C H A P T E R - 4

S C I E N T I F I C R E S U L T S

C O N T E N T S

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CHAPTER IV

SCIENTIFIC RESULTS

Two series of experiments were carried out. In the first series, the algae used were a mixture of four algal specimens. They were mostly Oscillatoria chalybea and Oscillatoria obscura (about 95%) and Spirulina sp. and Chlorella vulgaris (about 5%). In the second series of experiments, the alga used was exclusively Anacystis nidulans.

The two algal-cultures were previously acclimatized to Baroda settled, strained raw sewage and were found growing luxuriently under the laboratory conditions after several transfers in raw sewage, each transfer period extending to 7 - 10 days before they were used in the experiments according to the procedures already described. The two algal cultures are referred to as (a) Oscillatoria spp. and (b) Anacystis nidulans.

In both the experiments raw sewage was used as a control along with the algae-treated sewage as already described and the experiments were done on two different occasions. The results of physical and chemical examination of the two series of experiments are shown

in Table I and Table II in the Appendix. The results are described in the following pages in the following order: first about Oscillatoria and then about Anacystis.

(A) Physical variables:

(i) Temperature of the liquid cultures:

In the case of Oscillatoria cultures the range of temperature was 27.5 to 28.0°C and in the case of Anacystis nidulans it was 27.5 to 29.0°C.

(ii) Colour and Turbidity:

In the case of two controls, the cultures were brownish and turbid. Brownish flocculant precipitates also were seen. But in the case of the algae-treated cultures they were greenish in the case of Oscillatoria and bluish in the case of Anacystis; and the cultures were comparatively clearer. No brownish precipitates were seen at the bottom.

(iii) pH:

In the case of two controls the pH values ranged from 7.8 to 8.3 and from 7.5 to 7.8. But more definite increases were noted in the algal-treated samples. In

the Oscillatoria culture, the pH ranged from 7.8 to 9.6 and in the case of Anacystis nidulans it was 7.5 to 9.7.

(B) Chemical variables:

The important results of chemical analysis are shown in Table No.1 and Table No.2, and they are described briefly below:

(i) Phenolphthalein alkalinity:

Little increase in the phenolphthalein alkalinity was found in the control flasks. But in the case of algae treated samples it shot up on the second day and gradually increased upto 6th day. This indicates that carbon dioxide from bicarbonates were also being used up for algal photosynthesis and as a result carbonates were thrown down and they were responsible for increase in phenolphthelein alkalinity and in increased pH too.

(ii) Ammonia nitrogen:

There was no significant change of ammonia nitrogen in the control flasks. But there was a sharp fall in the case of the two algae. On the second day 86 - 93%

reduction was found in the case of Oscillatoria spp., and 58.7% of reduction in the case of Anacystis nidulans; and on the 6th day 95.8% reduction was found in the case of Oscillatoria spp. and 80.2% reduction in the case of Anacystis nidulans. These changes would seem to indicate that ammonia nitrogen was used up as a nutrient for the algal growths and no specificity was found for the different algae. The algae seem to bring about 80 to 96% reduction of ammonia nitrogen present in sewage within six days.

(iii) Nitrate and Nitrite nitrogen:

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

(iv) Orthophosphate (PO_4)

No appreciable change was found in the control flasks. In the case of Oscillatoria spp. 76.4% and in the case of Anacystis nidulans 62.8% decrease were shown on 2nd day and on the sixth day Oscillatoria spp. showed 91.5% and Anacystis nidulans 74.3% reductions.

In general the algae seem to have brought about 70 to 92% of decrease in orthophosphate showing that orthophosphate from sewage is being utilised as a nutrient for algal growths.

(v) BOD_5 at 20°C:

65 to 74% reduction of BOD_5 was found in the control flasks within 6 days. But this amount of reduction of BOD_5 was found within two days in the two algae-treated samples. Within 6 days Oscillatoria spp. brought about 92% and Anacystis 92% reductions. This might show that organic matter from sewage has been rapidly removed by algae and the amount removed was to a greater extent when it was compared with the control flasks.

(vi) COD:

64 to 66% reduction was found in control flask within 6 days and about the same amount of reduction was found in the algal flasks within 2 days. Within 6 days Oscillatoria spp. showed 87.6% and Anacystis nidulans 92.14% reduction.

These results confirm that bio-degradable organic matter is rapidly removed and that too, to a greater extent in the algal samples.

64 to 74% reduction of COD and BOD in the control raw sewage has to be atrributed to the phenomena of mechanical flocculation, bio-flocculation and bio-precipitation which are of common occurrence in nature

(Heukelekian 1941). In the case of the two algal samples the increased percentage reduction has to be ascribed to photosynthetic oxygen furnished to bacteria as a result of algal bacterial symbiotic reactions in the algal growth cultures.

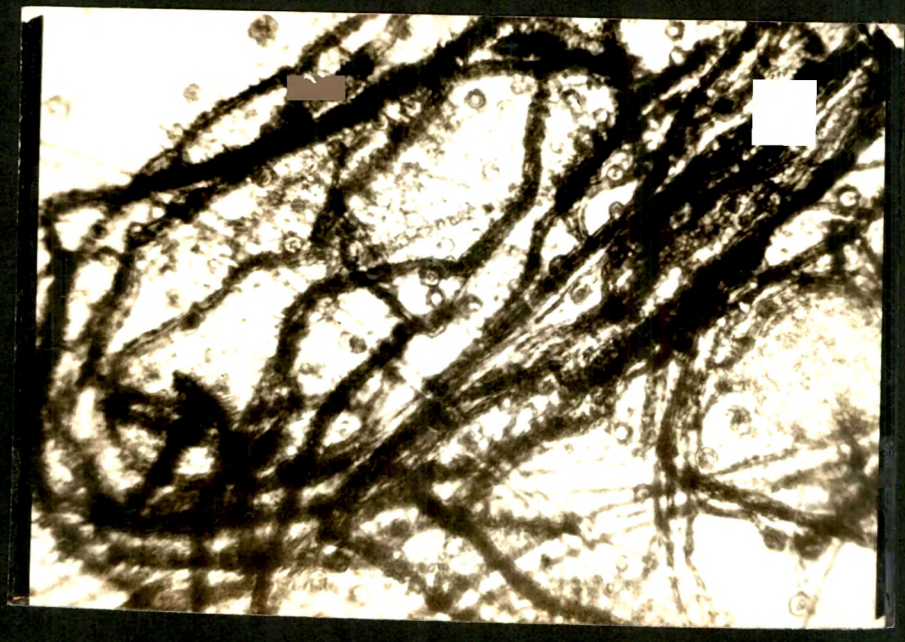
(C) Biochemical variables (Vide Fig. 12 to 16)

The results are tabulated below showing the percentage reductions of the various constituents on 0, 2, 4 and 6 days of the detention period.

P.T.O.

Percentage reduction of Biochemical variables on the experiment using Scenedesmus obliqus and Baroda strained raw sewage:

Detention period in(days)	Control - Raw sewage Found	Used	% reduction	Raw sewage + Scenedesmus obliqus Found	Used	% reduction
Free sugar mg/l						
0	41.8	-	-	41.8	-	-
2	35.6	6.2	14.8	29.6	12.2	29.2
4	22.2	19.6	46.9	14.8	27.0	65.5
6	18.7	23.1	55.3	5.6	36.2	86.6
Total sugar mg/l						
0	110.8	-	-	120.4	-	-
2	96.5	14.3	12.9	89.4	31.0	25.75
4	70.3	40.5	36.5	53.3	67.1	55.8
6	45.2	65.6	59.1	20.8	99.6	82.7
Protein mg/l						
0	6.1	-	-	6.0	-	-
2	4.8	1.3	21.3	3.8	2.2	36.7
4	4.5	1.6	25.6	3.2	2.8	46.7
6	2.7	3.4	55.7	2.0	4.0	66.7
Amino-acid nitrogen mg/l						
0	5.1	-	-	5.3	-	-
2	3.8	1.3	25.5	3.0	2.3	43.4
4	3.1	2.0	39.2	2.6	2.7	50.9
6	2.4	2.7	52.9	2.1	33.2	60.3
Volatile acid						
0	82.8	-	-	84.0	-	-
2	46.8	36.0	43.5	44.8	39.2	46.7
4	30.0	52.8	63.8	15.0	69.0	82.0
6	15.6	57.2	69.1	10.0	74.0	88.1



Dark brown filaments resembling Leptothrix ochracea

(D) Biological changes using Oscillatoria spp.

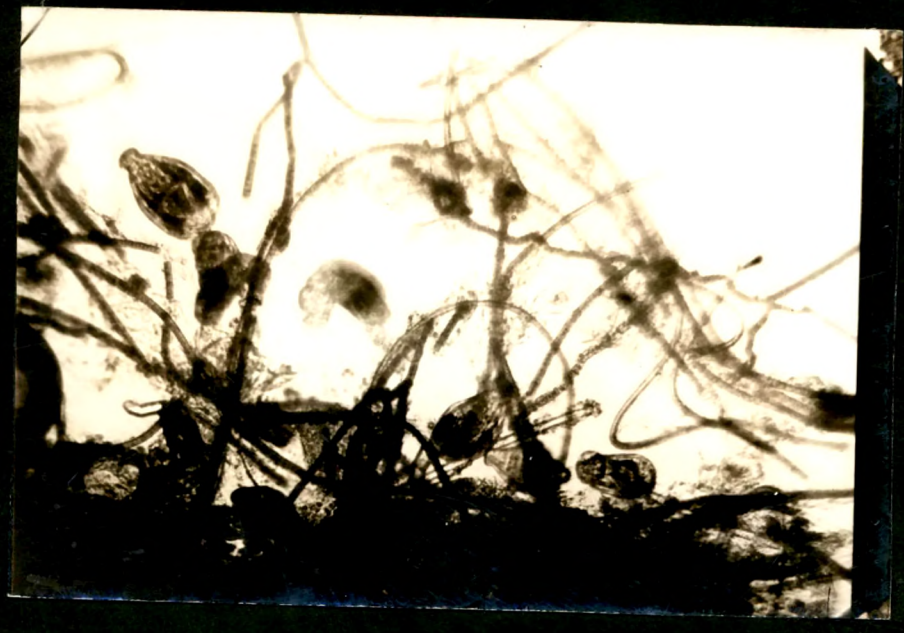
The biological changes noted are shown in Table. III & V.

It will be seen from a study of the table that (a) dark brown filaments resembling Leptothrix ochracea were always seen in varying numbers in both the control and algae-treated flasks on all days.

(b) Brownish flocculant precipitates were seen either in suspension or at the bottom of the control flasks only but not in algal-treated flasks where they were seen intermixed with algal flora but in considerably smaller amounts as organic debris.

(c) Paramoecium caudatum was seen in both the culture flasks, along with Spathidium spathula. Aspidisca costata was seen in larger numbers in the algae-treated culture flasks. Vorticella spp. (Smaller sizes) were seen in greater number in the control flasks. The rotifer Lucane sp. was seen in the algae-treated flasks only. There were some times seen in the green algal forms inside their stomach but mostly with the brownish organic debris.

(E) Bacteriological examination (Sanitary aspect) in high-rate aerobic oxidation pond using Oscillatoria spp. (Table - VI)



Rotifer Lucane sp. in the algal sample

Detention period in days	Control : Raw sewage Coliform	Total colonies count	High-rate : Algae-treated Coliform	Total colonies count
0	16.9×10^{12}	10.2×10^{11}	16.09×10^{10}	17.9×10^{10}
2	90*	81.7*	$+144.81 \times 10^{10}$	$+66.42 \times 10^{10}$
4	99.99*	99.99*	99.98*	99.63*
6	99.99*	99.99*	99.99*	99.99*

Shows exact increase in number. * Shows % reduction.

Control: Raw Sewage

A reduction of about 90 percent on 2nd day and 99.99 percent on the 4th and 6th day is seen. But at the end of 6th day still 46.0×10^{4} coliforms are present.

Regarding total colonies count a decrease of 81.7 percent on the 2nd day and 99.99 percent on 4th and 6th day are seen. Still 14.1×10^{6} residual bacteria are present at the end of 6th day.

High-rate (Algae treated)

In this case, coliform organisms have increased on the 2nd day by about 144.81×10^{10} but on the following 4th and 6th day the reduction is 99.98 and

99.99 percent respectively. Still 18 x 10^4 coliform colonies are present on the 6th day.

Regarding total colonies count, it increases on 2nd day by 66.42 x 10^{10}. While on 4th and 6th day there is a decrease of 99.63 and 99.99 percent respectively. It is evident that still there are 14.6 x 10^5 bacteria at the end of the 6th day.

(ii) Distribution of a few important attributes among the 200 dominant isolates:

Table VII shows the distribution of a few important properties of the dominant bacterial isolates in the experiments using Oscillatoria. In this case, the predominant isolates were all rods, mostly whitish and gram-negative.

Citrate utilizers on the 4th and 6th day of the detention period were almost half the number of those recorded for 0 day and 2 days for Oscillatoria treated samples.

In the case of Oscillatoria, the gelatin hydrolysis showed that the number of bacteria was nearly the same on 0 and 2nd day and 'nil' on the 4th and 6th day.

The distribution of tributyrin hydrolysers was also similar to protein hydrolysis although the percentages of positives on 0 and 2nd day were nearly one half of those recorded for tributyrin.

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

In table VIII are shown some of the important biochemical characteristics of the dominant bacteria as revealed in this experiment. In this experiment, the percentages of positives showing 'acid' or 'acid and gas' on the 0 and 2nd day were similar but they were reduced to nearly one-half on the 4th and 6th day. Nitrate reducers were nearly 50% on all the four detention periods, and those producing H_2S being less than 15% on the 0 and 2nd day only. "Oxidative" reactions were nearly 50% on 0 and 2nd day and their numbers were reduced to nearly half on the 4th and 6th day. But the "fermentative" reactions were only about 30% throughout while those wshowing "neutral" were double on the last two days; and also were marked by a significant percentage of "alkaline" reactions.

(iii) Dominant Micro-organisms in High-rate aerobic pond:

The bacteria found in high-rate oxidation pond grown on domestic sewage belong predominantly to gram-negative, non-spore forming rods, affecting sugars and - tentatively determined as belonging to the genera which varies in dominance on the different detention periods. The dominant genera are recorded in Table 7 from which, it will be seen that the dominant genera on 2nd day were Aerobacter, Alcaligens, Comamonas, Pseudomonas. On 4th and 6th days, the dominant genera were Aerobacter, Alcaligens, Comamonas and Zoogloea which were common in both 4th and 6th days also. Comamonas constitute the major part of the predominant bacteria of high-rate oxidation pond of domestic sewage, Alcaligens, Zoogloea, Aerobacter and Pseudomonas found to be the main generic bacteria constituents of high-rate aerobic pond.

Most of the gram-negative rod shaped strains did produce acid from glucose, though some like Alcaligens and Comamonas did not utilize glucose.

Colourless organisms attacking glucose with acid production are Achromobacter, Bacillus, and

and Zoogloea. Acid and gas in glucose are produced by the strains like Aerobacter, Aeromonas, E. coli, Proteus and Zymomonas (Bergey, 1957).

The strains which react oxidatively in Hugh and Leifson's glucose medium are Achromobacter and Zoogloea. Those which act fermentatively are: Aerobacter, Aeromonas and E. coli and those which are neutral are Alcaligens and Bacillus. And the strains which is alkaline in the glucose medium is Comamonas.

(iv) Microorganisms classified tentatively as representing "assimilatory" and "endogenous" phases:

The genera identified as the dominant bacteria in this experiment are shown in Table IX and they are tentatively classified as "assimilitory" and "endogenous" in Table No. X based on the detention periods. From a consideration of the species in the system, it is reasonable to assume that the species present in super abundance are the most active in the ecosystem (Hungate, 1962). No significant difference in composition of the bacterial flora can be found as

will be evident from those recorded for the 2nd, 4th and 6th day below:

Oscillatoria-treated experiments

2nd day	4th day	6th day
Aerobacter	Aerobacter	Aerobacter
Alcaligenes	Alcaligenes	Alcaligenes
Comamonas	Comamonas	Comamonas
Pseudomonas	Zoogloea	Zoogloea