the sixth day the green and blue-green colour became intense due to maximum algal growth.

ii) Temperature:

The temperature of water varied between 27.5 to 29.5°C in control and algal treated flasks.

iii) pH :

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

Detention time	Control (raw sewage)		Algae-treated	
	pH	Difference over zero day	pH	Difference over zero day
Scenedesmus obliqus				
0 day	7.85	-	7.85	-
2 days	7.9	+0.05	8.1	+0.25
4 days	8.0	+0.15	8.4	+0.55
6 days	8.3	+0.45	9.2	+1.35

..... contd.

Detention time	Control (raw sewage) pH	Difference over zero day	Algae-treated pH	Difference over zero day
Microcystis aeruginosa				
0 day	7.8	-	7.8	-
2 days	7.85	+0.05	8.3	+0.5
4 days	8.0	+0.2	8.8	+1.0
6 days	8.2	+0.4	9.8	+2.0

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 9.2 to 9.8 on the sixth day for both the algal samples. The increase has to be attributed mainly to phenolphthalein alkalinity resulting from photosynthetic activities of the algae.

b) Chemical conditions : (Vide Tables 1 and 2 Appendix)

Phenolphthalein alkalinity, ammonia nitrogen, phosphate, BOD_5 and COD were determined in the two series of experiments. The results are shown in the Tables 1 and 2 (Appendix).

i) Phenolphthalein alkalinity:

Phenolphthalein alkalinity in all the flasks have been summarised below:

Detention period	Control(raw sewage)		Algae-treated	
	Phenolphthalein alkalinity (mg/l)	Increase over zero day	Phenolphthalein alkalinity (mg/l)	Increase over zero day
Scenedesmus obliqus:				
0 day	25	-	25	-
2 days	35	10	50	25
4 days	40	15	65	40
6 days	42	17	80	55
Microcystis aeruginosa				
0 day	15	-	15	-
2 days	26	11	38	23
4 days	30	15	62	47
6 days	33	18	84	69

It will be seen from the above table that comparatively less increase in the phenolphthalein alkalinity was found in the control flasks. But in the case of algal treated flasks it shot up on the second day and gradually increased upto the sixth day. This shows that the CO_2 from the bicarbonates was

used up for algal photosynthesis resulting in increase in phenolphthalein alkalinity and in pH.

ii) Ammonia nitrogen:

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

Detention period	Control(raw sewage)		Algae-treated	
	Ammonia-nitrogen (mg/l)	% of reduction	Ammonia-nitrogen (mg/l)	% of reduction
Scenedesmus obliqus				
0 day	28.8	-	31.8	-
2 days	11.8	59	4.9	84.6
4 days	9.0	68.8	4.1	87.0
6 days	5.6	80.6	3.5	90.0
Microcystis aeruginosa:				
0 day	33.0	-	33	-
2 days	30.0	9.1	11.4	65.4
4 days	28.6	13.3	7.2	78.2
6 days	26.4	20.0	4.4	86.6

There was comparatively less reduction in the control flasks. 80.6 and 20% of reduction in control flasks have been found after 6 days. But there was a

sharp fall in the case of algal treated flasks. 65.4 to 84.6% of reduction in algal treated flasks, have been found within 2 days and 86.6 to 90.0% within 6 days.

The data indicate that ammonia nitrogen was used as a nutrient for the algal growth.

iii) Phosphate :

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

Detention period	Control(raw sewage) Phosphate (mg/l)	% of reduction	Algae-treated Phosphate (mg/l)	% of reduction
Scenedesmus obliqus				
0 day	12.5	-	12.5	-
2 days	15.7	-25.6	4.1	67.2
4 days	14.8	-18.4	2.6	79.2
6 days	14.0	-12.0	2.1	83.2
Microcystis aeruginosa				
0 day	17.5	-	17.5	-
2 days	16.0	8.6	10.0	42.9
4 days	14.0	20.0	8.9	49.1
6 days	13.5	22.9	6.8	61.1

No appreciable change was found in the control flasks. But algal treated flasks showed 42.9 to 67.2% of reduction within 2 days and 61.1 to 83.2% within 6 days indicating that phosphates from sewage were utilized as a nutrient for algal growth and also lost as precipitates on account of higher pH.

iv) BOD_5 at 25°C:

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

Detention period	Control (raw sewage) BOD_5 (mg/l)	% of reduction	Algae-treated BOD_5 (mg/l)	% of reduction
Scenedesmus obliqus				
0 day	280	-	280	-
2 days	191	31.8	67	76.1
4 days	125	55.4	43	84.6
6 days	83	70.4	35	87.5
Microcystis aeruginosa				
0 day	196	-	196	-
2 days	156	20.4	70	64.3
4 days	124	36.7	42	78.6
6 days	98	50.0	18	90.8

50.0 to 70.4% reduction in BOD_5 was found in the control flasks within 6 days. But more than this

amount of reduction (64.3 to 76.1%) was found in the algal flasks within 2 days and 87.5 to 90.8% within six days.

The results show that organic matter from sewage has been used up more rapidly in the algal flasks than in the controls.

v) COD :

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

Detention period	Control (raw sewage) COD (mg/l)	% of reduction	Algae treated COD (mg/l)	% of reduction
Scenedesmus obliqus				
0 day	372	-	372	-
2 days	294	21.0	96	74.2
4 days	200	46.2	72	80.6
6 days	132	64.5	56	84.9
Microcystis aeruginosa				
0 day	336	-	336	-
2 days	250	25.6	106	68.5
4 days	198	44.0	66	80.4
6 days	156	53.6	30	91.1

53.6 to 64.5% reduction of COD was found in the control flasks within 6 days. But more than this

amount of reduction (68.5 to 74.2%) was found in algal flasks within 2 days and 84.9 to 91.1% within 6 days.

The results confirm that biodegradable organic matter is used up to a greater extent during algal-bacterial symbiosis.

50 to 70% reduction of COD and BOD_5 in the control flasks has to be attributed to the phenomena of mechanical flocculations, bioflocculation and bioprecipitation which are of common occurrence in nature (Heukelekian, 1941) and also to the comparatively slower surface reaeration phenomenon. But in the case of the algal flasks 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.

c) Biological conditions: (vide Table 3, Appendix):

Algal dry weights were estimated in the two series of experiments for each detention period. The results are shown in the Tables 1 and 2 (Appendix). It will be seen from the tables that

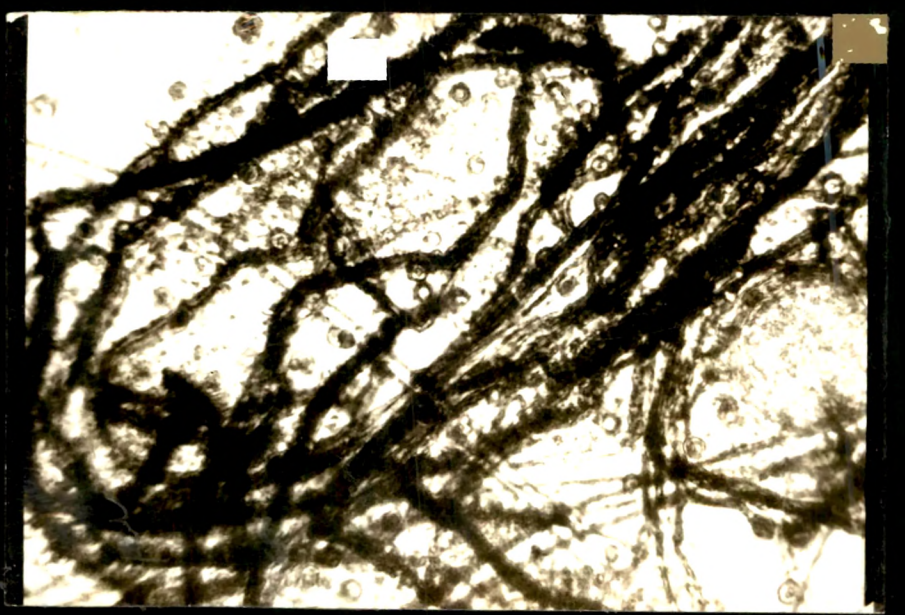


Fig. A: Dark brown filaments resembling Leptothrix ochracea.

algal dry weight on second day ranged between 224 to 275 mg/l. 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 236 to 282 mg/l.

Other biological changes were observed in the first series of experiment dealing with Scenedesmus obliqus. The results are shown in the Table 3 (Appendix).

It would be seen from a study of the table that:

i) dark brown to light brown filaments resembling the iron bacterium Leptothrix ochracea were always seen in both the cultures but in varying numbers on all the days. (Fig. A).

ii) 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.

Fig. B: Rotifer Lucane Spp. in the algal sample.

iii) Paramoecium caudatum was seen in both the control and algal-treated flasks but in larger numbers in the former case.

iv) Spathidium spathula was seen in smaller numbers in the control flasks only, as well as Paramoecium spp.

v) Aspidisca costata was seen in both the culture flasks but in comparatively larger numbers in the algae-treated flasks.

vi) Vorticella spp. were seen mostly in the control flasks and fewer in the algae-treated flasks.

vii) The rotifer "Lucane spp. was characteristic of algae-treated flasks. They showed brownish flocculant precipitates inside their bodies and on some occasions greenish algal growths indicating that they were living upon them. (Fig. B)

viii) Algae were dominant in the algae-treated flasks.

(d) Bacteriological results: (vide Tables 5 to 8, Appendix).

Coliforms and total colonies count show 99.9% reduction on the 6th day. Still the number of bacterial flora of heterotrophic, non-photosynthetic type are present in fairly larger number. An attempt at classifying and typing the organisms present on different detention periods has been made.

Table - 5 shows the distribution of a few important properties of the dominant bacterial isolates in the experiment using the Scenedesmus alga. The predominant isolates were all rods, mostly whitish, and Gram-negative.

Citrate utilizers on the zero and second day of detention period, were almost double the number of those recorded for the 4th and 6th day.

The pattern of distribution of starch hydrolysers on the 4th and 6th day were double the number of those recorded on 0 and 2nd day of the detention period.

There was a striking fact in the case of gelatin hydrolysers which were nearly the same on 0 and 2nd day and 'nil' on 4th and 6th day.

The distribution of tributyrin hydrolysers was also similar to protein hydrolysers.

All the 200 isolates were catalase positive and also contained the reserve food materials used in endogenous respiration like glycogen, lipid inclusions, and volutin.

In Table - 6 are shown some of the important biochemical characteristics of the dominant bacteria as revealed in the experiment. The percentages of isolates showing "acid" or "acid and gas" on the 0 and 2nd day were almost similar, but the numbers were reduced to nearly one-half on the 4th and 6th day of detention. Nitrate reductions were nearly similar on all the days; and those showing H_2S production were comparatively lower. The positive percentages in Hugh and Leifson's glucose medium showing "oxidative" reaction on 0 and 2nd day were almost double those showing "fermentative" reaction on the 4th and 6th day.

The generic identities of the dominant bacteria are shown in Table - 7 and they are tentatively classified as "assimilating" and "endogenous" in Table- 8 based on the detention periods and on the availability of food materials. From a consideration

of the species in the eco-system, it is reasonable to assume that the species present in superabundance are the most active in the ecosystem(Hungate, 1962). Significant difference in composition of the bacterial flora can be found as will be evident from those recorded from the 2nd, 4th and 6th day below:

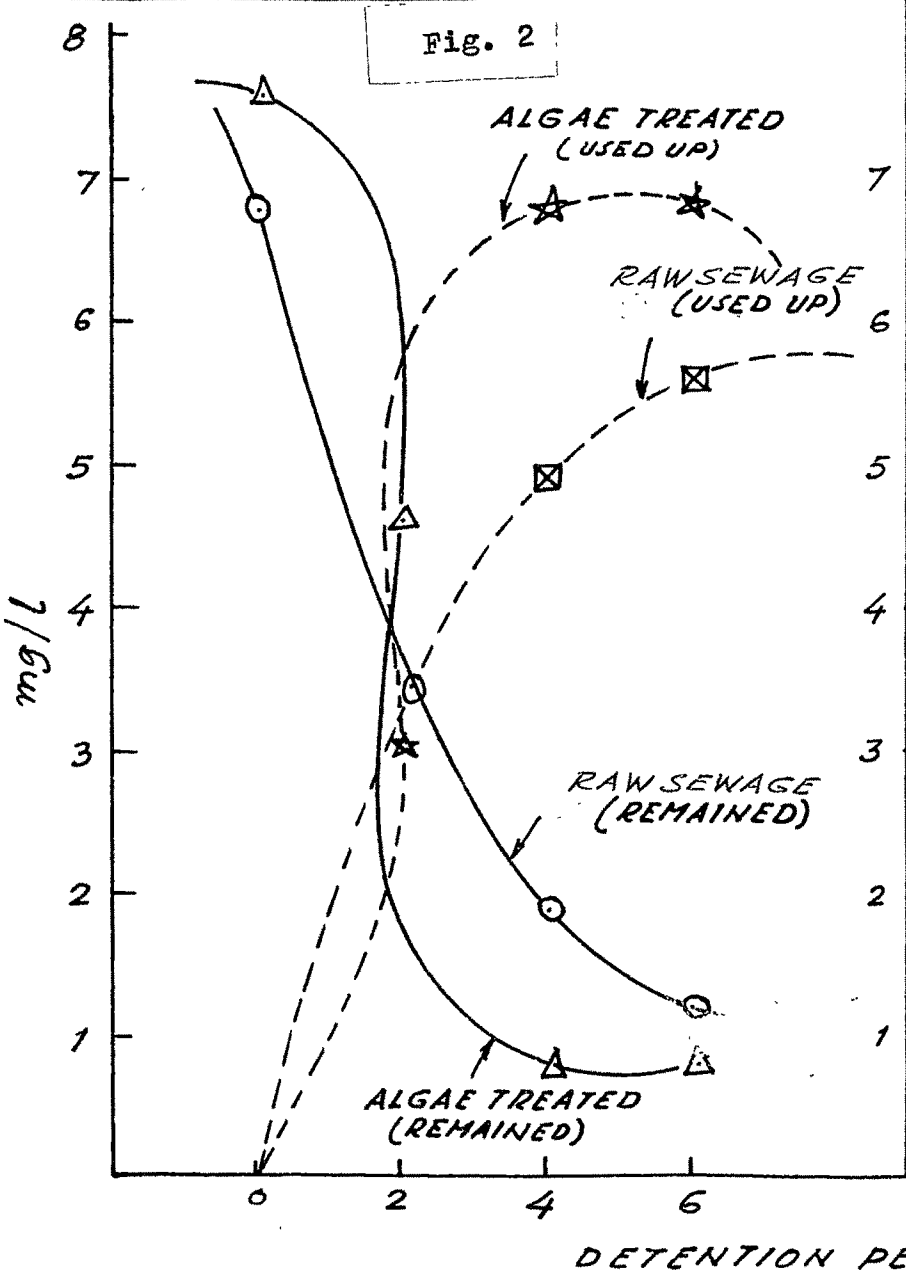
2nd day	4th day	6th day
Achromobacter	Aeromonas	Aerobacter
Aeromonas	Alcaligens	Alcaligens
Bascillus	Comamonas	Comamonas
Proteus	--	--
Pseudomonas	--	--
Zoogloea		

e) Biochemical conditions: (Vide Table-9, Appendix)

The estimations of protein, amino acid nitrogen, Total sugar, free sugar and volatile acid (lower organic fatty acids) were done only in the case of mixed algae. The results are shown in the Table -9 (Appendix).

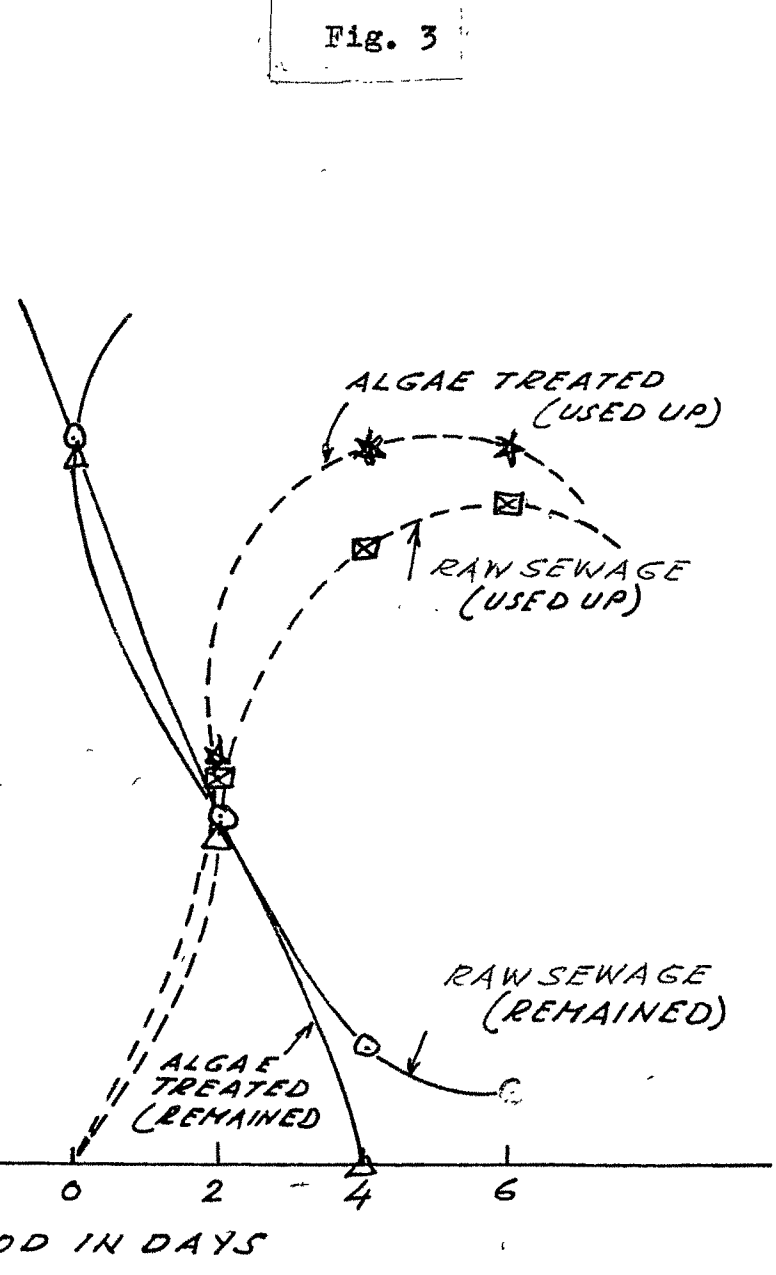
PROTEIN USED AND REMAINING IN THE EXPERIMENT WITH MIXED ALGAE

Fig. 2



AMINO-NITROGEN USED AND REMAINING IN THE EXPERIMENT WITH MIXED ALGAE

Fig. 3



DETENTION PERIOD IN DAYS

i) Protein:

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

Detention period	Control (raw sewage) Protein (mg/l)	% of reduction	Algae-treated Protein (mg/l)	% of reduction
0 day	6.8	-	7.6	-
2 days	3.4	50.0	4.6	39.5
4 days	1.9	72.1	0.8	90.0
6 days	1.2	82.35	0.8	90.0

The values for protein decreased in both the control and algal flasks. But the degree of variation differed. In the control flask about 82.35% reduction was found within 6 days and in the algae-treated flasks 90.0% reduction in the same time. The amounts of protein used up and remaining in the ecosystem for different detention periods are diagramatically represented in Fig. 2.

ii) Amino acid nitrogen:

The amounts of amino acid nitrogen present in the control and in the algal flasks have been

Fig. 4

TOTAL SUGAR REMAINED AND USED UP IN THE EXPERIMENT USING MIXED ALGAE

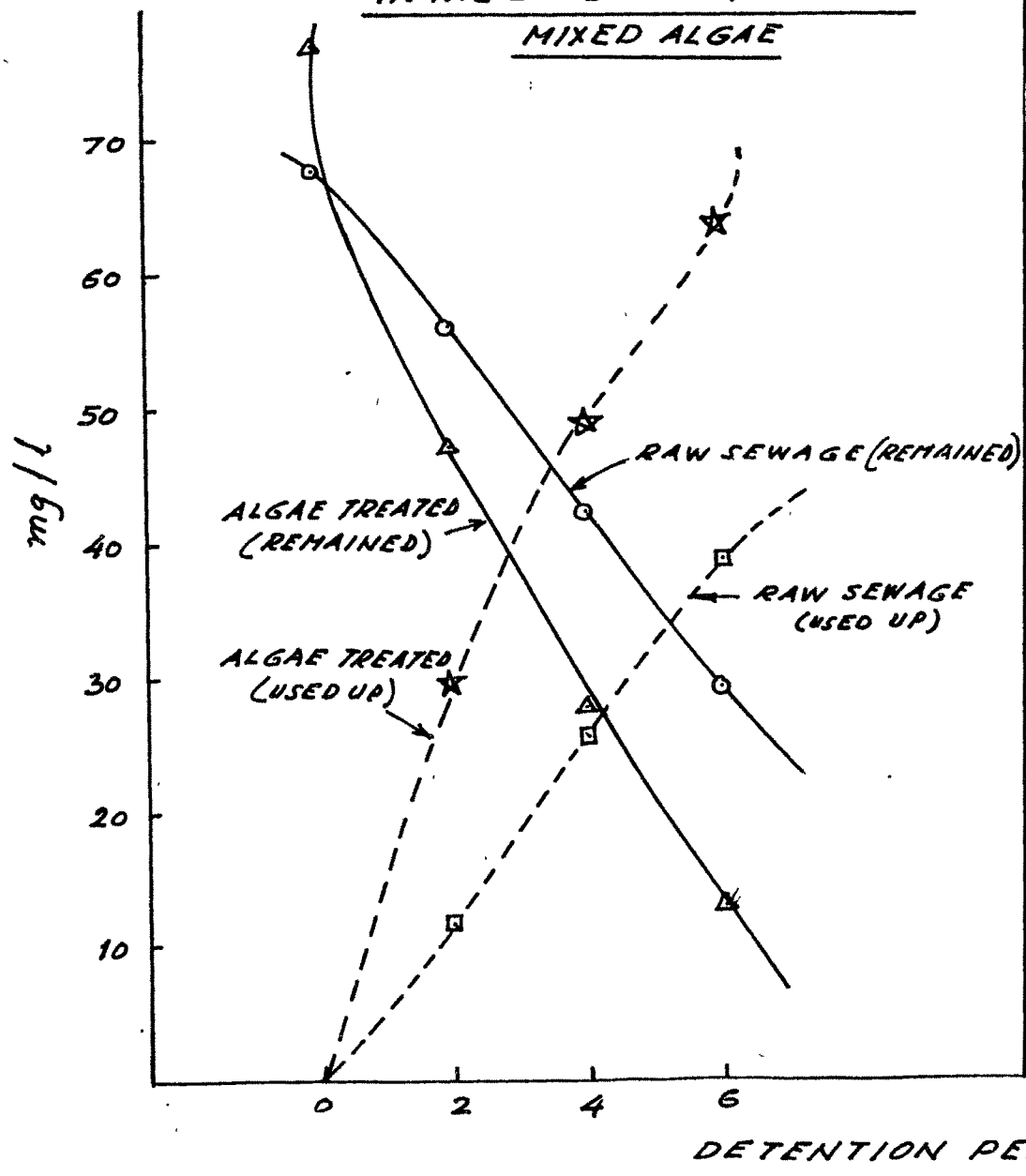
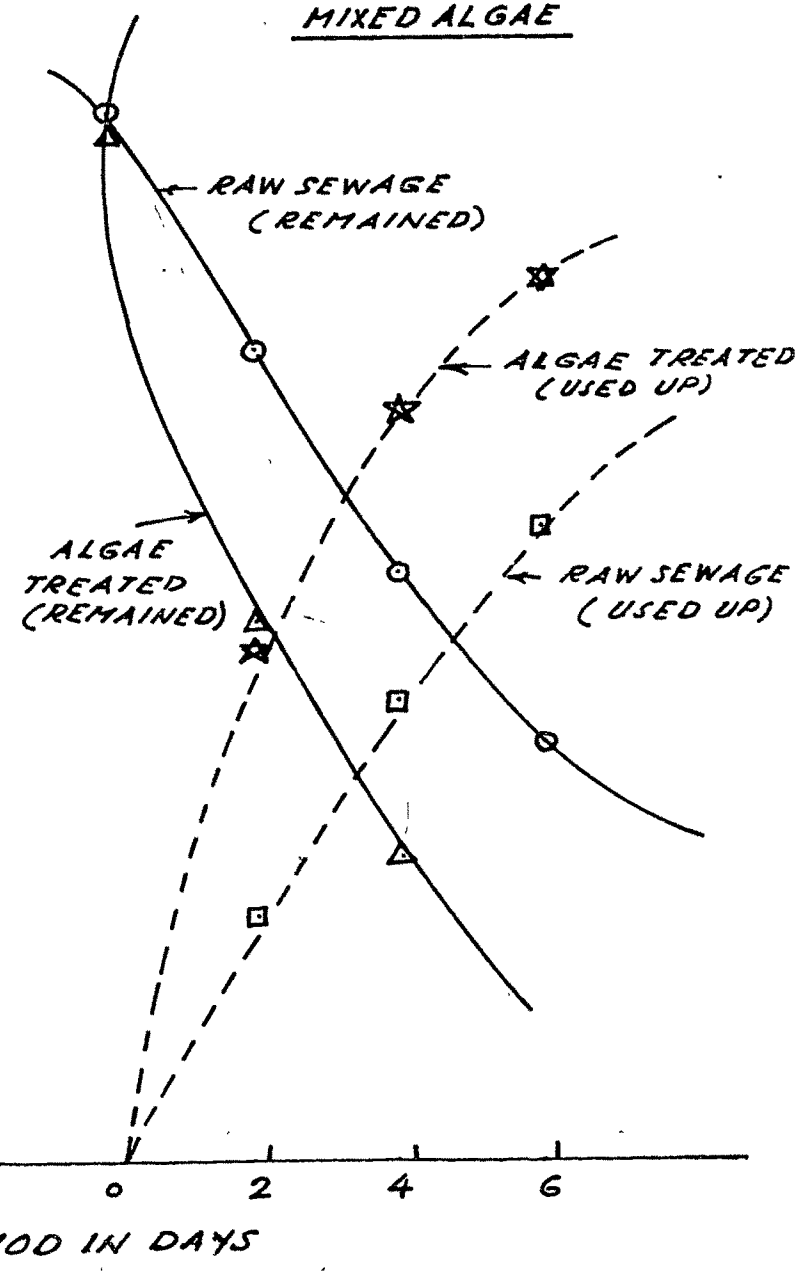


Fig. 5

FREE SUGAR REMAINED AND USED UP IN THE EXPERIMENT USING MIXED ALGAE



DETENTION PERIOD IN DAYS

summarised below:

Detention period	Control(raw sewage) Amino acid nitrogen (mg/l)	% of reduction	Algae treated Amino acid nitrogen (mg/l)	% of reduction
0 day	5.1	-	5.0	-
2 days	2.4	52.9	2.3	54.0
4 days	0.8	84.3	nil	100
6 days	0.5	90.2	nil	100

The data show a higher percentage of reduction in algae treated flasks. The control flasks showed 90.2% reduction in 6 days while the algae treated flask 100% reduction in 4 days. The results are shown in Fig. 3.

iii) Total sugar:

The amounts of Total sugar in the control and in the algal flasks have been summarised below in a tabular form:

Detention period	Control(raw sewage) Total sugar (mg/l)	% of reduction	Algae-treated Total sugar (mg/l)	% of reduction
0 day	68.25	-	77.25	-
2 days	56.44	17.4	47.5	38.6
4 days	42.55	37.6	28.1	63.6
6 days	29.55	56.7	13.3	82.8

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 56.7% decrease within 6 days while the algae-treated flask 63.6% decrease within 4 days and 82.8% 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.

iv) Free sugar:

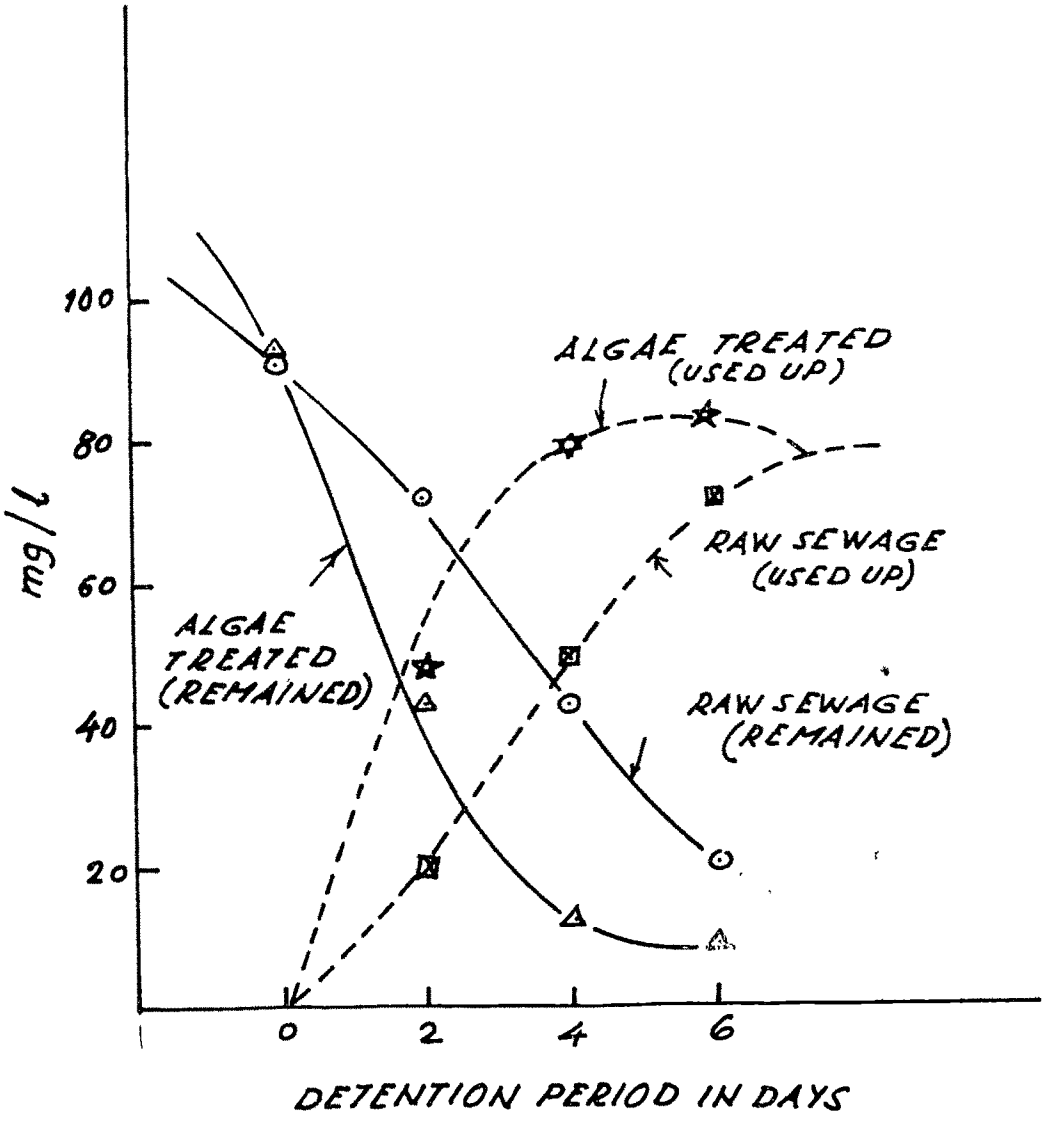
The amounts of free sugar present in the control and in the algal flasks have been summarised below:

Detention period	Control(raw sewage)		Algae-treated	
	Free sugar (mg/l)	% of reduction	Free sugar (mg/l)	% of reduction
0 day	36.55	-	36.55	-
2 days	28.22	23.0	18.8	48.6
4 days	20.6	43.7	10.6	71.0
6 days	14.6	60.0	5.8	84.1

The results show a gradual decrease and a higher percentage of decrease in the algal treated flasks. The control flasks showed 60.0% decrease within 6 days. But more than this amount of reduction

Fig. 6

VOLATILE ACIDS USED AND REMAINING IN THE EXPERIMENT WITH MIXED ALGAE



(71.0%) was found in the algal treated flasks within 4 days and 84.1% reduction within 6 days. The results are shown in Fig. 5.

v) 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(raw sewage) Volatile acids (mg/l)	% of reduction	Algae-treated Volatile acids (mg/l)	% of reduction
0 day	90.0	-	90.0	-
2 days	70.8	21.3	42.0	53.3
4 days	42.0	53.3	12.0	86.7
6 days	20.0	77.7	8.2	91.0

77.7% reduction was found in the control flasks within 6 days. But the algae-treated flasks showed 86.7% reduction in 4 days and 91.0% within 6 days. The results 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 has helped in providing oxygen to bacteria for degradation of organic matter. But the higher percentage of reduction in the algal-treated samples has to be attributed to the greater and more intimate availability of photo-synthetic oxygen than reaeration.