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EXPERIMENTAL STUDIES ON ALGAL-BACTERIAL SYMBIOSIS

Pure culture studies are made for the important physico-chemical factors such as turbidity, pH, relative stability, acid $KMnO_4$ value, ammoniacal nitrogen and phosphate with : (a) the 10 selected bacterial strains; (b) three fast growing algae; (c) three slow growing algae; (d) mixture of 10 selected bacterial strains with the three fast growing algae; and (e) mixture of 10 selected bacterial strains with the three slow growing algae. The results of these studies are discussed below.

Results of the Ten Selected Bacterial Strains

Turbidity. The results of turbidity test for each of the ten selected strains and the mixture are shown in table 8-1 and in the Fig. 8-1. From a study of the table and figure, it will be seen that all the 10 selected strains show good reduction in turbidity. Six of them viz. *Micrococcus* sp. (R-66), *Flavobacterium* sp. (0-140), *Corynebacterium* sp. (0-149), *Micrococcus* sp. (0-195) and *Brevibacterium* sp. (0-201) are able to effect as high as 80-90% reduction in 21-28 days like the mixture of bacteria while three others (*Brevibacterium* sp. (0-96), *Corynebacterium* sp. (0-137), and *Brevibacterium* sp. (0-166) are able to effect only about 50-56% during the same period. But *Brevibacterium* sp. (0-143) although gives about 70% reduction on the 14th day, is found to produce more turbidity on the last two days, so that the overall reduction amounted only to about 40%. Generally speaking, all the

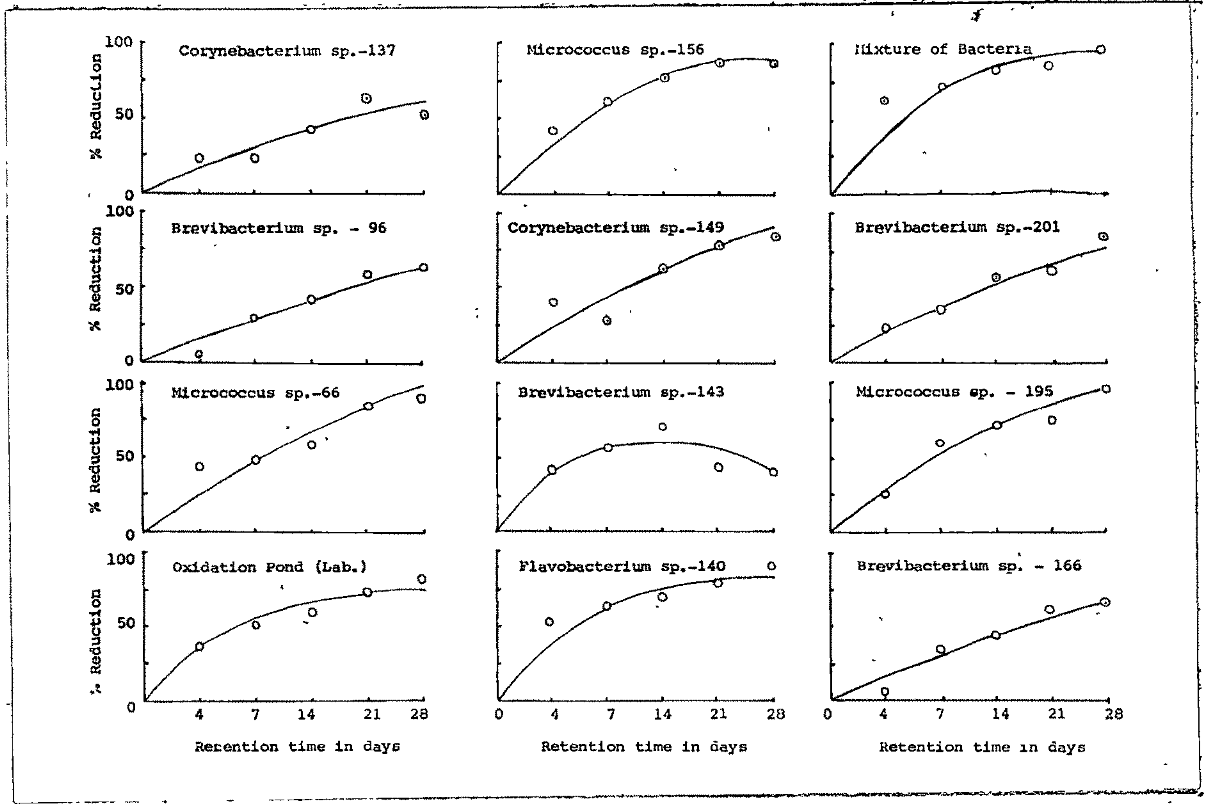


Fig.8-1: Turbidity changes noted with different strains and mixture of bacteria

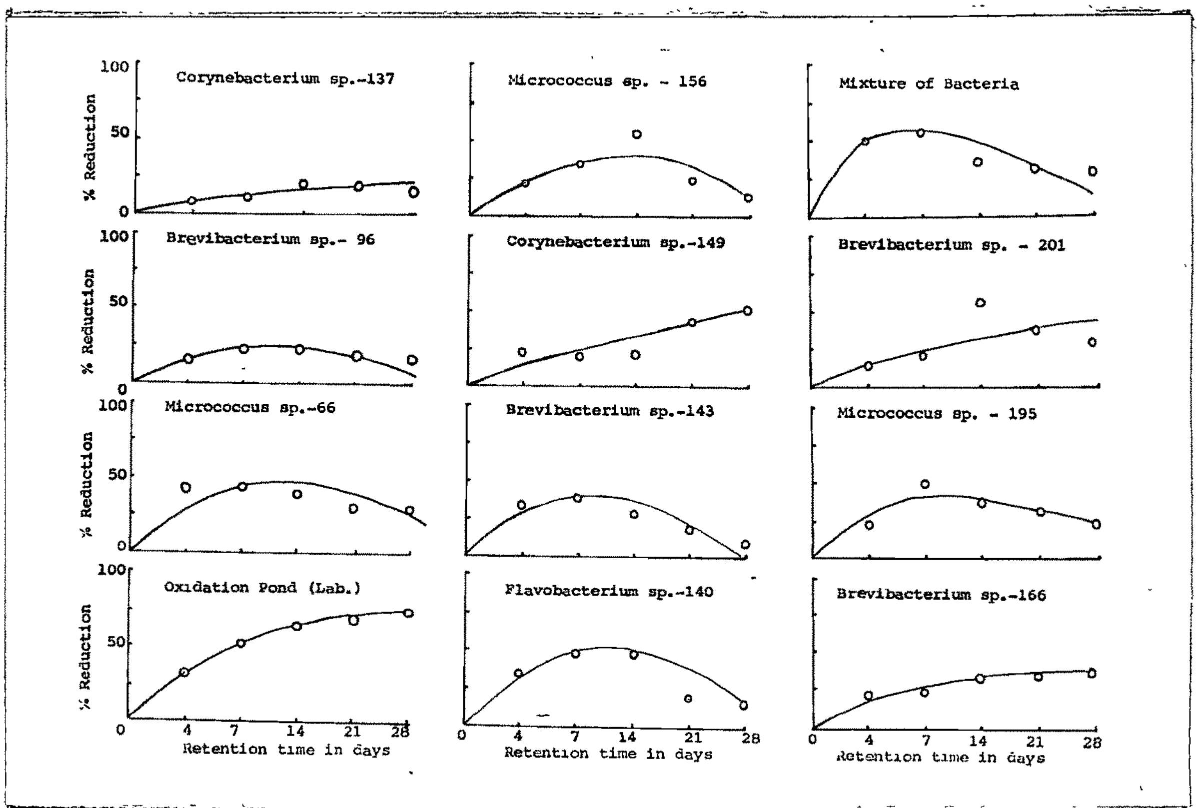


Fig.8-2: Changes in acid $KMnO_4$ (4 hours) value noted with different strains and mixture of bacteria

bacteria and the mixture show comparatively less reduction during 4 to 7 days and more during 14-28 day phase.

Hydrogen ion concentration (pH). The results of this test for all the 10 bacterial strains are shown in table 8-2. It can be seen from the table that there is a gradual increase in pH with all the bacterial strains from 8.6[illegible] to 9.00. Comparatively the pH reach in all the cases is less in 4-7 days and more in 7-28 days.

Relative stability. The results of this test given in table 8-3 show that the 9 strains, excepting *Brevibacterium sp.* (0-96), gives above 75% stability values. The latter gives only 21%. The higher stability values are perhaps due to endogenous respiration.

Acid $KMnO_4$ values (4 hours). The results of this test are shown in table 8-4 and in Fig. 8-2. Only the two bacteria: *Corynebacterium sp.* (0-149) and *Brevibacterium sp.* (0-166) show consistent reduction. In the former case the final reduction is about 51% and in the latter case only 38%. In all other cases, though there are definite reductions upto 7 to 14 days the values are found to increase during the rest of the period. A similar reaction is found to take place in the case of the mixed strains also. But in the case of the laboratory model, the values were found to decrease consistently. Sp, exact reason for the increase in the case of the pure

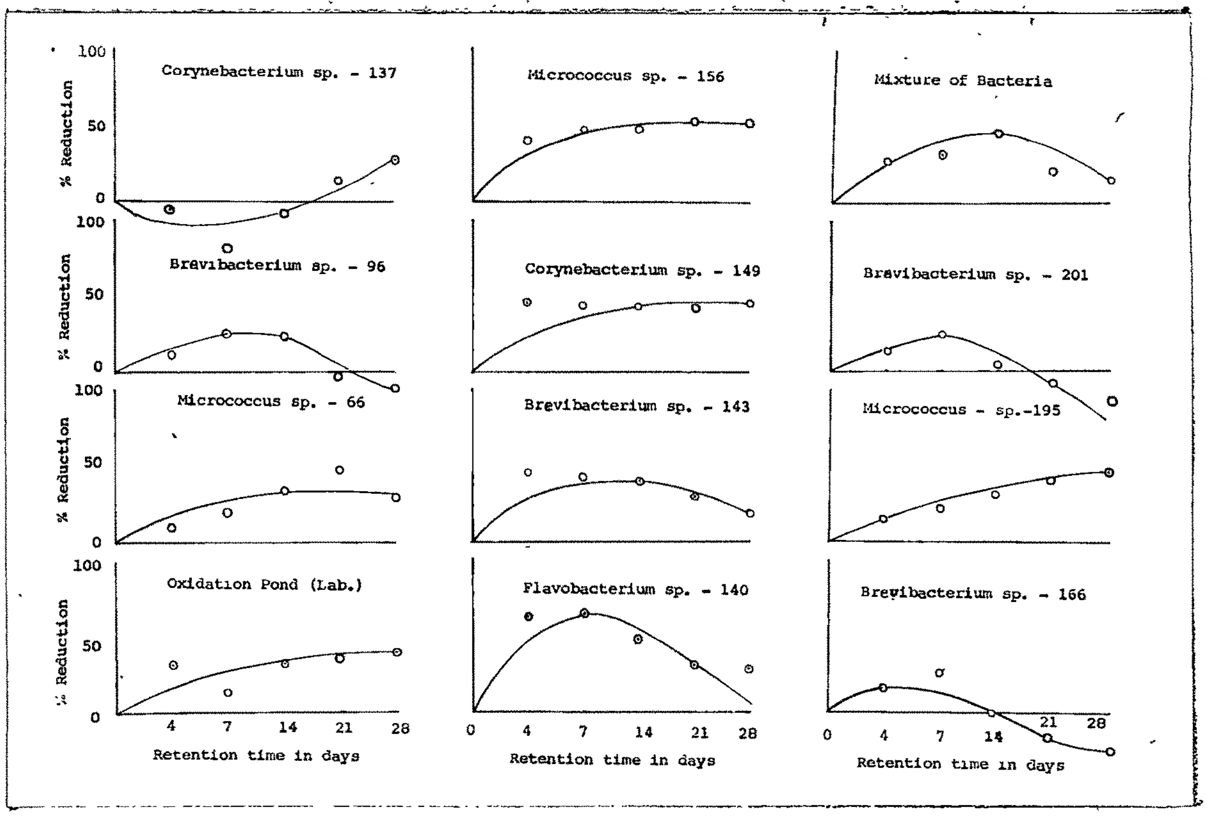


Fig.8-3: Changes in Orthophosphate content noted with different strains and mixture of bacteria

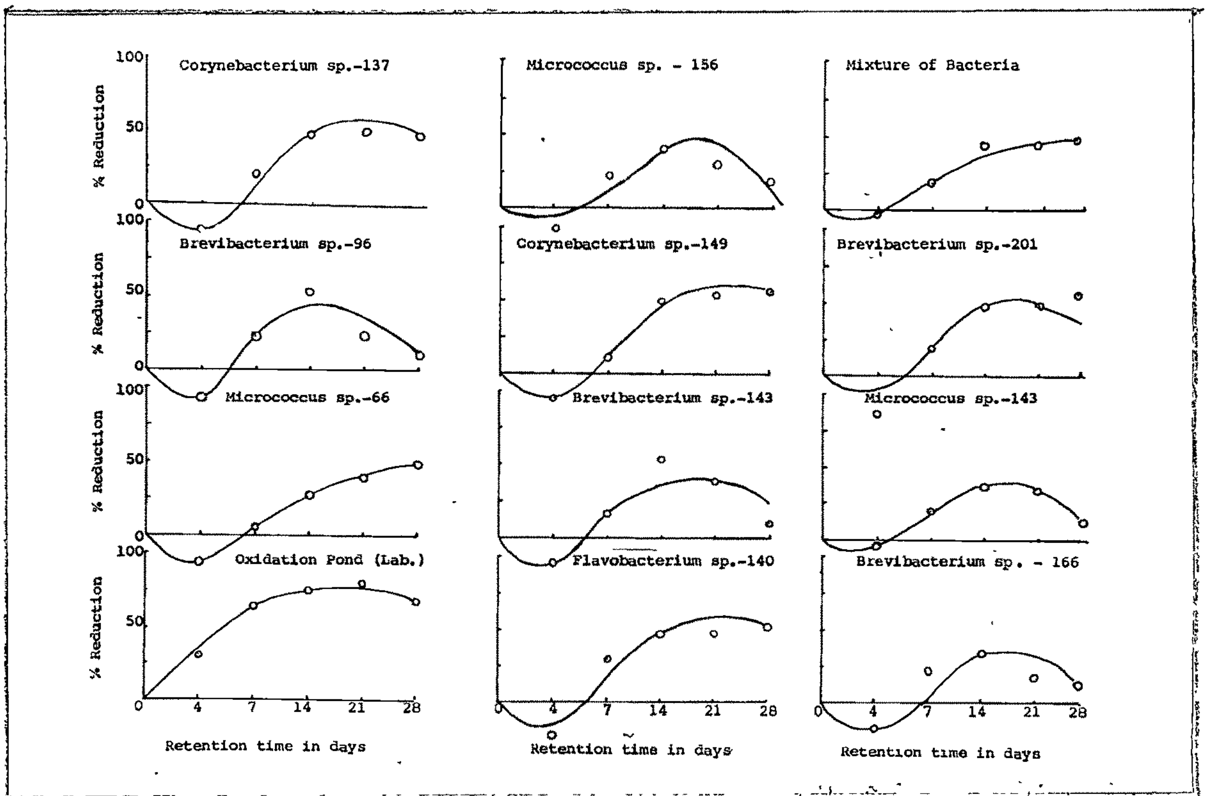


Fig.8-4: Changes in Ammonia-Nitrogen content noted with different strains and mixture of bacteria

cultures is not known. Most probably, the bacteria died and added to the organic matter content.

Ortho-phosphate. The results of this test are shown in table 8-5 and in Fig. 8-3 from which the following conclusions are derived. The reactions of the 10 bacterial strains can be grouped under three heads : (a) bacteria showing consistent reduction throughout. They are the three species of *Micrococcus*. showing reductions ranging between 30 to 53%. (b) Bacteria showing initial reductions and then increases. They are all the 4 *Brevibacterium* species, one *Flavobacterium* sp, the mixed strains and one *Corynebacterium* sp. (0-149). Out of the six individual organisms, three show increase in value over the 0 day ih the final stage and it amounted to 10 to 28%.

In the laboratory model oxidation pond the overall reduction was only 47%.

Ammonia - nitrogen. The data for this factor are shown in table 8-6 and in Fig. 8-4. A study of the table and figure, reveals that all the strains show increased figures for the 4th day and later decreased. The bacterial reactions can be grouped roughly under two heads : (a) bacterial strains showing consistent reduction throughout (about 50%). The organisms are: *Micrococcus* sp. R-66, *Corynebacterium* sp. (0-137); *Flavobacterium* sp (0-140); *Corynebacterium* sp (0-149); *Brevibacterium* sp (0.201) and the mixture of all bacteria. (b) The other bacterial strains

Table :8-1: Turbidity changes noted with Different Strains and Mixture of Bacteria.

Retention time in days		0	4	7	14	21	28
Bacteria		Klett Units at 660 m µ	.... % Reduction				
Type	Strain No.						
Micrococcus sp	R-66	47 (38-51)	43.0 (40.0-45.5)	47.0 (45.0-50.0)	58.0 (54.0-60.0)	85.0 (82.0-90.0)	89.0 (87.0-95.0)
Brevibacterium sp	0.96	"	4.2 (3.0-4.9)	30.0 (24.5-33.7)	42.5 (37.0-45.0)	58.5 (54.0-60.0)	63.0 (60.0-66.0)
Corynebacterium sp	0.137	"	23.4 (21.5-25.9)	23.4 (22.0-27.0)	42.5 (41.0-46.5)	63.9 (60.4-71.8)	51.3 (48.3-54.6)
Flavobacterium sp.	0.140	"	53.8 (49.2-58.0)	63.0 (60.0-66.0)	70.0 (65.0-75.0)	79.2 (76.4-82.8)	91.5 (87.0-96.5)
Brevibacterium sp.	0.143	"	40.4 (38.0-42.5)	55.2 (50.0-60.4)	70.3 (66.0-75.6)	44.4 (42.0-46.8)	40.7 (37.0-44.4)
Corynebacterium sp	0.149	"	40.0 (34.5-47.5)	27.6 (25.0-28.4)	63.8 (62.0-66.4)	78.8 (72.4-81.6)	83.0 (80.0-86.0)
Micrococcus sp.	0.156	"	42.5 (38.5-47.0)	60.5 (53.0-65.0)	87.2 (83.0-90.4)	87.2 (81.0-93.4)	87.2 (85.0-89.6)
Brevibacterium sp.	0.166	"	6.4 (3.2-10.0)	34.0 (31.5-37.5)	44.7 (40.1-49.3)	61.0 (57.0-66.5)	66.3 (64.0-69.6)
Micrococcus sp.	0.195	"	25.5 (20.0-31.0)	59.5 (55.0-64.5)	72.5 (70.0-75.0)	74.3 (71.0-78.0)	95.7 (89.0-99.4)
Brevibacterium sp.	0.201	"	23.4 (20.2-27.0)	36.1 (34.0-39.0)	57.4 (53.0-60.8)	63.8 (61.2-67.4)	85.1 (80.0-89.4)
Mixture of all the above bacteria	R-66 to 0-201	"	64.0 (59.0-70.5)	72.3 (70.0-73.4)	83.0 (80.5-85.0)	87.0 (81.0-91.5)	93.0 (90.0-96.0)

7-18

Table :8-2: pH Changes Noted with Different Strains and Mixture of Bacteria

Retention Time in Days		0	4	7	14	21	28
Bacteria		Observed pH values					
Type	Strain No.						
Micrococcus sp.	R-66	8.62 (8.50-8.75)	8.50 (8.40-8.60)	8.52 (8.48-8.60)	8.60 (8.55-8.70)	8.86 (8.70-8.90)	8.90 (8.85-8.98)
Brevibacterium sp.	0-96	"	8.65 (8.62-8.78)	8.65 (8.62-8.70)	8.65 (8.60-8.70)	8.78 (8.70-8.85)	8.92 (8.90-8.96)
Corynebacterium sp.	0-137	"	8.60 (8.58-8.62)	8.60 (8.58-8.64)	8.65 (8.60-8.68)	8.75 (8.72-8.78)	9.00 (8.85-9.92)
Flavobacterium sp.	0-140	"	8.55 (8.50-8.60)	8.60 (8.58-8.68)	8.68 (8.65-8.75)	8.72 (8.68-8.80)	9.00 (8.85-9.25)
Brevibacterium sp.	0-143	"	8.55 (8.50-8.60)	8.70 (8.68-8.75)	8.72 (8.70-8.75)	8.72 (8.68-8.80)	8.98 (8.90-9.18)
Corynebacterium sp.	0-149	"	8.62 (8.55-8.70)	8.68 (8.65-8.70)	8.62 (8.58-8.65)	8.68 (8.64-8.75)	8.90 (8.84-8.98)
Micrococcus sp.	0-156	"	8.55 (8.50-8.58)	8.85 (8.78-8.95)	8.75 (8.70-8.80)	8.70 (8.68-8.75)	8.60 (8.55-8.70)
Brevibacterium sp.	0-166	"	8.70 (8.65-8.78)	8.72 (8.68-8.78)	8.75 (8.70-8.78)	8.78 (8.72-8.85)	8.85 (8.82-8.90)
Micrococcus sp.	0-195	"	8.65 (8.62-8.70)	8.68 (8.65-8.72)	8.75 (8.72-8.80)	8.70 (8.68-8.75)	8.80 (8.72-8.85)
Brevibacterium sp.	0-201	"	8.70 (8.65-8.75)	8.70 (8.62-8.74)	8.75 (8.72-8.80)	8.80 (8.78-8.84)	8.85 (8.82-8.90)
Mixture of all the above Bacteria	R-66 to 0-201	"	8.50 (8.42-8.54)	8.65 (8.60-8.68)	8.70 (8.68-8.74)	8.78 (8.75-8.80)	8.85 (8.80-8.92)

Table:8-3: Changes in Relative Stability noted with Different Strains and Mixture of Bacteria

Retention Time in Days		0	4	7	14	21	28
Bacteria				... % Relative Stability			
Type	Strain No.						
Micrococcus sp	R-66	8.0 (6-12)	16 (12-20)	25 (20-30)	55 (48-60)	68 (60-75)	> 75 > 75
Brevibacterium sp	0.96	"	8 (6 -14)	16 (12-18)	19 (16-24)	19 (16-24)	21 (17-24)
Corynebacterium sp	0.137	"	8 (6 -12)	16 (12-20)	55 (48-60)	> 75 > 75	> 75 > 75
Flavobacterium sp.	0.140	"	8 (6 -12)	19 (16-22)	70 (60-75)	75 (70-80)	> 75 > 75
Brevibacterium sp.	0.143	"	8 (6 -12)	35 (24-39)	39 (30-45)	59 (55-64)	> 75 > 75
Corynebacterium sp.	0.149	"	8 (6 -12)	19 (16-22)	70 (60-75)	> 75 > 75	> 75 > 75
Micrococcus sp.	0.156	"	8 (6 -12)	19 (16-22)	44 (39-50)	> 75 > 75	> 75 > 75
Brevibacterium sp.	0.166	"	16 (12-22)	25 (20-30)	39 (30-45)	>75 >75	> 75 > 75
Micrococcus sp.	0.195	"	16 (12-22)	24 (20-30)	70 (60-75)	>75 >75	>75 >75
Brevibacterium sp.	0-201	"	16 (12-22)	35 (24-39)	39 (30-45)	> 75 > 75	>75 >75
Mixture of all the above Bacteria	R-66 to 0-201	"	24 (19-36)	24 (19-36)	60 (55-64)	>75 >75	>75 >75

Table: 8-4: Changes in Acid $KMnO_4$ (4 hrs.) value noted with Different Strains and Mixture of Bacteria

Retention Time in Days		0	4	7	14	21	28
Bacteria		mg. per 100 ml	% Reduction				
Type	Strain No.						
Micrococcus sp.	R-66	10.5 (6.7-12.8)	42.5 (30.0-40.5)	43.8 (40.2-46.4)	38.5 (34.5-40.5)	30.5 (27.2-34.8)	30.5 (27.0-33.0)
Brevibacterium sp.	0-96	"	15.2 (9.6-18.8)	23.8 (18.5-27.6)	22.8 (20.8-25.4)	20.0 (17.4-22.8)	17.8 (13.6-20.5)
Corynebacterium sp.	0-137	"	7.6 (3.4-10.8)	11.4 (9.6 -14.8)	20.5 (15.5-25.0)	19.2 (17.8-22.0)	16.9 (12.4-21.8)
Flavobacterium sp.	0-140	"	33.8 (29.7-38.2)	48.5 (45.0-50.9)	47.6 (42.6-51.4)	19.6 (17.4-22.8)	16.0 (14.0-19.0)
Brevibacterium sp.	0-143	"	33.8 (29.6-38.0)	38.1 (35.0-42.4)	23.8 (20.5-26.8)	18.1 (17.1-20.2)	10.5 (8.2-12.7)
Corynebacterium sp.	0-149	"	21.0 (18.0-25.5)	20.0 (18.5-22.4)	21.0 (17.0-26.5)	43.8 (40.5-47.0)	50.8 (46.8-55.5)
Micrococcus sp.	0-156	"	21.4 (18.4-25.0)	33.8 (29.9-36.5)	53.3 (52.1-55.4)	23.8 (20.5-27.6)	13.3 (9.4-17.0)
Brevibacterium sp.	0-166	"	22.8 (19.6-26.7)	24.7 (22.5-26.8)	34.3 (30.3-37.0)	35.2 (33.1-38.4)	37.6 (35.4-39.9)
Micrococcus sp.	0-195	"	22.8 (19.5-26.4)	49.5 (47.2-52.8)	36.5 (34.6-38.9)	32.8 (29.7-35.0)	23.8 (20.7-27.0)
Brevibacterium sp.	0-201	"	14.8 (11.7-18.0)	20.0 (18.5-23.0)	56.2 (54.2-59.1)	38.4 (34.6-43.7)	31.1 (29.5-34.7)
Mixture of all the Bacteria	R-66 to 0-201	"	50.5 (47.1-52.9)	54.2 (52.4-56.8)	34.3 (33.1-36.8)	31.4 (28.4-35.0)	29.0 (27.0-33.4)

Table:8-5: Changes in Orthophosphate Content Noted with Different Strains and Mixture of Bacteria

Retention Time in Days		0	4	7	14	21	28
Bacteria		mg per 1000ml	% Reduction				
Type	Strain No.						
Micrococcus sp.	R-66	17.2 (13.5-20.8)	10.4 (7.6-14.0)	20.3 (17.3-24.5)	34.6 (32.5-37.0)	47.7 (43.4-50.1)	30.2 (29.1-32.4)
Brevibacterium sp.	0-96	10.4 "	10.4 (7.4-12.9)	25.5 (23.5-28.0)	24.3 (22.4-26.8)	+1.7 * (-2.4-+3.5)	+10.0 * (+7.0-+14.5)
Corynebacterium sp.	0-136	"	+10.4 * (+7.2-+14.5)	+31.4 * (+29.4-34.0)	+31.4 * (+8.5-+15.0)	-14.4 (-12.5-16.4)	-29.2 (26.2-32.9)
Flavobacterium sp.	0-140	"	63.9 (60.5-65.9)	66.8 (63.8-70.5)	47.6 (46.2-50.0)	31.7 (28.4-33.9)	29.2 (27.4-32.0)
Brevibacterium sp.	0-143	"	46.5 (45.5-48.5)	42.5 (39.5-46.0)	42.0 (40.5-43.0)	30.0 (28.5-33.3)	19.0 (17.5-22.0)
Corynebacterium sp.	0-149	"	46.5 (44.4-49.0)	45.3 (42.5-47.0)	43.2 (42.5-45.9)	43.6 (40.6-45.9)	47.0 (45.5-49.4)
Micrococcus sp.	0-156	"	41.8 (39.7-44.0)	49.0 (47.4-52.0)	49.0 (48.0-51.5)	53.5 (51.5-56.0)	53.0 (52.4-54.9)
Brevibacterium sp.	0-166	"	15.1 (13.5-18.0)	26.7 (24.5-29.4)	+11.6 * (+9.5-+12.9)	+16.3 * (+14.7-+18.5)	+28.3 * (+27.5-+30.5)
Micrococcus sp.	0-195	"	15.1 (13.4-18.5)	25.5 (22.5-28.4)	30.2 (29.1-32.8)	44.5 (41.5-46.8)	45.7 (43.3-48.9)
Brevibacterium sp.	0-201	"	10.4 (7.4-12.9)	23.8 (20.9-25.8)	5.7 (3.2-8.4)	+3.5 * (+0.5-4.7)	+18.7 * (+16.7-+21.0)
Mixture of all the Bacteria	R-66 to 0-201	"	27.1 (25.6-30.1)	32.6 (29.8-34.1)	47.8 (46.9-49.2)	23.8 (21.4-26.0)	17.4 (13.9-20.5)

*(+) Sign shows the % increase over 0-day value

Table :8-6: Changes in Ammonia Nitrogen Content noted with Different Strains and Mixture of Bacteria

Retention Time in Days		0	4	7	14	21	28
Bacteria		mg. per 1000ml	% Reduction				
Type	Strain No.						
Micrococcus sp.	R-66	16.2 (13.8-20.2)	+20.5 * (+18.2-+23.0)	-5.0 (-2.0--8.4)	26.0 (25.1-28.0)	38.0 (35.5-42.4)	47.8 (45.6-49.5)
Brevibacterium sp.	R-96	"	+20.5 * (+18.5-+23.0)	-22.2 (-20.5--24.5)	53.0 (50.4-56.8)	23.0 (22.4-25.8)	11.7 (9.8-13.6)
Corynebacterium sp.	0-137	"	+17.2 * (+14.9-+20.8)	-21.0 (-19.8--23.4)	49.3 (48.8-50.4)	51.1 (48.5-55.0)	48.0 (46.4-50.9)
Flavobacterium sp.	0-140	"	+26.5 * (+24.7-+28.0)	-30.5 (-28.4--32.7)	47.0 (45.8-49.6)	48.1 (46.9-50.5)	52.0 (50.4-55.6)
Brevibacterium sp.	0-143	"	+17.3 * (+14.9-+19.8)	-17.3 (-14.4--20.8)	53.8 (51.4-56.4)	39.5 (37.3-41.8)	11.7 (9.8-14.4)
Corynebacterium sp.	0-149	"	+17.2 * (+15.8-+20.7)	-11.1 (-9.2--13.7)	50.0 (46.5-55.0)	53.6 (50.4-56.8)	56.7 (53.4-58.9)
Micrococcus sp.	0-156	"	+14.0 * (+12.4-+17.4)	-23.4 (-21.0--26.0)	41.3 (39.2-44.0)	30.3 (28.3-33.3)	18.5 (17.1-20.9)
Brevibacterium sp.	0-166	"	+16.0 * (+14.5-+17.4)	-23.2 (-21.7--25.9)	34.3 (32.1-37.4)	20.9 (17.8-22.7)	17.1 (14.4-19.8)
Micrococcus sp.	0-195	"	+4.9 * (+1.7-+7.7)	-20.4 (-18.5-23.7)	37.0 (34.0-39.8)	34.5 (30.5-37.4)	14.5 (12.5-17.0)
Brevibacterium sp.	0-201	"	+26.5 * (+24.5-+28.9)	-19.7 (-16.4-22.7)	48.0 (47.0-49.5)	49.7 (47.5-51.4)	55.0 (51.5-57.5)
Mixture of all	R-66 to 0-201	"	+1.9* (-1.8-+3.5)	-21.0 (-19.2--24.5)	44.4 (42.6-47.5)	45.5 (43.2-47.4)	47.5 (45.5-49.4)

* (+) shows % increase over 0-day value

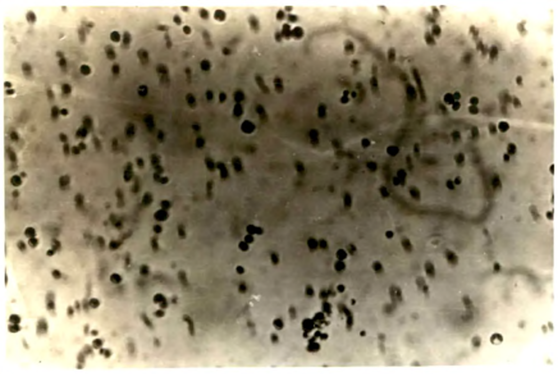
show initial reductions upto 14-21 days and on the 28th day the values are found to increase though the increases do not go above the 0-day value.

Results of the Experiments with Three Fast-growing Algae (Chlorella, Oscillatoria obscura and O.chalybea) alone and with the mixture of the Ten Selected Strains of Bacteria

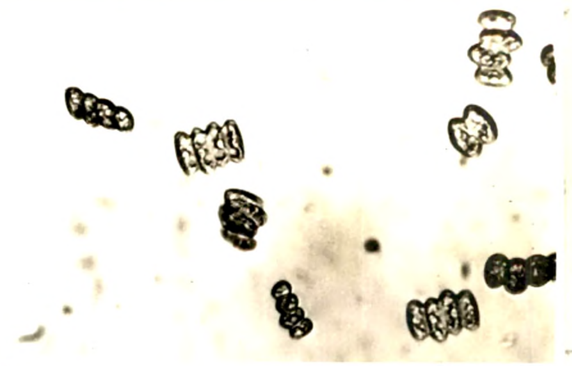
Since it was found that the two species of Oscillatoria turned yellowish after 14 days of culturing; it was decided to continue the experiments upto 14 days only. The results of each of the three species alone and in combination with the bacterial mixture are discussed below.

Turbidity. The results of this test are shown in table 8-7 and in Fig. 8-5. It will be seen from a study of the table that the two algae cultures (Oscillatoria) alone show good reduction in turbidity in 4-7 days. On the 14th day they begin to turn yellowish with the resultant increase in turbidity. In the case of Chlorella cultures, as the organism passes through the cotton filter, the turbidity values are found to increase with the increased growth of Chlorella.

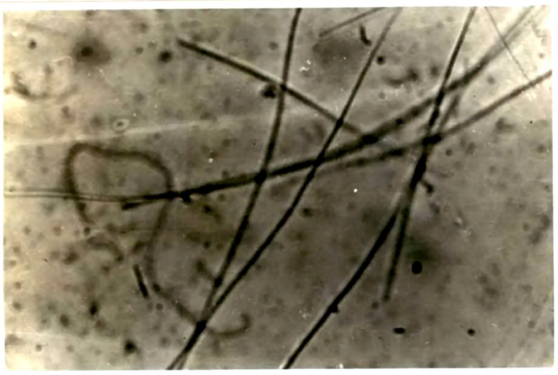
Comparatively, better results are obtained in the case of algae cultures when grown in the absence of bacteria. While the mixed bacterial culture alone gives 72%, the algal-bacterial mixed cultures are showing 88% reduction; and the mixed algae alone are showing 92% on the 7th day and less later due to their death and disintegration.



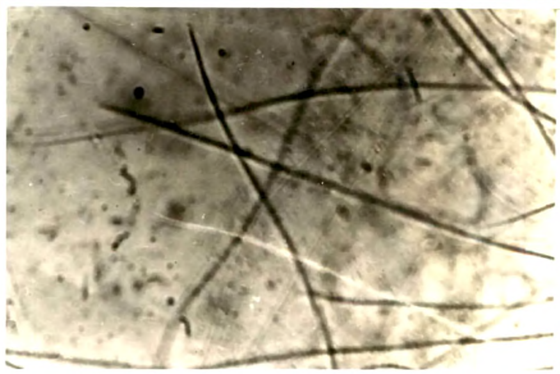
A: Chlorella Vulgaris



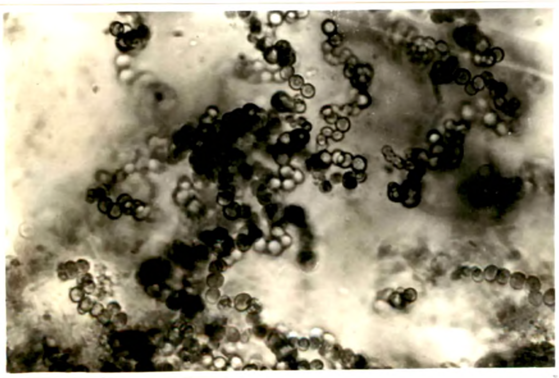
B: Scenedesmus quadricauda



C: Oscillotoria obscura



D: Oscillatoria chalybea



E: Nostoc pyriformis

F: Aulosira fertilisima

Fig.8-5: Photomicrographs of the Algae used in pure culture studies

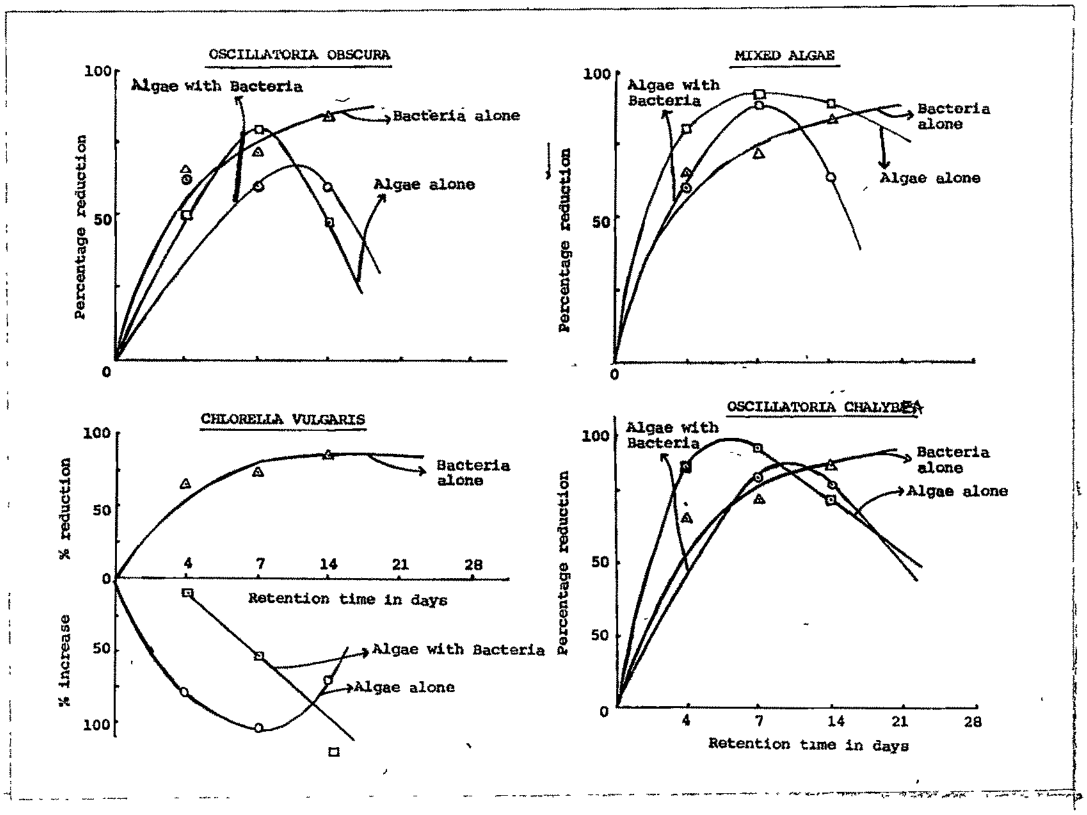


Fig.8-6: Turbidity changes noted with different fast-growing and mixed algae with and without bacteria

Hydrogen ion concentration (pH). The results of this test are shown in table 8-8, from which the following conclusion is drawn.

The algal cultures alone or the algal cultures in the presence of bacteria show definite increase in pH from 8.6 to 10.90-11.20. On the other hand, with the bacterial mixture alone, the pH ranges from 8.62 to 8.70 only. The higher increase in the case of algae has to be attributed to photosynthetic activities of the algae.

Relative stability. The results of this test are shown in table 8-9. All the three algae individually or in combination with bacteria produced effluents of high stability in 4 days amounting to 55-75%. This value is maintained upto 7 days but the two *Oscillatoria species* alone were found to turn yellowish after 7 days when the stability was reduced to about 50%. In contrast, the bacterial cultures grown under identical conditions produce effluents of 24% stability only in 7 days, but in 14 days the stability increases to 60% and to more than 75% in 28 days (vide table 8-3).

The increased stability values with algae in as short a period as 4 days may be attributed to oxygen production by the algae, which is indicated by the high figures for alkalinity. (Vide table 8-10). But it is found that the stability figures are higher with algae alone than as with algae and bacteria. It would appear, therefore, that the algae alone without bacteria are able to bring about this stability. In fact, there are references to

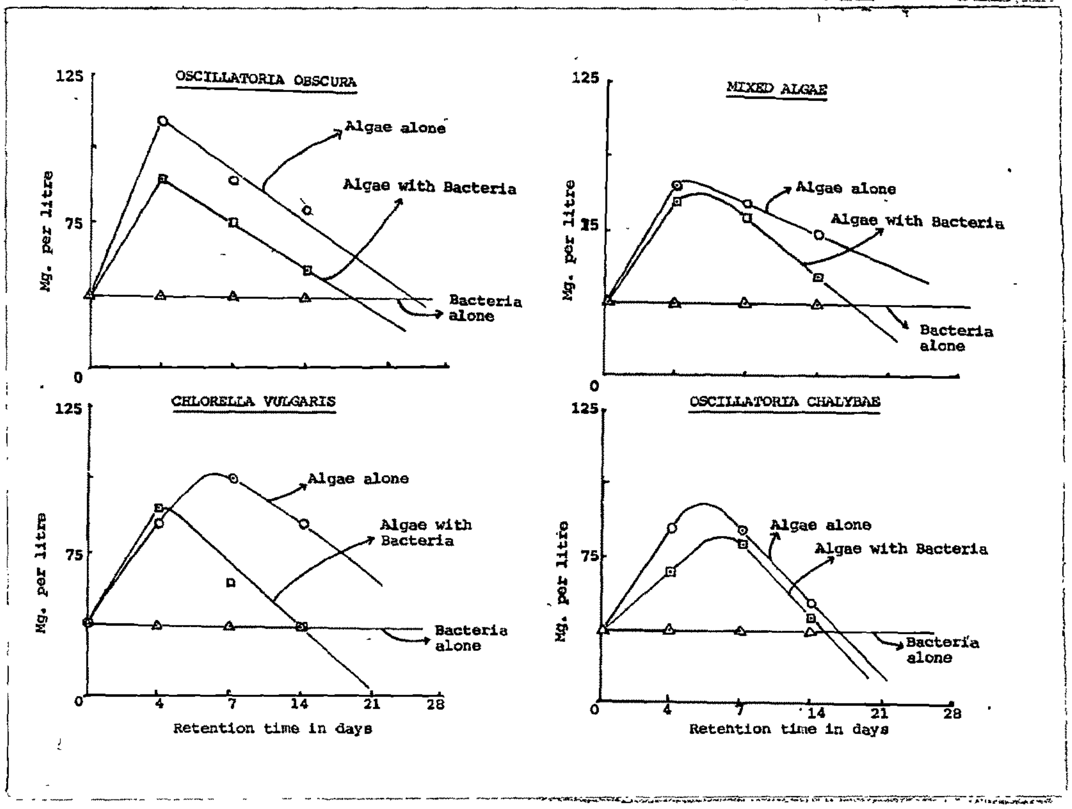


Fig.8-7: Changes in Phenolphthalein alkalinity noted with different fast-growing and mixed algae with and without bacteria

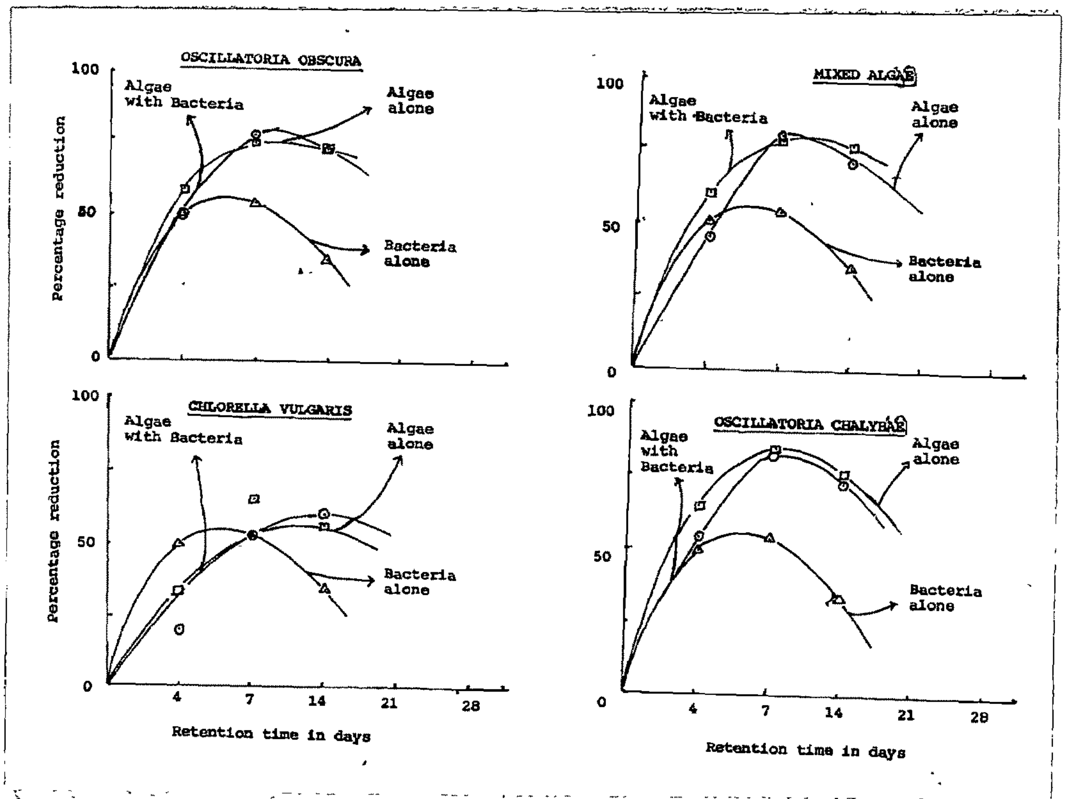


Fig.8-8: Changes in acid KMnO (4 hr.) value noted with different fast-growing and mixed algae with and without bacteria

show that the algae utilise organic substances (Fogg 1960) during their metabolic activities and may thus bring about stability in media containing decomposing organic matter.

Phenolphthalein alkalinity. The results of this test shown in table 8-10 and in Fig. 8-7 indicate that in the case of all the three algae, phenolphthalein alkalinity is gradually increasing upto 4-7 days and then is found to decrease evidencing that with increased algal production, there is simultaneous increase in oxygen content and phenolphthalein alkalinity, as indicated by Atkins and Harris (1924). But with bacteria alone there is no such increase in phenolphthalein alkalinity.

The decrease in phenolphthalein alkalinity after 4-7 days is due to the death of the algae.

Acid $KMnO_4$ value (4 Hours). The values are shown in table 8-11 and Fig. 8-8 from which the following inferences are drawn.

In the case of the algae alone maximum reduction of 65-84% is obtained in 7 days, and later the values increase which may be accounted for as being due to the death of the algae. Comparing the algae themselves, it is seen that the two species of *Oscillatoria* give better results than *Chlorella*.

The algal-bacterial mixed cultures, do not show any improvement over the algae alone. The reductions due to bacteria alone are comparatively lower showing that algae are able to show a better performance.

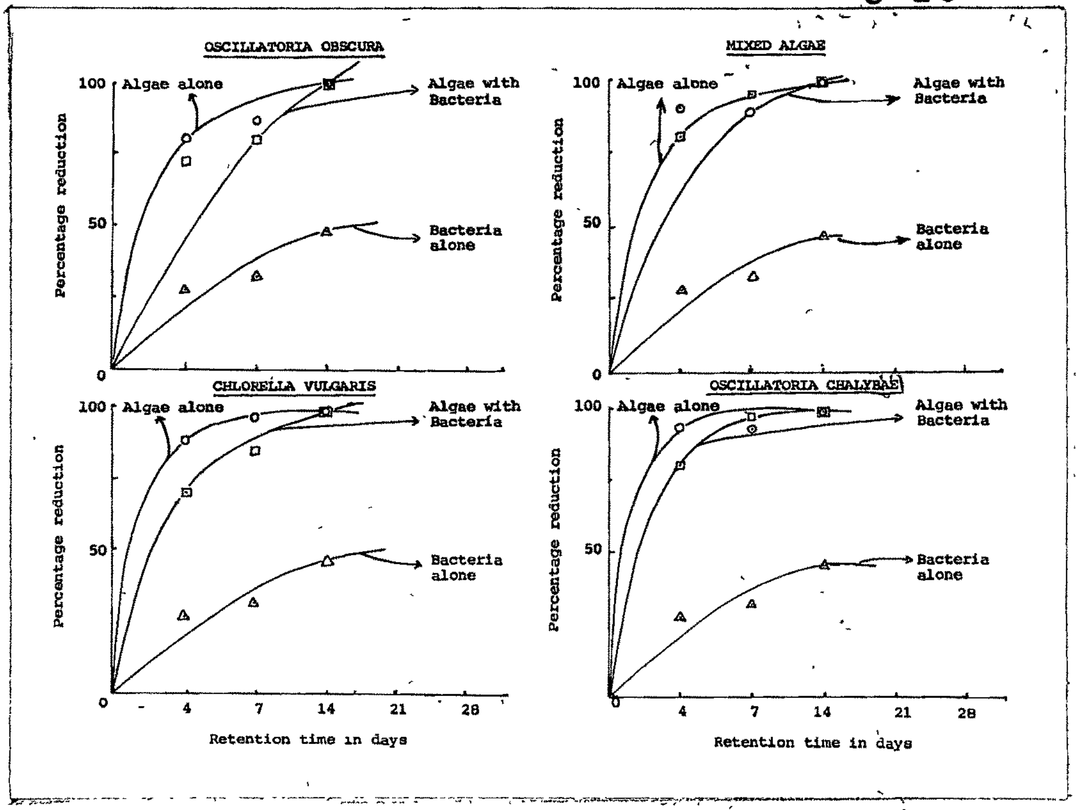


Fig.8-9: Changes in orthophate content noted with different fast-growing and mixed algae with and without bacteria

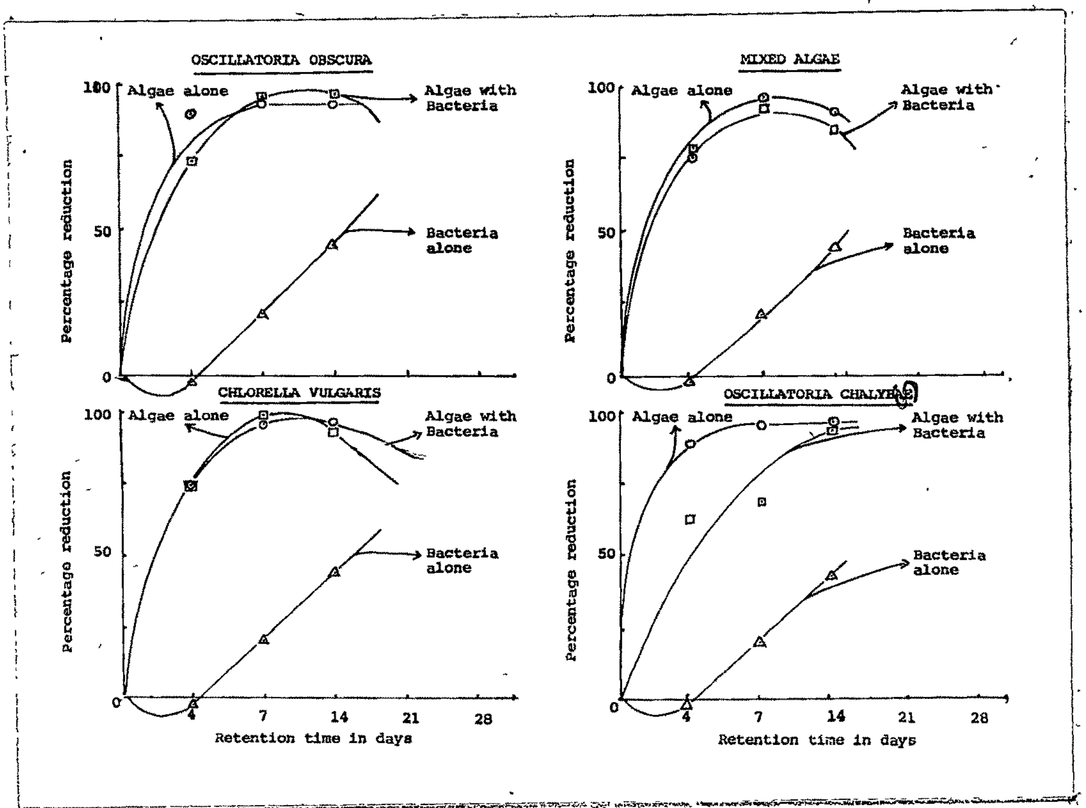


Fig.8-10: Changes in Amm-N. content noted with different fast-growing and mixed algae with and without bacteria

Ortho-phosphates. The results of this test are shown in table 8-12 and in Fig. 8-9. It is found that there is almost a complete removal of phosphates in all the cases excepting the bacteria alone when only about 48% reduction is indicated during the same period.

Ammonia-nitrogen. The results of this test are shown in table 8-13 and in Fig. 8-10. Just as in the case of phosphates, a very high reduction in ammonia-nitrogen content is seen in all the cases excepting the bacteria alone where the reduction amounted to only about 45%.

Tests are also carried out for the presence of nitrites and nitrates, but they are not detected at any time, showing that all the ammonia nitrogen content is used up by the algae.

Results of the Experiments with the Three Slow-growing Algae alone and with the mixture of the Ten Selected Strains of Bacteria

The same type of experiments as is done with the fast-growing algae is done in this case as well, but the experiments are continued for 28 days. The results of these experiments are discussed below.

Turbidity. The results of this test are shown in table 8-14 and in Fig. 8-11. The results show that there is reduction upto 14th day and thereafter there is an increase in turbidity. Bacteria alone show gradual reduction upto 28 days. Algae alone

Table :8-7: Turbidity Changes Noted with Different Fast-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14
Algae	Klett units at 660 m μ	% Reduction		
Chlorella vulgaris	47 (38-51)	+80 * (+77-+84)	+104 * (+95-+110)	+70 * (+68-+73)
Chlorella vulgaris with <u>Bacteria</u>	"	+10.* (+7.5-+14.0)	+54 * (+50-+57)	+136 * (+134-+139)
Oscillatoria obscura	"	50 (48.0-52.5)	78 (76.4-81.3)	48 (46.5-50.8)
Oscillatoria obscura with <u>Bacteria</u>	"	62 (60-65)	60 (57.5-64.0)	60 (58.4-63.0)
Oscillatoria chalybea	"	82 (79.2-84.4)	88 (86.8-90.5)	72 (70.4-75.0)
Oscillatoria chalybea with <u>Bacteria</u>	"	82 (80.5-85.0)	78 (77.4-79.8)	76 (73.0-80.0)
Mixture of above three algae	"	80 (78.5-83.0)	92 (90.5-94.7)	88 (85.7-92.0)
Mixture of above three algae with <u>Bacteria</u>	"	60 (58.4-64.2)	88 (86.4-90.5)	64 (60.8-66.4)
Bacteria only	"	64 (59.0-70.5)	72 (70.0-73.5)	83.5 (80.5-85.0)

* (+) shows the % increase over 0-day value

Table :8-8: pH Changes noted with Different Fast-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14
Algae		Actual Values		
Chlorella vulgaris	8.62 (8.50-8.75)	10.55 (10.40-10.70)	11.10 (10.98-11.28)	11.00 (10.90-11.22)
Chlorella vulgaris with Bacteria	"	10.35 (10.15-10.55)	10.90 (10.80-11.00)	11.10 (10.92-11.40)
Oscillatoria obscura	"	10.95 (10.82-11.98)	11.50 (10.45-11.65)	10.90 (10.85-11.00)
Oscillatoria obscura with Bacteria	"	10.90 (10.88-10.98)	11.00 (10.95-11.05)	11.00 (10.92-11.15)
Oscillatoria chalybea	"	10.90 (10.80-11.00)	10.80 (10.75-10.95)	11.20 (11.00-11.35)
Oscillatoria chalybea with Bacteria	"	10.45 (10.30-10.50)	10.55 (10.48-10.60)	10.95 (10.90-11.00)
Mixture of above three algae	"	10.54 (10.35-10.58)	11.30 (11.10-11.50)	11.00 (10.95-11.25)
Mixture of above three algae with Bacteria	"	10.75 (10.60-10.85)	11.15 (11.00-11.35)	10.95 (10.88-11.00)
Bacteria only	"	8.50 (8.42-8.54)	8.65 (8.60-8.68)	8.70 (8.68-8.74)

Table :8-9: Changes in Relative Stability noted with Fast-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14
Algae	% Relative Stability			
Chlorella vulgaris	8 (6-12)	>75 (>75 - >75)	>75 (>75 - >75)	>75 (>75 - >75)
Chlorella vulgaris with Bacteria	"	60 (55 - 70)	70 (65 - >75)	>75 (>75 - >75)
Oscillatoria obscura	"	> 75 (>75 - >75)	>75 (>75 - >75)	50 (40 - 60)
Oscillatoria obscura with Bacteria	"	65 (55 - 70)	70 (65 - 75)	50 (48 - 64)
Oscillatoria chalybea	"	>75 (>75 - >75)	>75 (>75 - >75)	50 (40 - 60)
Oscillatoria chalybea with Bacteria	"	55 (50 - 60)	65 (60 - 75)	55 (48 - 64)
Mixture of all the three algae	"	>75 (>75 - >75)	>75 (>75 - >75)	60 (55 - 64)
Mixture of all the three algae with Bacteria	"	70 (60 - 75)	>75 (>75 - >75)	50 (40 - 60)
Bacteria only	"	24 (19 - 36)	24 (19 - 36)	60 (55 - 64)

Table :8-10: Changes in Phenolphthalein Alkalinity Value noted with Different Fast-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14
Algae	Actual Values in PPM			
Chlorella vulgaris	50 (45-65)	90 (85-95)	100 (95-110)	85 (80-95)
Chlorella vulgaris with <u>Bacteria</u>	"	85 (75-90)	65 (55-75)	50 (45-60)
Oscillatoria obscura	"	110 (100-115)	90 (85-100)	80 (75-95)
Oscillatoria obscura with <u>Bacteria</u>	"	90 (85-100)	75 (70-80)	60 (50-65)
Oscillatoria chalybea	"	85 (80-95)	85 (75-90)	60 (55-70)
Oscillatoria chalybea with <u>Bacteria</u>	"	70 (65-80)	80 (75-90)	55 (50-65)
Mixture of all the three algae	"	90 (80-95)	85 (80-95)	75 (65-80)
Mixture of all the three algae with <u>Bacteria</u>	"	85 (80-90)	80 (75-90)	60 (50-65)
Bacteria only	"	50 (35-60)	50 (55-60)	50 (40-60)

Table :8-11: Changes in Acid $KMnO_4$ (4 hrs.) Value noted with Different Fast-growing and Mixed Algae - With and Without Bacteria

Retention Time in Days	0	4	7	14
Algae	mg. per 1000ml.	% Reduction		
Chlorella vulgaris	10.5 (6.7-12.8)	33.9 (30.5-34.7)	65.0 (63.5-68.4)	56.5 (54.5-59.0)
Chlorella vulgaris with <u>Bacteria</u>	"	20.0 (18.5-22.6)	54.8 (53.4-56.0)	60.0 (57.8-64.5)
Oscillatoria obscura	"	58.7 (56.7-60.0)	76.1 (74.8-78.0)	73.9 (70.5-76.9)
Oscillatoria obscura with <u>Bacteria</u>	"	50.4 (48.7-52.6)	75.2 (72.8-77.0)	73.9 (71.9-75.5)
Oscillatoria chalybea	"	65.2 (63.8-68.0)	83.9 (80.7-86.5)	76.5 (74.5-79.0)
Oscillatoria chalybea with <u>Bacteria</u>	"	54.3 (52.6-57.0)	82.2 (80.5-85.0)	74.8 (73.6-77.0)
Mixture of all the three algae	"	45.0 (43.5-48.0)	79.1 (77.8-81.4)	72.6 (70.5-74.9)
Mixture of all the above algae with <u>Bacteria</u>	"	60.0 (58.4-62.8)	78.2 (76.8-80.9)	77.7 (75.5-80.9)
<u>Bacteria</u> only	"	50.5 (47.1-52.9)	54.2 (52.4-56.8)	34.3 (33.1-36.8)

Table :8-12: Changes in Orthophosphate Content noted with Different Fast-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14
Algae	mg.per 1000ml.	% Reduction		
Chlorella vulgaris	17.2 (13.5-20.8)	88.0 (84.0-91.5)	96.7 (94.3-99.0)	99.9 (99.9-99.9)
Chlorella vulgaris with Bacteria	"	70.6 (68.5-73.0)	89.1 (86.0-91.7)	99.9 (99.9-99.9)
Oscillatoria obscura	"	80.2 (77.5-82.9)	86.8 (85.0-88.2)	99.9 (99.9-99.9)
Oscillatoria obscura with Bacteria	"	72.8 (70.4-74.8)	80.4 (78.5-83.7)	99.9 (99.9-99.9)
Oscillatoria chalybea	"	93.0 (90.5-95.4)	97.0 (95.4-100.0)	99.9 (99.9-99.9)
Oscillatoria chalybea with Bacteria	"	80.4 (78.5-83.6)	93.1 (91.8-95.0)	99.9 (99.9-99.9)
Mixture of all the three algae	"	90.0 (86.8-93.5)	96.3 (94.4-92.8)	99.9 (99.9-99.9)
Mixture of all the three algae with Bacteria	"	80.9 (78.4-82.7)	91.0 (88.4-99.5)	99.9 (99.9-99.9)
Bacteria only	"	27.1 (25.6-30.1)	32.6 (29.8-34.1)	47.8 (46.9-49.2)

Table :8-13: Changes in Ammonia Nitrogen Content noted with Different Fast-growing and Mixed Algae- with and without Bacteria

Retention Time in Days	0	4	7	14
Algae	mg per 1000ml	% Reduction		
Chlorella vulgaris	16.2 (13.8-20.0)	74.5 (72.0-76.8)	98.6 (97.8-99.9)	94.4 (92.2-97.0)
Chlorella vulgaris with Bacteria	"	74.5 (72.5-77.4)	96.5 (93.5-98.6)	97.8 (95.4-99.8)
Oscillatoria obscura	"	90.0 (87.8-91.5)	95.8 (91.4-98.9)	94.5 (92.9-96.4)
Oscillatoria obscura with Bacteria	"	74.4 (72.0-75.9)	93.5 (91.5-95.8)	94.2 (92.4-95.8)
Oscillatoria chalybea	"	89.7 (88.7-92.0)	96.7 (93.9-98.7)	97.2 (95.4-99.4)
Oscillatoria chalybea with Bacteria	"	63.4 (61.8-65.9)	69.6 (68.1-71.8)	96.7 (95.6-98.0)
Mixture of all the three algae	"	76.5 (74.4-78.0)	97.6 (95.4-99.0)	91.7 (88.6-93.9)
Mixture of all the three algae with Bacteria	"	77.2 (75.8-80.4)	95.9 (93.4-97.6)	81.5 (79.8-84.2)
Bacteria only	"	+1.9 * (-1.8-+3.5)	-21.0 (-19.2--24.5)	44.4 (42.6-47.5)

*(+) shows the % increase over 0 day-value

and algae plus bacteria produce almost similar results.

The reductions are similar in both the groups of algae.

Hydrogen ion concentration (pH). The results of this test are shown in table 8-15.

Similar increase as in the case of the first group of algae is noted but a lower maximum value is reached in this case.

Relative stability. The results are shown in table 8-16 and the individual alga behaves differently in each case.

Aulosira fertilisima produces maximum stability in 4 days and is retained upto the 14th day. Later there is decrease in stability which is lowest on 28th day.

On the other hand, Nostoc and Scenedesmus behave almost alike. They effect the maximum stability in 7-14 days and thereafter there is a decline.

Aulosira, Nostoc and Scenedesmus with or without bacteria behave alike.

Algal mixture alone produces maximum stability in 4 days but later on it is found to decrease. The algal mixture with bacteria, on the other hand, behaves differently. Maximum stability is produced after 14 days.

With bacteria alone stability is found to increase slowly reaching above 75% on 28th day.

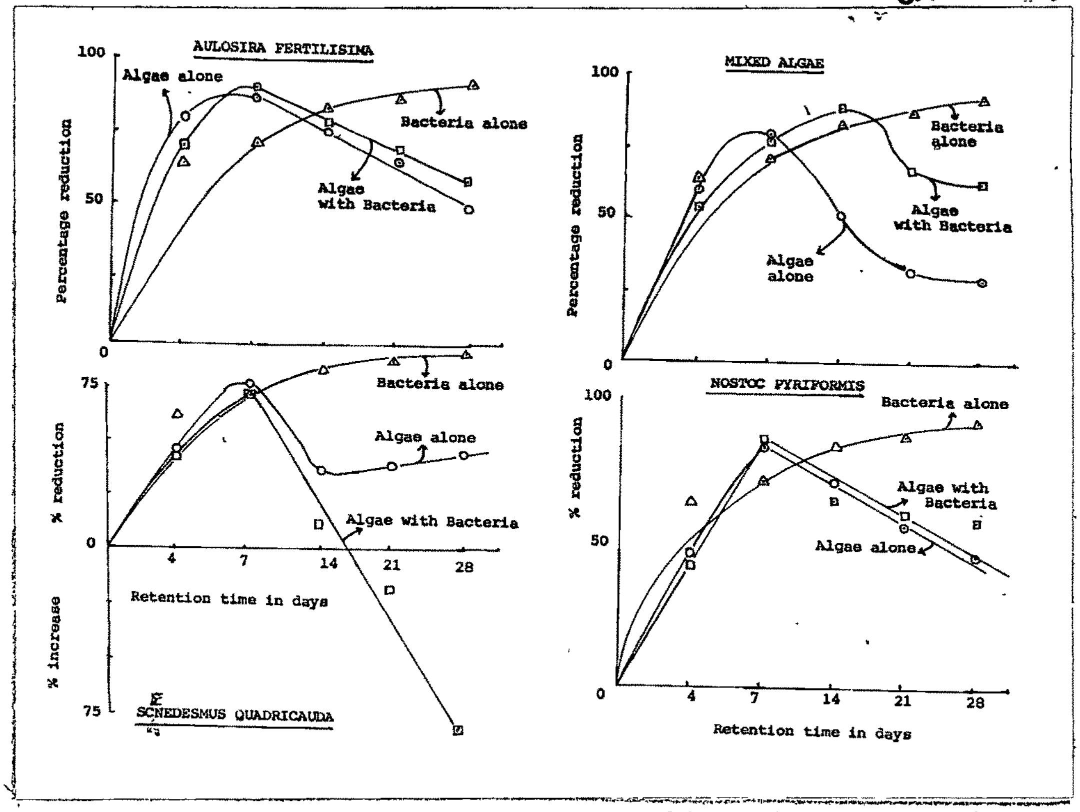


Fig.8-11: Turbidity changes noted with different slow-growing and mixed algae with and without bacteria

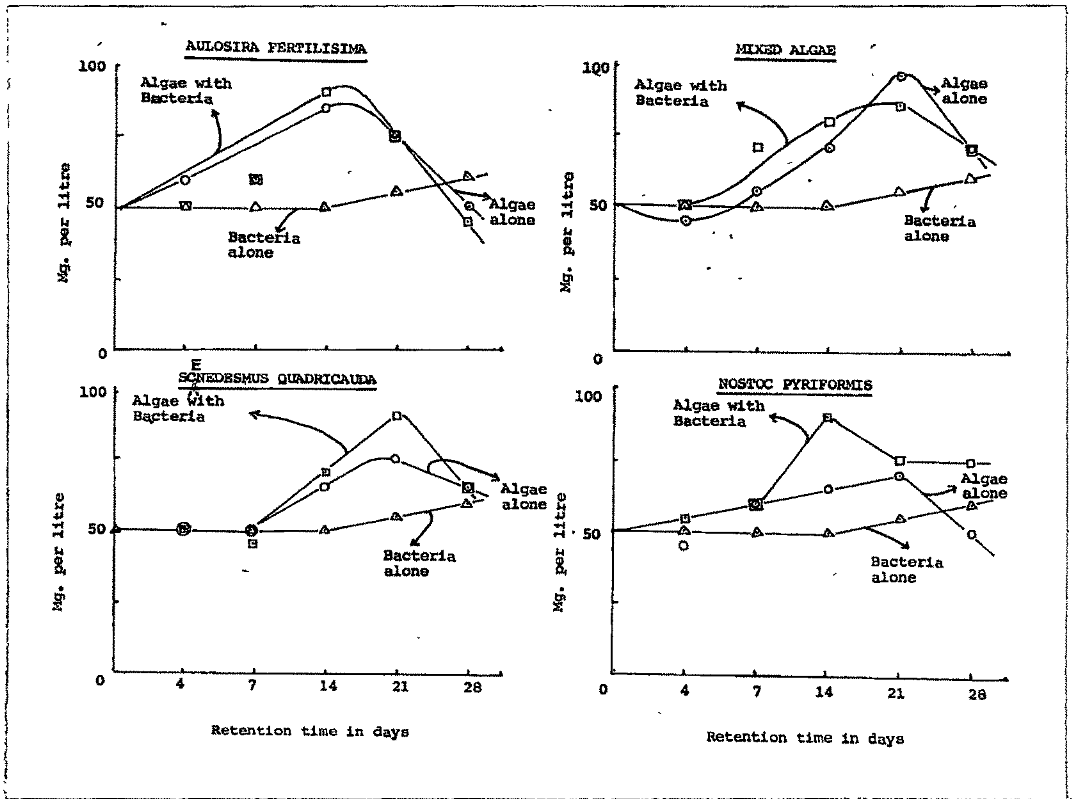


Fig.8-12: Changes in Phenolphthalein alkalinity value, noted with different slow-growing and mixed algae with and without bacteria

Phenolphthalein alkalinity. The results are shown in table 8-17 and in Fig. 8-12.

The three algae show a gradual increase upto 7 days. Then the values increase rapidly reaching the maximum on 14-21 days, and later decrease on 21-28 days.

Another observation made is that higher values are obtained when the algae are grown along with the bacteria.

The only point of difference between the two groups of algae is that in the former case, as the algae are fast growing, maximum values are obtained earlier (4-7 days).

Acid $KMnO_4$ value (4 Hours). The results of this test are shown in table 8-18 and in Fig. 8-13.

From a study of the table and figure, it will be seen that the maximum reductions are reached on different days with the different alga. *Scenedesmus* gives maximum reduction in 14 days, *Aulosira* in 4 days, *Nostoc* in 7 days and the mixed algae in 4 days and later on the values are found to decrease.

Individual alga with bacterial mixture is found to behave in a way similar to that with the algae alone.

The algal mixture with bacteria on the other hand shows a consistent reduction throughout.

Compared to the first algal group, the performance of this algal group is comparatively poor.

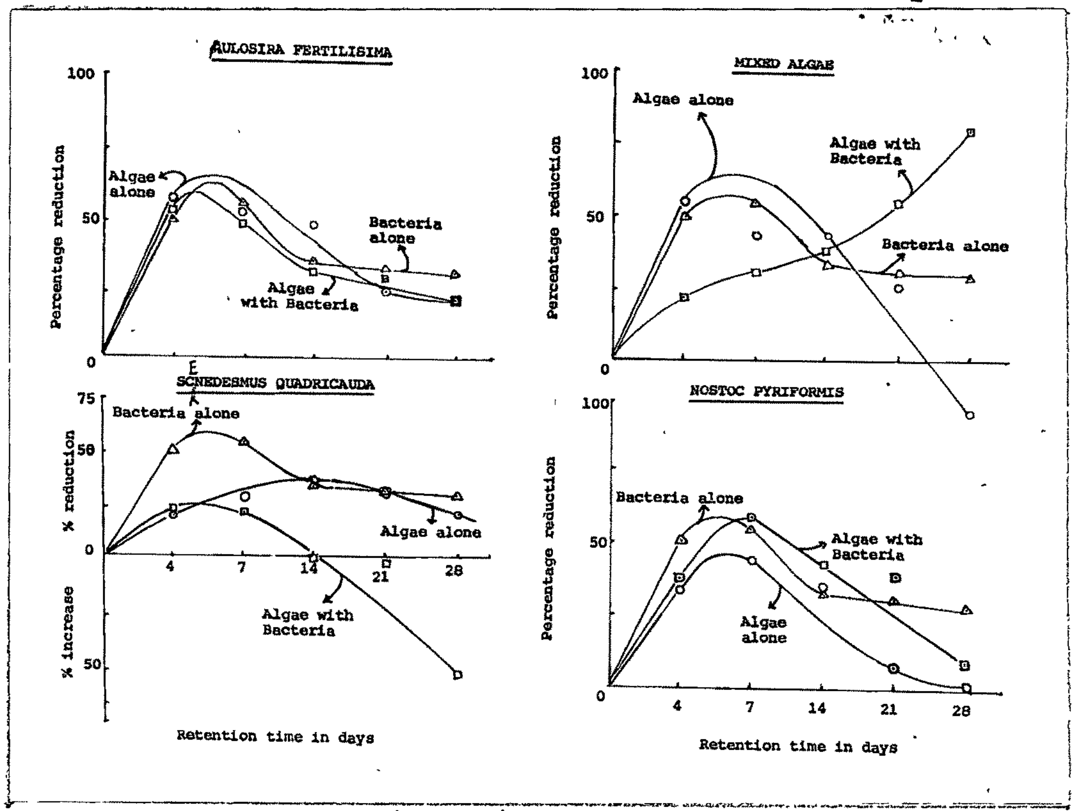


Fig.8-13: Changes in acid $KMnO_4$(4 hr.) value noted with different slow-growing and mixed algae with and without bacteria

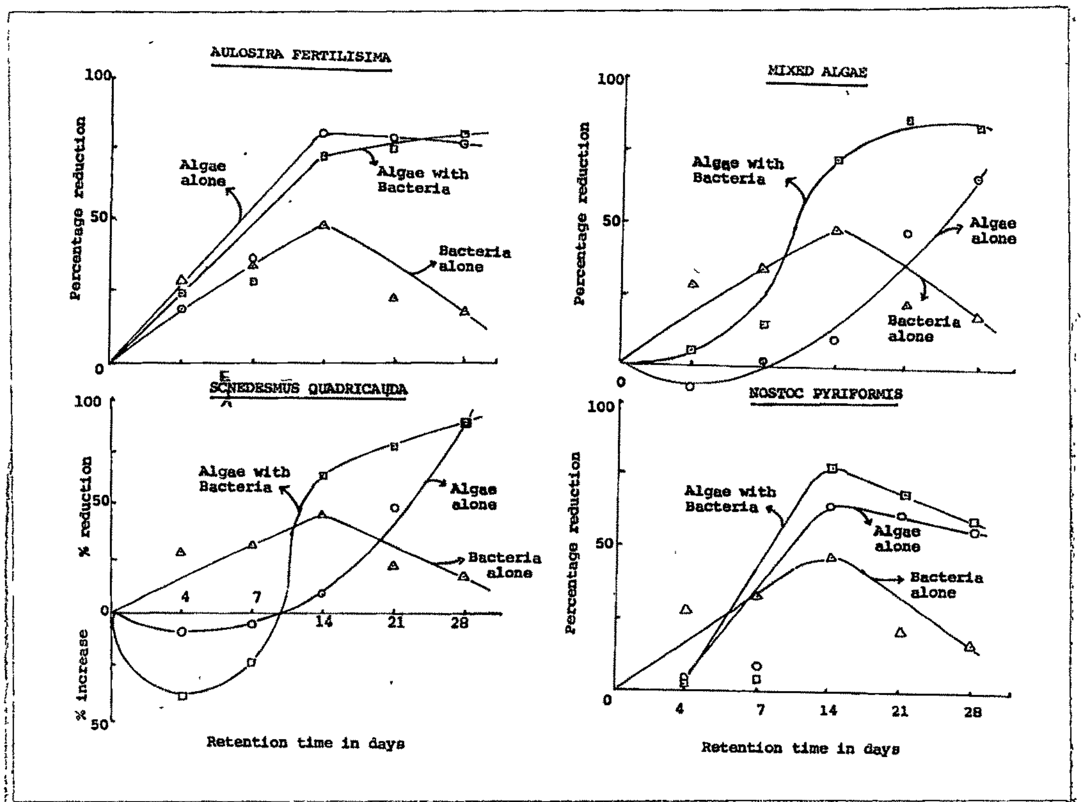


Fig.8-14: Changes in orthophosphate content noted with different slow-growing and mixed algae with and without bacteria

Ortho-phosphates. A study of the results shown in table 8-19 and Fig. 8-14 shows that in the case of Scenedesmus, there is first an increase in value upto 7 days and then decrease. Maximum reduction is obtained in 28 days. Similar results are obtained when the alga is grown with bacteria though the increase is greater.

Aulosira and Nostoc behave alike giving maximum reduction of about 80% in 14 days. Later the values are found to increase. Similar results are obtained when the algae are grown with the bacteria.

The algal mixture, on the other hand, is found to show consistent reduction throughout. With bacteria higher reductions are obtained. With bacteria only, maximum reduction of about 48% is obtained on 14th day and thereafter the values are found to increase.

Compared to the first algal group where almost complete removal of phosphates is observed, in the second group, the consumption of phosphates is much less. (about 77%).

Ammonia-nitrogen. The results of this test are shown in table 8-20 and in Fig. 8-15.

The three algae have behaved differently in this case. Taking Scenedesmus, it is found that the values for this test are reduced gradually reaching the maximum reduction of about 98% in 28 days. The same alga with bacteria, removes 98% in 14 days. Aulosira, on the other hand shows 80% reduction in 14 days.

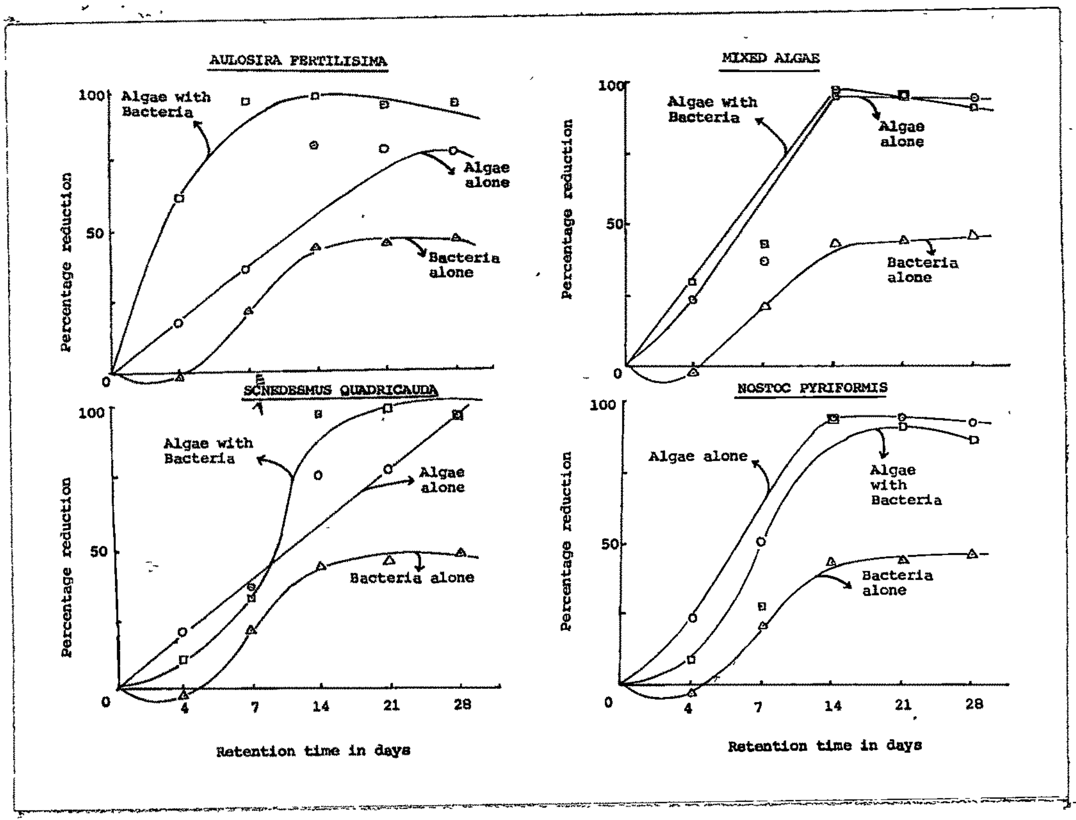


Fig.8-15: Changes in Amm-N. content noted with different slow-growing and mixed algae with and without bacteria

Table :8-14: Turbidity Changes noted with Different Slow-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14	21	28
Algae	Klett units at 660 mμ	% Reduction				
Scenedesmus quadricauda	47 (38-51)	46.0 (44.0-47.0)	76.0 (73.5-79.0)	40.0 (38.4-43.6)	42.0 (38.5-45.5)	46.0 (43.0-49.0)
Scenedesmus quadricauda with Bacteria	"	44.0 (41.5-47.4)	70.0 (68.9-71.7)	14.0 (10.8-17.0)	+16.0 * (+13.8-+19.0)	+80.0 * (+78.0-+815
Aulosira fertilisima	"	80.0 (77.8-82.9)	92.0 (89.0-94.6)	76.0 (74.3-78.9)	66.0 (63.5-70.4)	50.0 (48.7-54.0)
Aulosira fertilisima with Bacteria	"	70.0 (68.0-73.0)	90.0 (87.5-93.3)	78.0 (75.4-80.8)	70.0 (67.8-73.5)	60.0 (58.2-63.0)
Nostoc pyriformis	"	48.0 (46.4-51.0)	84.0 (81.8-87.0)	72.0 (70.7-75.0)	56.0 (52.9-59.2)	48.0 (47.4-50.4)
Nostoc pyriformis with Bacteria	"	44.0 (40.5-46.5)	86.0 (82.4-89.8)	64.0 (62.5-67.0)	60.0 (57.8-62.9)	58.0 (56.4-60.8)
Mixture of all the three algae	"	60.0 (57.4-62.8)	80.0 (78.4-83.4)	52.0 (50.5-55.0)	32.0 (28.8-34.7)	30.0 (28.4-32.9)
Mixture of all the three algae with Bacteria	"	54.0 (52.5-55.6)	78.0 (76.5-80.4)	88.0 (85.9-90.4)	68.0 (64.0-71.8)	64.0 (63.0-66.8)
Bacteria only	"	64.0 (59.0-70.5)	72.3 (70.0-73.4)	83.0 (80.5-85.0)	87.0 (81.0-91.5)	93.0 (90.0.96.0)

* (+) shows the % increase over 0-day value

Table :8-16: Changes in Relative Stability noted with Different Slow-growing and Mixed Algae – with and without Bacteria

Retention Time in Days	0	4	7	14	21	28
Algae			% Relative Stability			
Scenedesmus quadricauda	8.0 (6-12)	24 (20-30)	60 (55-64)	>75 (>75->75)	60 (55-64)	40 (30-48)
Scenedesmus quadricauda with Bacteria	"	36 (30-39)	60 (55-64)	>75 (>75->75)	>75 (>75 ->75)	60 (55-64)
Aulosira fertilisima	"	>75 (>75->75)	>75 (>75->75)	>75 (>75- >75)	60 (55-64)	35 (30-39)
Aulosira fertilisima with Bacteria	"	>75 (>75->75)	>75 (>75->75)	60 (55- 64)	60 (55-64)	40 (36-44)
Nostoc pyriformis	"	36 (30-39)	>75 (>75->75)	>75 (>75->75)	60 (55-64)	24 (20-30)
Nostoc pyriformis with Bacteria	"	24 (20-30)	60 (55-64)	>75 (>75->75)	>75 (>75->75)	30 (24-36)
Mixture of all the three algae	"	>75 (>75->75)	>75 (>75->75)	60 (55-60)	40 (36-64)	24 (20-30)
Mixture with all the three algae with Bacteria	"	24 (20-30)	60 (55-64)	>75 (>75->75)	>75 (>75->75)	>75 (>75->75)
Bacteria only	"	24 (19-36)	24 (19-36)	60 (55-64)	60 (55-64)	>75 (>75->75)

Table :8-17: Changes in Phenolphthalein Alkalinity Value noted with Different Slow-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14	21	28
Algae			mg. per 1000 ml.			
Scenedesmus quadricauda	50 (45-65)	50 (40-60)	50 (45-60)	65 (60-75)	75 (70-85)	65 (60-70)
Scenedesmus quadricauda with <u>Bacteria</u>	"	50 (45-64)	40 (35-50)	70 (65-80)	90 (80-95)	65 (60-70)
Aulosira fertilisima	"	60 (50-65)	60 (55-70)	85 (80-95)	75 (70-80)	50 (40-55)
Aulosira fertilisima with <u>Bacteria</u>	"	50 (40-55)	60 (50-65)	90 (80-100)	75 (65-80)	45 (35-60)
Nostoc pyriformis	"	45 (40-55)	60 (55-65)	65 (55-75)	70 (65-80)	50 (40-60)
Nostoc pyriformis with <u>Bacteria</u>	"	55 (45-60)	60 (55-70)	90 (80-100)	75 (70-80)	75 (65-80)
Mixture of all the three algae	"	45 (40-60)	55 (45-60)	70 (65-80)	95 (85-100)	70 (65-80)
Mixture of all the three algae with <u>Bacteria</u>	"	70 (60-80)	50 (40-65)	80 (75-85)	85 (80-95)	70 (65-75)
<u>Bacteria</u> only	"	50 (45-65)	50 (45-65)	50 (45-65)	55 (45-60)	60 (55-70)

Table :8-18: Changes in Acid $KMnO_4$ (4 hrs.) Value noted with Different Slow —with and without Bacteria

Retention Time in Days	0	4	7	14
Algae	mg.per 1000 ml	% Reduction		
Scenedesmus quadricauda	10-5 (6.7-12.8)	19.4 (17.8-21.4)	29.9 (26.9-32.9)	35.8 (33.9-36.8)
Scenedesmus quadricauda with Bacteria	"	24.0 (22.8-25.9)	22.0 (19.3-24.9)	0 (0 - 0)
Aulosira fertilisima	"	56.7 (54.8-58.7)	53.7 (51.3-56.4)	47.7 (43.2-49.9)
Aulosira fertilisima with Bacteria	"	52.2 (51.8-54.6)	47.7 (44.4-50.8)	31.3 (28.4-33.8)
Nostoc pyriformis	"	34.3 (32.1-36.4)	44.8 (41.4-46.8)	35.8 (33.4-37.8)
Nostoc pyriformis with Bacteria	"	37.3 (34.6-40.9)	58.2 (56.4-61.8)	43.3 (41.8-45.6)
Mixture of all the three algae	"	55.2 (53.8-58.0)	43.3 (40.2-45.6)	44.8 (41.8-46.9)
Mixture of all the three algae with Bacteria	"	22.4 (20.8-25.0)	31.3 (30.2-32.9)	38.8 (36.5-39.9)
Bacteria only	"	50.5 (47.1-52.9)	54.2 (52.4-56.8)	34.3 (33.1-36.8)

* (+) shows % increase over 0.day value

Table :8-19: Changes in Orthophosphate Content noted with Different Slow-growing and Mixed Algae — with and without Bacteria

Retention Time in Days	0	4	7	14	21	28
Algae	mg per 1000ml			% Reduction		
Scenedesmus quadricauda	17.2 (13.5-20.8)	+8.2 * (+6.2-+10.5)	+4.9 * (+2.4-+7.4)	-9.8 (-6.9--11.8)	49.1 (47.5-51.4)	86.9 (82.5-87.4)
Scenedesmus quadricauda with Bacteria	"	+36.0 * (+34.5-38.7)	+23.0 * (+20.5-+25.5)	- 60.6 (-58.4--63.4)	78.6 (77.2-80.5)	86.6 (82.6-87.4)
Aulosira fertilisima	"	18.0 (16.5-21.0)	36.0 (34.4-37.8)	80.3 (77.3-82.3)	78.6 (76.6-80.4)	77.0 (75.4-80.4)
Aulosira fertilisima with Bacteria	"	24.6 (22.8-25.9)	27.8 (25.9-29.8)	72.1 (70.4-75.8)	75.4 (73.8-77.8)	78.4 (76.4-80.9)
Nostoc pyriformis	"	4.9 (1.8-6.8)	9.8 (7.8-12.4)	65.5 (62.4-67.8)	62.3 (60.5-64.9)	57.3 (54.3-60.4)
Nostoc pyriformis with Bacteria	"	3.3 (1.5-5.9)	6.5 (4.5-8.7)	78.6 (76.4-81.0)	70.4 (68.3-73.4)	60.6 (58.8-63.8)
Mixture of all the three algae	"	+8.2 * (+7.5-+9.8)	-1.6 (+1.5--4.5)	- 9.8 (-6.8--11.8)	47.5 (45.4-49.8)	65.5 (63.4-68.8)
Mixture of all the three with Bacteria	"	4.9 (1.8-7.8)	14.7 (12.5-15.9)	72.1 (70.5-74.6)	86.8 (84.4-88.7)	83.6 (81.4-85.9)
Bacteria only	"	27.1 (25.6-30.1)	32.6 (29.8-34.1)	47.8 (46.9-49.2)	23.8 (21.4-26.0)	17.4 (13.9-20.5)

* (+) shows % increase over 0-day value

Table :8-20: Changes in Ammonia-Nitrogen Content noted with Different Slow-growing and Mixed Algae - with and without Bacteria

Retention Time in Days	0	4	7	14	21	28
Algae	mg per 1000 ml	% Reduction				
Scenedesmus quadricauda	16.2 (13.8-20.2)	20.7 (17.7-22.8)	36.8 (35.4-38.8)	75.9 (73.8-77.9)	77.0 (74.5-80.8)	97.7 (95.8-99.8)
Scenedesmus quadricauda with Bacteria	"	11.5 (8.4-13.8)	33.3 (30.3-35.5)	97.7 (95.8-99.4)	98.8 (97.8-99.5)	96.5 (94.3-99.0)
Aulosira fertilisima	"	18.0 (15.8-20.9)	36.0 (34.5-39.0)	80.3 (78.4-83.5)	78.6 (76.5-80.4)	77.0 (75.8-78.9)
Aulosira fertilisima with Bacteria	"	62.0 (60.5-64.8)	97.0 (95.2-99.0)	97.7 (96.4-99.9)	95.4 (93.2-98.0)	95.4 (92.8-97.6)
Nostoc pyriformis	"	23.0 (20.8-25.4)	51.7 (49.5-54.0)	95.4 (93.4-98.0)	95.4 (94.3-97.0)	94.2 (92.8-95.9)
Nostoc pyriformis with Bacteria	"	8.0 (6.5-10.4)	27.6 (25.5-29.8)	96.5 (93.8-98.4)	92.0 (90.5-95.0)	87.3 (85.4-89.0)
Mixture of all the three algae	"	24.1 (21.8-26.9)	37.9 (35.8-39.4)	96.5 (93.8-99.9)	96.5 (92.5-98.8)	95.4 (92.4-98.5)
Mixture of all the three algae with Bacteria	"	30.0 (27.8-32.6)	43.9 (41.8-46.9)	97.7 (95.8-99.0)	96.5 (93.0-98.5)	94.4 (92.5-96.0)
Bacteria only		+1.9 * (-1.8-+3.5)	-21.0 (-19.2--24.5)	44.4 (42.6-47.5)	45.5 (43.2-47.4)	47.5 (45.5-49.4)

* (+) show % increase over 0-day value

With bacteria this alga is able to utilize 97% in 7 days. Nostoc alone is able to use 95% in 14 days and along with the bacteria, similar results are obtained, but the values are found to increase later. When all the three algae are grown together, about 96% is utilized in 14 days. Similar results are obtained when the mixed algae are grown in the presence of bacteria. Bacteria alone can utilize about 47% only in 28 days.

DISCUSSION

A comparative study of the results obtained for the ten bacteria, the three fast-growing algae with and without the bacteria, and the three slow-growing algae with and without bacteria is made below. All the algal and bacterial cultures were grown under the same conditions of light and temperature for comparable results.

Turbidity

(a) Bacteria. Reductions in turbidity are found to be greater during 7-28 days than during 4-7 days. In the latter case, the reductions varied between 23.4% and 63.0% and in the former case between 40.7% and 95.7%. In the case of the mixed bacteria the reductions are comparatively greater.

The greatest reductions in turbidity during 7-28 days has to be attributed to endogenous respiration and the reduction during 4-7 days to oxidative assimilation of the soluble organic matter.

(b) Fast growing algae. In the case of Chlorella alone, the turbidity values are higher due to the fact that the minute cells easily passed through cotton. In the other two cases the reductions in turbidity are greater with the algae alone than with the algae with bacteria. Similar results are recorded in the case of the mixture of algae with and without the bacteria, thus, showing the anti-bacterial activity of Chlorella and Oscillatoria species.

(c) Slow growing algae. Reductions are greatest during 4-7 days and much less during 7-28 days. This has to be attributed to the death and disintegration of the algae which had contributed to the turbidity.

The individual alga with and without bacteria shows more reduction than the bacteria alone.

Hydrogen ion concentration (pH)

Individual bacterium or the mixture of bacteria do not show any reduction in pH indicating that the bacteria are not producing any CO_2 or sufficient CO_2 or organic acids to reduce the pH either to the neutral or acidic sides.

Maximum values are reached in 7 days retention time in the case of all the fast growing algae and the value is highest in the case of Chlorella alone. Chlorella-bacterial mixture shows slightly less reduction. Oscillatoria obscura alone shows comparatively higher pH than Oscillatoria obscura with the ten bacteria. Oscillatoria chalybea also behaves in a similar manner. After 14 days there is a fall in pH indicating death and destruction of the algae on 21st and28th days in the preliminary experiments which are not reported here.

A considerable increase in pH to 11.0 has to be attributed to the vigorous photosynthetic activity of the algae. The presence of bacteria in the algae-bacteria culture does not decrease the pH values considerably.

In the case of the slow-growing algae an increase similar to that of the fast growing algae is noted in 28 days retention time but the values are comparatively lower indicating less activity. The presence of bacteria also does not result in any lowering of the pH values.

So, it would appear that the alga Chlorella vulgaris is the most vigorous and yielding more chlorophyll in sterile sewage rather than in the presence of bacteria.

Relative Stability

Excepting Brevibacterium sp (0-137), all the other bacterial strains show low values for 4-7 days, but thereafter the values increase during 7-28 days, including the mixture of bacteria. In the case of Brevibacterium sp (0-137) the value is very low.

In the case of the three fast growing algae the values for 4-7 days are much greater than in the case of the bacteria. But the individual alga with bacteria shows less stability values than [illegible] the corresponding values for the individual alga, showing anti-bacterial activity of the three alga.

The three slow growing algae, on the other hand, do not show values as high as the three fast-growing algae but take the intermediate position between the bacteria and the fast growing algae. No anti-bacterial effect is seen in the case of these three algae.

Phenolphthalein alkalinity

(a) This test was not done by mistake for the bacterial cultures.

(b) Fast growing algae. Of the three algae of this group *Chlorella vulgaris* alone shows the highest value indicating again, more vigorous and rapid algal synthesis in sterile sewage. But when bacteria are present with the alga, the value is appreciably lower indicating either inhibition of the activity of *Chlorella* by the bacteria present or due to inhibition of the bacteria by the anti-bacterial organic substances released by the alga. From the observations of other workers about which we have already referred on pages 46 and 47 of chapter 4, it would appear that it is the former case.

In the case of the other two algae, the values are comparatively lower than that for *Chlorella* alone, but higher than the values obtained with bacteria. Thus symbiotic activity results in lower values for these two algae also. The same is the case with the three mixed algae and bacteria.

Maximum values are obtained on the 4th day and less thereafter on account of their senescence. The values for this test are indications of the degree of vigorous photosynthesis. The more vigorous is the photosynthesis, the greater is the oxygen production, and the greater is the phenolphthalein alkalinity, all the three factors being closely inter-related. Usually photosynthesis is vigorous during short retention periods. Oxygen production and cellular material production appear to be greatest in the case of <u>Chlorella vulgaris</u>.

(c) <u>Slowing growing algae</u>. The values are generally found to be lower than in the case of the fast-growing algae. The maximum value is reached in 14 days retention time and thereafter there is a gradual decrease in values indicating decline in growth and activity, when there would be greater respiration than oxygen production. Thirdly, the individual alga with the bacteria seems to produce more phenolphthalein alkalinity than with the algae alone in all the three cases. In this respect, the three slow-growing algae differ from the three fast-growing algae. There does not seem to be any anti-bacterial activity.

The algal cells are affected by long retention periods, thereby their Chlorophyll content is reduced and in consequence, phenolphthalein alkalinity and oxygen produced are reduced.

<u>Acid $KMnO_4$ Value</u> (<u>4 hours</u>)

(a) Bacteria. During short retention periods of 4-7 days, the percentage reduction of individual bacterium is great in most cases. On other days (7-28 days) the percentage of reduction is comparatively lower. The organism Corynebacterium sp (0-137) alone is an exception and shows comparatively less reduction. The mixture of all the bacteria shows the maximum reduction of 54.2%. Next, come Micrococcus sp (0-195) with 49.5%, Flavobacterium (0-140), with 48.5%, the rest showing varying degrees of reduction.

(b) Fast growing algae. The algae, individually, produced the greatest reduction in 7 days. The mixture of algae produces greater reduction than with the algae plus bacteria. The individual alga in combination with bacteria also shows less reduction. So, it would appear that there is bacterial inhibition by the three fast-growing algae.

(c) Slow-growing algae. The values are comparatively lower than in the case of the fast growing algae. The percentage of reduction is greater during 4-7 days than during 7-28 days. During the later stage, there was less reduction on account of their death. The mixture of algae with the bacteria produces greater reduction than with the bacteria alone.

Orthophosphates

(a) Bacteria. In this case also, reductions during 4-7 days are comparatively greater than during other days. Individual bacterium produces varying amounts of phosphate reduction. Flavobacterium sp (0-140) produces 67% reduction and Micrococcus sp (R-66) the least (0-20%) in 7 days retention time. But

Corynebacterium (0-136) alone shows an increase upto the 14th day and reduction later. The reason for this is not clear. A similar increase is recorded for Brevibacterium spp (0-166 and 0-201) during 7 to 28 days of retention period.

(b) Fast growing algae. Chlorella vulgaris alone produces nearly 97% reduction and Chlorella with bacteria only 89%. Similar results are recorded in the case of the two Oscillatoria species as well.

The highest reduction in phosphates may be due to the higher phenolphthelein alkalinity, pH and their utilisation by the alga when the pH ranged between 10 and 11 in most cases in this group of fast growing algae.

(c) Slow growing algae. Generally, the three algae produce maximum reduction on the 14th day and thereafter the reductions are less. Aulosira fertilisima produces maximum reduction of 80.3%. In a few cases the maximum reduction is also recorded on the 28th day. The percentages of reduction are also not as great as in the case of the three fast growing algae as the values for pH and phenolphthalein alkalinity are also not correspondingly great. The mixture of algae and bacteria produces more reduction than the algal mixture alone.

Ammonia-Nitrogen

In 4-7 days, Flavobacterium sp (0-140) consumes a maximum of 30.5% and the mixture of bacteria only about 21.0%; while Micrococcus (R-66) only 5%.

During 7-28 days, more ammonia-nitrogen is used up. A maximum of 56.7% is used by the <u>Corynebacterium</u> <u>sp</u> (0-145) and a minimum of 14.5% only by the <u>Micrococcus</u> <u>sp</u>(0-195). The mixture of bacteria shows a reduction of 47.5% only.

The reductions are considerably higher in the case of the three fast growing algae. <u>Chlorella</u> alone shows a reduction of 98.6%, while the same alga with bacteria shows less i.e. 96.5%. <u>Chlorella</u> consumes the highest amount due to more vigorous photosynthesis than the other two algae.

<u>Oscillatoria obscura</u> alone shows a reduction of 95.5% and with bacteria 93.8%. Corresponding values for <u>O.chalybea</u> are 96.7% and 69.6% respectively. The algal mixture alone shows 97.6% and with bacteria 95.9%, showing anti-bacterial effect of the three alga together.

The three slow growing algae, on the other hand, consume more nitrogen in the presence of bacteria than when alone. The consumption of this nutrient is much less during 4-7 days than during 7-28 days. Of the three algae, <u>Scenedesmus</u> consumes the maximum of 86.9%, and <u>Nostoc</u> the lowest i.e. 57.3%. The algal mixture with bacteria consumes 83.6% and without the latter only 65.5% showing definitely that there is no anti-bacterial effect.

Reviewing, it will be seen that during 4-7 days, there is oxidative assimilation by bacteria resulting in the utilisation of dissolved organic compounds, nitrogen containing substances and ortho-phosphates. The pH is always on the alkaline side. Algae also multiply during this period. Later on, the period 7-28 days appears to be the period of endogenous respiration, when autodigestion of cellular materials takes place and the medium becomes clearer. Chlorella is the fastest growing and produces more algal cells in sterile sewage in the shortest time. The anti-bacterial activity is most conspicious during this period. The log phase of Chlorella growth is 4-7 days, when the maximum amount of oxygen is produced. After the log phase, oxygen depletion occurs. During long retention period, higher respiration results in more consumption of oxygen than its liberation; less chlorophyll production and more endogenous respiration.

Short retention periods favour quicker algal growth, more dissolved oxygen, more phenolphthalein alkalinity, more reductions in phosphates and in ammonia-nitrogen than compared to long retention periods. Fast growing algae should be preferred for algal-bacterial symbiosis and for reducing the period of retention to less than 2 days.

So, for quicker purification of sewage in less than 2 days, the factors influencing algal-bacterial symbiosis are: select algal and bacterial species and their overlapping from the very starting, nutrients, and light intensity. The shorter retention period will be helpful in removing as quickly as possible the extra-cellular products of algae which may be anti-bacterial.

The relationship between organisms has been described in several ways. One of them is designated as 'Symbiosis' to describe the intimate and constant association of two organisms with reciprocal benefits; and there are three types of symbiotic relationships such as neutralistic, mutualistic and antagonistic (De Lay, 1956). "Neutralistic" symbiosis is the relationship existing between two organisms when they have little or no effect on one another. If one or both participants benefit from the relationship without any injurious effects on the other, this relationship is called "mutualistic". In "antagonistic" symbiosis one of the two participants may or may not derive any benefit from the relationship or one of them is deleterious to the other. So, it will be seen from the above definition, that the algal-bacterial symbiosis in the case of the three fast growing algae is "antagonistic symbiosis" and that in the case of the other three slow-growing algae appears to be of the "mutualistic" type. Further work is necessary with pure cultures of different green and blue-green algae and diatoms to find out the type of symbiotic relationship existing in sewage oxidation pond.

SUMMARY

1. Pure culture studies of 10 strains of bacteria and 6 algae with and without bacteria confirm the findings recorded in Chapter 4.

2. Several types of bacteria with differing rates of activity are found to utilise the soluble organic and other nutrient substances in sewage during 4-7 days by oxidative assimilation.

3. Chlorella vulgaris, Oscillatoria obscura, and Oscillatoria Chalybea carry on "antagonistic symbiosis " while Scenedesmus quadricauda, Nostoc pyriformis and Aulosira fertilisima carry on " mutualistic symbiosis."

4. Final purification takes place; and it results in considerable reduction in turbidity, BOD (corresponds to relative stability); Organic matter, ammonia nitrogen and phosphates during 7-28 days as a result of endogenous respiration. There is no accumulation of sludge as in activated sludge process.

5. Chlorella vulgaris is a very fast growing alga in sewage producing comparatively more chlorophyll, dissolved oxygen, pH and phenolphthalein alkalinity. As a result of the latter, almost all ortho-phosphates and Am-N are removed from the medium.
