CHAPTER

IV

THE ECO-SYSTEM

EXPERIMENTAL DATA

Setting up the laboratory model

In the laboratory model oxidation pond i.e. the glass aquarium, raw settled sewage was filled upto a depth of 9.5" and was left undisturbed for about 30 days for the growth and development of algal organisms. Periodically samples were withdrawn aseptically from the liquid medium for the several groups of tests as already described in Chapter 3. Within the first few days of storage a viscous or slimy layer of discrete particles was also found to develop on the surface and sides of the vessel and also some settled at the bottom. It was also found to disintegrate and to disappear within a week or so. The viscous scum was also separately studied and is reported in the next Chapter. (Chapter 5). Three series of experiments were done and the results of the typical experiment are discussed.

THE ENVIRONMENT

Studies on the changes taking place in the liquid medium

Physico-chemical variables. The important results are shown in table 4-1 and in Fig. 4-1. Each of these factors is described briefly below :

Colour. The colour gradually turned from brown on the 0 day to greenish yellow towards the end of the bacterial

Table 4-1. Important physico-chemical results of the samples drawn from the laboratory model oxidation pond on different days

Description	Retention time in days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
A : Physical						
1. Temperature (^{o}C)	26.6	27.0	27.3	27.5	28.0	28.5
2. Colour	Brown	Light Yellow	Greenish Yellow	Greenish	Green	Yellowish
3. Turbidity	55	35	27	22	15	11
4. pH	6.9	7.3	7.6	8.1	8.3	8.6
B : Chemical						
5. Phenolphthalein alkalinity(mg/l)	Nil	Nil	Nil	Nil	100	10
6. Total alkalinity (mg/l)	475	325	580	620	540	975
7. Dissolved oxygen (mg/l)	Nil	Nil	Nil	0.8	3.6	0.6
8. 5-day BOD at $20^{o}C$ (mg/l)	365	125	91.4	65	30.0	12.5
9. Acid $KMnO_4$ value -4 Hours(mg/l)	22.3	15.0	10.7	8.2	6.8	6.0
10. Orthophosphate (mg/l)	31.3	21.3	27.0	21.0	20.0	18.7
11. Ammonia-Nitrogen (mg/l)	60.5	42.0	21.4	15.1	12.4	19.6
12. Nitrite-Nitrogen (mg/l)	Nil	Nil	Nil	Nil	0.13	0.24
13. Nitrate-Nitrogen (mg/l)	Nil	Nil	Nil	Nil	Nil	Nil

phase-I and thereafter it became gradually green, and towards the end yellowish.

Turbidity. The decrease in turbidity during the bacterial phase was less (51%) than during the algal phase (59%). On the whole, the percentage reduction was 80%. Determination of turbidity is, according to Ecker and Lockhart (1961), a more sensitive method for estimating bacterial growth than counting of cell numbers.

pH. The pH was found to increase from 6.9 to 7.6 during the bacterial phase I and from 7.6 to 8.6 during the algal phase II. The increase has to be attributed mainly to algal activities.

Alkalinity. The phenolphthalein alkalinity was noted only on the last two occasions of the algal phase.

Total alkalinity was found to be fluctuating throughout. Comparatively the values were higher during the algal phase II than during the bacterial phase - I.

Dissolved oxygen. During bacterial phase I there was no estimatable dissolved oxygen although the liquid was greenish-yellow. But later on during the algal phase the oxygen content increased to a maximum on the 21st day (3.6 mg/l) and than decreased to 0.6 mg/l on 28th day as the algae started decaying.

Biochemical oxygen demand. (5 days at 20°C). During the bacterial phase I there was a reduction of 75% and during

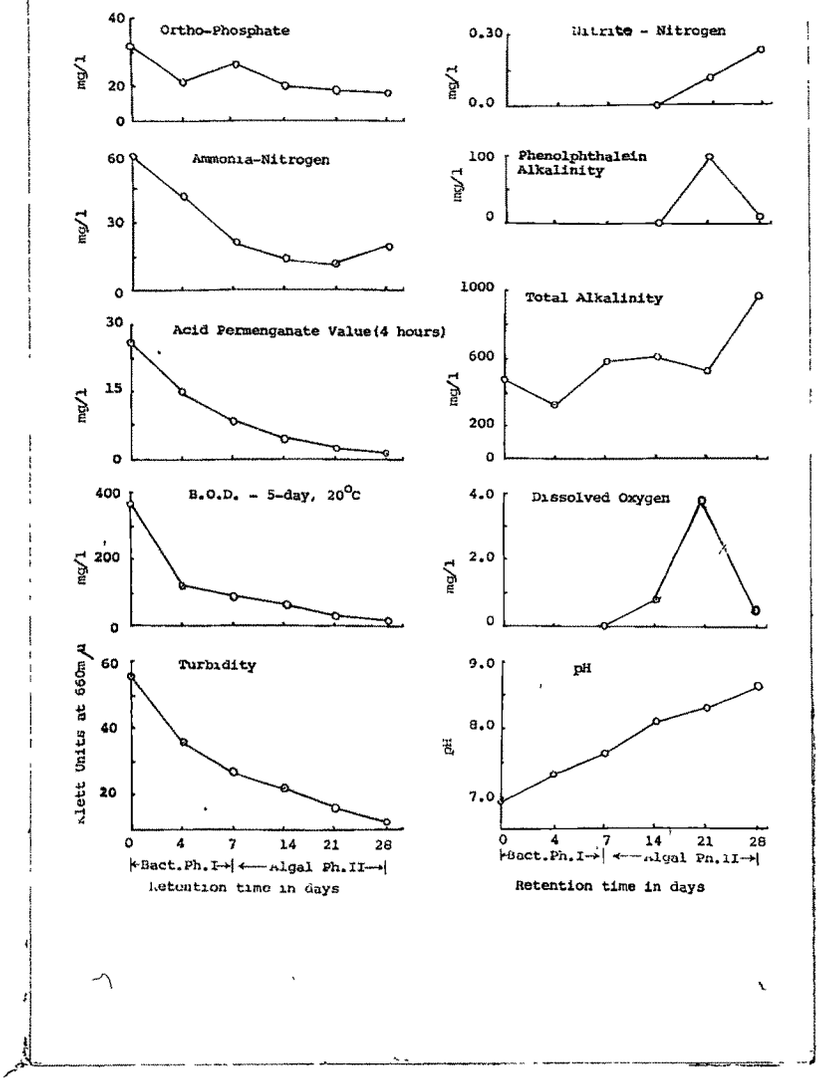

Fig.4-1: Changes in Physico-chemical variables

the algal phase II 86.8%. On the whole, the reduction was 97.0%. Similar reduction values have been reported by Oswald and Gotaas (1957).

Four hours acid $KMnO_4$ value. The values for this factor show more or less a similar trend to that of BOD. During bacterial phase I the reduction was 52% as against 43.8%. during the algal phase II. On the whole the reduction was 73%.

Ortho-phosphate. A slight reduction of about 14% is noticeable during the bacterial phase I but later on there is an increase and then a decrease during the algal phase II. The overall reduction was only 40.2%

Ammonia-nitrogen. In this case, there was a sharp fall during the bacterial phase I amounting to 65% reduction and later on the decrease was gradual during the phase II which amounted to 8.3%. The total reduction in both the phases taken together was 67.7%.

Nitrite-nitrogen. This factor was practically nil till the 14th day; and thereafter there was a slight increase which reached a maximum of 0.24 mg/l on the 28th day.

Nitrate-nitrogen. This was not detected at any time.

From a careful study of the Fig. 4-1, it will be seen that there is an inverse correlationship among turbidity, 5 day BOD, and 4 hours $KMnO_4$ value on the one hand and among pH,

dissolved oxygen and total alkalinity on the other hand.

Changes in the Soluble Organic Constituents

The results are shown in Table 4-2 and fig. 4-2.

Free Sugar. The value for free sugar increased nearly thrice on the fourth day and thereafter decreased to nil upto the 14th day, and later on it was not detected.

Total sugar. This also increased on the 4th day to nearly double its original value and later on decreased gradually.

Amino acid nitrogen. This also showed a similar increase, on the 4th day and later on decreased gradually.

Protein. Also increased on the 4th day and later on decreased gradually.

Total fat. In contrast to the above four constituents, the total fat showed a decrease on the 4th day, and increased gradually upto the 14th day when nearly three times the original zero day value was reached. Then, it decreased on the 21st day, but increased on the 28th day.

In short the first four constituents showed almost a similar trend, while the values for the total fat ran in the opposite direction.

Chlorophyll -a. Towards the end of the bacterial phase when the liquid became greenish-yellow chlorophyll-a was found to be 46.60 mg/l; and thereafter there was a rapid increase to a maximum of 105.00 mg/l on the 21st day and a decline thereafter. Senescent algae are yellowish and low in chlorophyll. They

Table 4-2 (a). Changes in the Soluble Organic Constituents in Samples drawn from the Laboratory Model Oxidation Pond on different days.

Description	Retention time in days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
1. Free sugar (mg/l)	0.73	2.10	0.93	Nil	Nil	0.09
2. Total sugar (mg/l)	2.10	4.90	3.50	2.35	1.20	0.60
3. Amino acid nitrogen(mg/l)	0.25	0.63	0.41	0.31	0.17	0.22
4. Protein (mg/l)	10.00	19.00	13.00	11.37	10.75	11.25
5. Total fat (mg/l)	18.40	12.50	31.16	60.15	23.60	44.20

Table 4-2 (b). Change in the Chlorophyll Content in Samples drawn from the Laboratory Model Oxidation Pond on different days.

Description	Retention Time in Days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
Chlorophyll-a (mg/l)	Nil	Nil	46.60	100.00	105.00	11.90

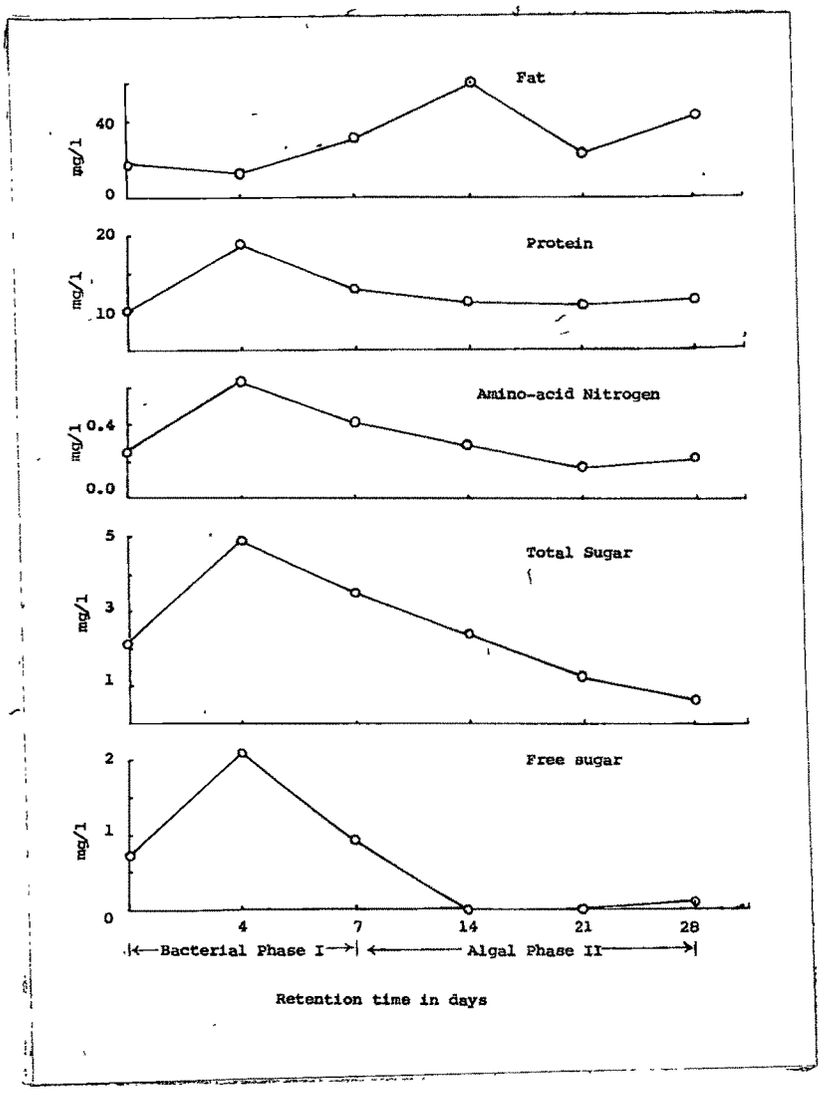

Fig.4-2: Changes in Soluble Organic Constituents

consume more oxygen and synthetise less. Autolysis of older cells may serve as a source of nutrition for new cells, but ultimate death will lead to putrification, and higher BOD of the effluent.

BIOLOGICAL CHANGES

Biological changes are reported in table 4-3. From the study of the table it will be seen that raw sewage was practically free from any protozoan or algal forms excepting for the stray occurrence (rrr) of the two forms Amoeba sp and Arcella vulgaris.

Significant changes were noted from the fourth day onwards. On that day, the most dominant protozoans seen were Astasia sp. (a flagellate) and Vorticella microstoma (a stalked ciliate). The common (C) forms were Bodo caudatus, Chilodonella uncinate, Colpodium colpoda, Paramecium caudatum and Tetrahymena pyriformis (Ehrenb). Those that were less common (r), rare (rr) or stray (rrr) were Polytoma, Chilodonella cucullula, Frontonia sp, Glaucoma scintillans, Spathidium spathula and Podophrya fixa.

On the 7th day, Chilodonella cucullula (a free swimming ciliate) was the only dominant form, Aspidisca costata, Chilodonella uncinata, Colpodium colpoda (Ehrenb), Spathidium spathula, Glaucoma scintillans (all free swimming ciliates) and Vorticella microstoma (a stalked ciliate) were the common forms. Epistylis plicatilis (Fig.2-3), Carchesium polypinum (Fig.2-3),

Table :4-3: Biological Changes

Description	Retention time in days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
A. Rhizopoda :						
1. Amoeba sp,	rrr	-	-	-	-	-
2. Arcella vulgaris	rrr	-	-	-	-	-
B. Flagellata :						
3. Bodo caudatus	-	c	rr	-	-	-
4. Astasia sp.	-	cc	r	-	-	-
5. Polytoma	-	r	rr	rr	-	-
C. Ciliophora (Free swimming)						
6. Aspidisca costata	-	-	c	c	rr	rr
7. Chilodonella uncinata	-	c	c	rr	-	-
8. Chilodonella cucullula	-	r	cc	c	r	-
9. Colpidium colpoda (Ehrenb)	-	c	c	r	-	-
10. Frontonia sp.	-	r	rr	-	-	-
11. Glaucoma scintillans (Ehrenb)	-	r	c	c	rr	-
12. Lionotus fasciola (Ehrenb)	-	-	r	rr	r	r
13. Paramecium caudatum	-	c	r	r	rr	-
14. Spathidium spathula	-	r	c	rr	-	-
15. Tetrahymena pyriformis(Ehrenb)	-	c	r	rr	-	-
D. Ciliophora (Stalked)						
16. Carchesium polypinum	-	-	-	-	-	-
17. Epistylis plicatilis(Ehrenb)	-	-	-	-	-	-
18. Intranstylum sp.	-	-	r	rr	-	-
19. Opercularia sp.	-	-	-	-	-	-
20. Podophrya fixa (O.F.Mull)	-	r	rr	rr	-	-
21. Vorticella microstoma	-	cc	c	r	rr	-
22. Vorticella sp.	-	-	-	rr	r	r
23. Vorticella campanulla(Ehrenb)	-	-	-	-	-	-
E. Rotatoria						
24. Brachionus sp.	-	-	-	-	r	rr
25. Notommata sp.	-	-	-	r	rr	r
F. Algae						
Chlorella vulgaris	-	-	r	ccc	ccc	r
Oscillatoria obscura	-	-	-	r	c	rr
Oscillatoria chalybea	-	-	rr	c	c	r
Nitzschia palea	-	-	rrr	r	rr	rr
Scenedesmus quadricauda	-	-	rr	r	r	rr

Opercularis sp. (Fig. 2-3 and 2-4), Vorticella campanula (Fig. 2-7), V.convellaria (Fig.2-6) and V.nebulifera (Fig. 2-8) were not found.

Chlorella vulgaris, Oscillatoria chalybea, and Oscillatoria obscura were the less common algae forms seen. Also Nitzachia palea was seen as a stray (rrr) form. Algae were just beginning to appear.

Thus, during bacterial phase I there were more of the free swimming ciliate and only one stalked ciliate i.e. Vorticella microstoma (Fig. 2-9) which was the most dominant form. Another flagellate which was equally dominant was Astasia sp.

By the 14th day most of the ciliates and flagellates had become rarer and Chlorella vulgaris (a green alga) became most common (ccc) along with Oscillatoria spp being common (c) or less common (r), forms. Of the few protozoans which were also common (c) were Aspidisca costata, Chilodonella cucullula and Glaucoma scintillans.

On the 21st day, flagellates and ciliates became less common (r), or rare (rr); and the rotifers Brachionus sp and Notommata sp were just beginning to appear as rare (rr) forms. The alga, Chlorella vulgaris became conspicuous by its dominance. Another alga, next in importance, which appeared

were Oscillatoria obscura and O.chalybea. Scenedesmus quadricauda and Nitzschia pales were either r or rr.

On the 28th day excepting for the stray occurrences of Aspidisca costata, Lionotus fasciola, Vorticella sp, Brachionus sp and Notommata sp, there were no other protozoan of any importance. Algal forms were seen dying in greater abundance and the liquid turned yellowish.

BACTERIAL CHANGES

Changes in Coliform Group Density

The variations in the four groups of organisms belonging to the coliform group are shown in table 4-4 and in Fig. 4-3.

Coliforms at 37°C. The coliforms showed a progressive reduction from 79 x 10^6 organisms per 100 ml to 780 per 100 ml in 4 weeks. The reduction in the bacterial phase I was 99.7% and in the algal phase II it was 99.9%. The overall reduction was 99.9%.

E.Coli Type I at 44°C. They also showed a similar reduction in the bacterial phase to that of coliforms. But in the algal phase the reduction was complete.

Faecal Streptococci at 45°C. In this case, the reduction during the bacterial phase I was 99% and in the algal phase II it was complete. The over-all reduction was 100%.

Table :4-4: Changes in Coliform Group Density and Total Colonies Count

Description	Retention Time in Days					
	Bacterial Phase I			Algal Phase 2		
	0	4	7	14	21	28
A. Coliform Group Density : (MPN per 100 ml)						
1. Coliforms at 37°C	$79x10^6$	$2x10^5$	$2.3x10^5$	$35x10^3$	$35x10^2$	780
2. E.Coli Type I at 44°C	$79x10^5$	$2x10^5$	-*	$11x10^2$	Nil	Nil
3. Faecal streptococci at 45°C	$4.5x10^5$	-*	49.10^2	$2x10^3$	$24x10^2$	Nil
4. Citrate utilizers at 37°C	$4.5x10^5$	$5.8x10^5$	$1.7x10^5$	$17x10^3$	$45x10^2$	$24x10^3$
5. Total colonies count per ml at room temperature after 10 days in sewage agar.	$36x10^9$	52.10^9	$11.9x10^9$	$1.09x10^9$	$11.5x10^6$	$7.2x10^9$

* As the dilutions used were higher; there was no reaction.

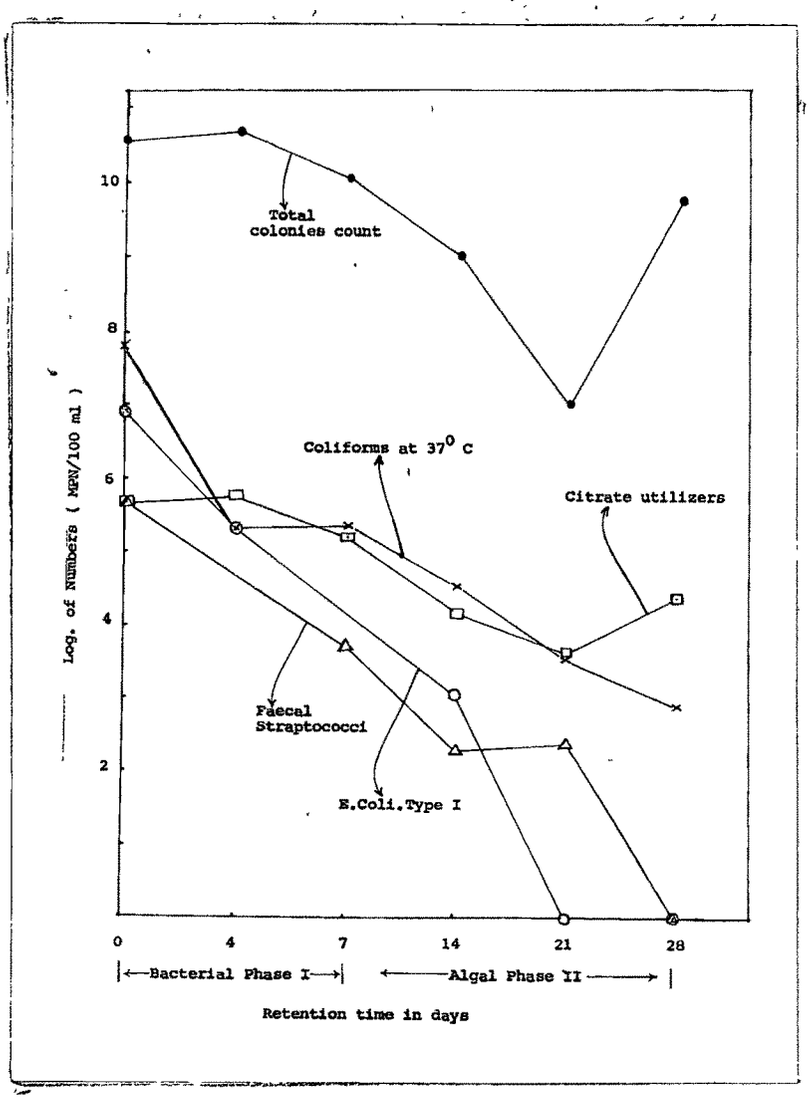

Fig.4-3: Changes in Coliform group Density and Total Colonies Count

Citrate Utilizers. During the bacterial phase I, the reduction was 62.3% and in the algal phase II there was a slight increase of 41.2%. The overall reduction was 94.7%. The noteworthy point is that there is an increase in the algal phase II even though there is reduction of about 95% on the whole.

Significance of the Results

Comparing all the results we find there are very high reductions in the case of coliforms, (Ecoli. Type I and faecal streptococci amounting to nearly 100%). But in the case of the citrate utilisers, the percentage reduction though about 95%, the numbers are nearly thirty fold higher than the coliforms present on 28th day.

Total Colonies count per ml

In this case, the percentage reduction in the bacterial phase was nearly 67 and in the algal phase II was 40 till the 28th day. But if we take into consideration the entire period, we find that there was a reduction of 86.6%. Compared to the reduction figures in coliforms and E. Coli type I, this reduction is much less. But the reduction is almost similar to the case of citrate utilisers.

Changes in the Indigenous flora

Cultural characteristics of the Bacteria isolated on different days (table 4-5, and fig. 4-4).

Gram Stain. On the whole; 296 isolates were studied; and

Table :4-5: Morphological Characteristics of Bacteria Isolated from the Laboratory Model Oxidation Pond on Different Days. (Results as percentages of the total Number of Positive Isolates.)

Characteristics	Retention Time in Days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
Total Number of Positive Isolates	40	55	60	48	42	51
A. Chromogenesis : White	60	27	32	12	21	12
Yellow	40	50	38	38	42	78
Pink	Nil	23	13	38	28	6
Brown	Nil	Nil	13	12	7	6
B. Gram Staining : Negative Rods	10	14	19	12	28	6
Positive Rods	20	49	58	60	42	54
Positive Cocci	70	36	19	12	28	42
C. Flagella : Polar	Nil	Nil	Nil	Nil	Nil	Nil
Peritrichous	Nil	7	7	12	Nil	18
Non-Motile	100	92	92	84	100	84
D. Burdon's Stain	20	100	100	48	87	90
E. Capsule stain	90	21	52	72	63	90
F. Spore Stain	Nil	Nil	13	Nil	Nil	Nil

the common cultural characteristics of these organisms are shown in table 4-5. Gram negative rods were found in maximum number on the 21st day (nearly three times the number seen for raw sewage) and again on the 7th day, the number was nearly twice as much as in raw sewage. On other days (4 and 14) the Gram negative rods remained nearly the same excepting on the 28th day when it was nearly half.

Gram positive rods were found in far larger numbers than Gram negative rods. They were only 20% on the 0 day and increased to 54% on the 28th day.

As for Gram positive Cocci they were found in largest percentage in raw sewage and decreased as days progressed. The lowest percentage of 12 was recorded on the 14th day; thereafter there was an increase to 42% on the 28th day.

None of the 296 isolates has single polar flagellum. A few percentages on 4th, 7th, 14th and 28th day showed the presence of peritrichous flagella.

All other organisms were mostly non-motile.

Chromogenesis. An interesting observation was that the whitish coloured colonies were found to decrease from 0 day to 28th day while the yellowish coloured colonies showed increasing percentages as the days progressed. Sixty percent of the colonies were whitish and 40% yellowish in raw sewage, while the lowest percentages of both the types were seen on the 14th day.

Pinkish and brownish colonies were absent in raw sewage on 0 day but they were found in varying percentages on other days. (6 to 28 for pink and 6 to 13 for brown).

Special staining reactions. For demonstrating the presence of intracellular fat in bacteria isolated from the ecosystem under examination, Burdon's (1946) staining technique was tried for all of them. The revised Burdon's technique is expected to show the intracellular lipid material in bacteria. The cellular lipid in most organisms takes up the characteristic blue-black or blue-grey colour almost at once. The colour distinction between the blue-black or blue-grey of the fat droplets and the pink of the counter stain is clearly visible.

While only 20% of the organisms on the 0 day showed Burdon's reaction, 100% of the isolates studied on 4th and 7th day gave positive results. The percentage was reduced to nearly half on the 14th day. On the last three days, the percentages were nearly doubled.

Ninety percent of the strains studied on the 0 day gave positive reaction for capsule staining but on the 4th day it was reduced to 21%, only to increase two to four times on the other days.

All the organisms tested gave negative results for the spore test, except on the 7th day when 13% gave positive results.

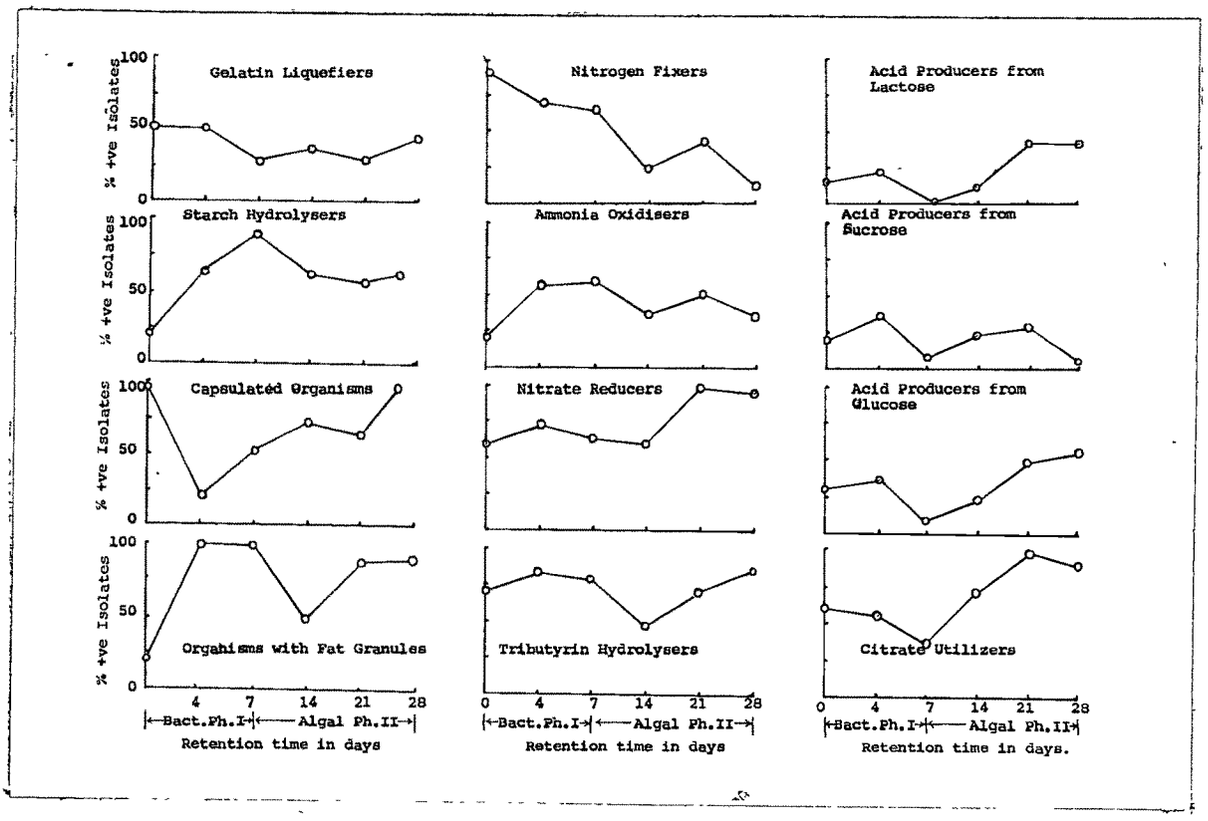

Fig.4-4: Biochemical Characteristics of Bacteria Isolated

Biochemical characteristics of the organisms

The results of the important physiological tests useful in identification of the bacteria upto the genus level are shown in table 4-6, and fig. 4-4.

Starch Hydrolysis. A striking observation has been made by Dias and Bhat (1964) in a large number of pure strains isolated from seven different activated sludge flocs. When the pure strains were grown on proteose-peptone-yeast extract agar containing starch and the colonies flooded with iodine solution, the medium and the colonies stained a deep blue. They state: " Normally bacterial colonies do not stain with iodine when grown on starch agar. The starch-accumulating bacteria, when judged by the usual criterion of the medium around the colony losing its iodophilic character, showed no diastatic action. However, when these bacteria were grown in PPYE broth containing 0.1% soluble starch for about a week the medium no longer stained a deep blue with iodine but violet. Moreover, the cells after harvesting by centrifugation and washing, stained a deep blue during the initial stages of growth; on subsequent incubation the cells lost their iodophilic nature. Whether the above observations represent a preferential utilization of amylose (leaving the amylopectin intact) or the formation of dextrins (Tilden and Hudson 1942) can be answered after further experimentation. However, they point out to the fact that an iodophilic substance is initially stored(absorbed)

Table :4-6: Biochemical Characteristics of Bacteria Isolated from the Laboratoty Model Oxidation Pond on different days. (Resubts expressed as percentages of the total number of Positive Isolates).

Characteristics	Retention Time in Days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
Total number of positive isolates	40	55	60	48	42	51
1. Starch hydrolysers	20	63	88	60	56	60
2. Gelatin liquefiers	50	50	26	36	28	42
3. Tributyrin hydrolysers	70	84	78	48	70	84
4. H_2S Producers	20	Nil	Nil	12	49	6
5. Indole producers	Nil	Nil	Nil	Nil	Nil	Nil
6. Methyl-red positive	10	14	Nil	Nil	21	Nil
7. Voges-Proskauer reaction	10	Nil	Nil	36	28	Nil
8. Litmus Milk :						
Acidic	10	42	38	36	28	18
Alkaline	20	28	38	Nil	28	42
Neutral	70	27	19	60	42	42
9. Nitrate reducers	60	72	64	60	100	96
10. Ammonia oxidisers	20	56	58	36	50	36
11. Nitrogen fixers	90	69	64	24	42	12
12. Citrate utilizers	60	56	38	72	100	84
13. Acid producers from glucose	30	36	7	24	49	54
14. Acid producers from sucrose	20	36	7	24	28	6
15. Acid producers from lactose	10	23	Nil	12	42	42

in the cells and subsequently metabolized though it seems unlikely that the amylose molecule would be taken up as such into the cell without prior degradation. "

" Some of the bacteria which accumulated iodophilic material together with a few which failed to do so were screened for their ability to synthesise iodophilic material from glucose, sucrose, maltose, lactose, dextrin, glycogen, glycerol, sodium acetate, sodium succinate and glucose-1-phosphate suitably incorporated (0.5% for all compounds other than glucose-phosphate which was used at 0.1% level; the latter substance was sterilised by filtration) into PPYE agar. In no instance was iodophilic material synthetised. Recently Carrier and Mcclesky (1962) described Corynebacteria that accumulated iodophylic poly-saccharide in their cells when grown only on starch and other closely related compounds. The present findings, as far as the author is aware, represent the first report on the existence of Gram-negative bacteria having a specific requirement for starch for the accumulation of intra-cellular starch-like material. "

In our case no such reaction took place during starch hydrolysis of our culture. The portion surrounding the colonies which was hydrolysed remained clear even after flooding with iodine while non-hydrolysed portions turned bluish.

Another important observation made was that starch hydrolysers were found maximum (88%) during the bacterial

phase I and were found to decrease gradually till the algal phase II.

Gelatin liquefiers. During the initial stage of the bacterial phase I, 50% of the isolates were gelatinolytic, then they were found to decrease towards the end of the bacterial and the beginning of the algal phase II. Thereafter there was an increase which reached 42% on the 28th day.

Tributyrin hydrolysers. They were found to be comparatively higher throughout.

Nitrate reducers. During the bacterial phase nitrate reducers were high and increased still further to (91-100%) on the 21st to 28th day.

Ammonia oxidisers. Compared to nitrate reducers ammonia oxidisers were found to be comparatively lower in numbers throughout the two periods of observation.

Nitrogen fixers. About 90% of the isolates from raw sewage were nitrogen fixers and these were gradually reduced in number during the two phases. On the 28th day there was a sudden drop to 12%.

Citrate utilizers. These were found to decrease gradually during the bacterial phase and to increase during the algal phase.

Fermentation reactions with sugars

Glucose. During bacterial phase, organisms producing acid from glucose decreased and later during the algal phase increased reaching 54% on the 28th day.

Sucrose and lactose. Organisms producing acid from sucrose and lactose were comparatively lower throughout. Barring this the general trend appeared to be similar to glucose.

Typing the Organisms

Using Bergey's Manual of Determinative Bacteriology (Breed, Murray and Smith, 1957) the organisms were identified up to the genus level and are shown in table 4-7. From a study of the table, it will be seen that :

Micrococcus spp were the dominant organisms (40%) on the 0 day, Brevibacterium spp were dominant (36%) on the 4th day; Corynebacterium sppwere dominant on the 7th and 14th days; Flavobacterium spp and Micrococcus spp became dominant on the 21st and 28th days; and Brevibacterium spp became sub-dominant on the 28th day.

Microccous spp which were found dominant on the 0 day, decreased in numbers during the bacterial phase I and then increased gradually during the algal phase II.

Sarcina spp. the next dominant bacterial form in raw sewage decreased gradually both during the bacterial and algal phases and disappeared finally.

Table :4-7: Types of Bacteria present in the Laboratory Model Oxidation Pond on different days. (Results expressed as Percentages of the total number of the Positive isolates).

Bacteria	Retention Time in Days					
	Bacterial Phase I			Algal Phase II		
	0	4	7	14	21	28
Total number of Positive Isolates	40	55	60	48	42	51
Alcaligenes spp.	Nil	7	Nil	Nil	Nil	Nil
Bacillus spp.	Nil	Nil	14	Nil	Nil	Nil
Brevibacterium spp.	20	36	14	24	21	36
Corynebacterium spp.	Nil	14	35	36	14	12
Flavobacterium spp.	10	7	21	12	28	6
Micrococcus spp.	40	28	7	12	28	42
Sarcina spp.	30	7	14	12	Nil	Nil
Nocardia spp.	Nil	Nil	Nil	Nil	7	Nil

Brevibacterium spp. which constituted 20% of the raw sewage flora increased on the 4th day to 36% and then decreased on 7th day to fourteen percent and increased nearly two to three times during the algal phase II.

Corynebacterium spp. which was not detected in raw sewage increased gradually during the bacterial phase I and later decreased during the algal phase II.

So, Micrococcus spp, Sarcina spp, Brevibacterium spp, Flavobacterium spp, and Corynebacterium spp, were found to be present throughout the period of investigation and the dominance was found to shift as follows :

Micrococcus --> Brevibacterium --> Corynebacterium --> Flavobacterium --> Micrococcus.

Achromobacter spp. Alcaligenes spp, Bacillus spp and Nocardia spp were seen occasionally.

Jasewicz and Pørges (1956) were able to distinguish a wide variation in microbial populations between assimilative and endogenous phases in their studies on the disposal of dairy wastes. They found 74% of the organisms to belong to the genus Bacillus in the assimilative phase and 8% of the organisms to the genus. Bacterium in the endogenous phase. They also found the endogenous sludge to consist of 42% of the proteolytic organisms Pseudomonas and Alcaligenes and 48% of

the sacehrolytic organisms Flavobacterium and Micrococcus.

If we consider the bacterial phase I as assimilative and the algal phase II as endogenous phase, a similar distinction as noted by Jasewicz and Proges is not seen. McKinney (1956b) has stated that most bacteria can flocculate under proper conditions, and so such a distinction cannot be made as in our case.

DISCUSSION

Nature and concentration of organic constituents of raw sewage

Raw sewage contains organic materials in all states of dispersion, chiefly coarsers, solids and liquid portion. The former will be removed earlier in presedimentation basins due to gravitational force leaving purely the liquid which contains a gradation of particles varying in size from collodial aggregates or finely suspended matter to true colloids and to material in molecular dispersion and in true solution.

The quantity of true colloidal matter in sewage is only about 6 to 10% according to Mills (1931) and Rudolf and Gehm (1939) and roughly about 35 to 45% of the organic material is in true solution according to Mills (1931, 1932) and Calvert (1932). But recently Rickert and Hunter (1967) using more refined methods were able to estimate the following four fractions in 24 hour composite samples of sewage from two activated sludge plants in U.S.A.

Description	Percentage of Solids in Influent	Effluent
(a) Settleable (> 100 μ)	14.0	1.0
(b) Supra collodial (1 to 100 μ)	11.0	5.0-6.0
(c) Colloidal (1 mμ to 1 μ)	6.0	1.0
(d) Soluble (< 1 mμ)	69.0	93.0

Sewage solids were 14% settleable, 11% Supra-Colloidal, 6% colloidal and 69% soluble. Effluent solids were 1% settleable, 6% Supra-Colloidal, 1% Colloidal and 93% soluble. So in purification of sewage, the soluble organic constituents constituting about 70% of the total dissolved solids will have to be removed by biological oxidation.

According to Placak and Ruchhoft (1947) the soluble organic constituents in sewage cover a wide range of compounds, namely Carbohydrates (l-xylose, glucose, maltose, lactose, sucrose, dextrin and soluble starch); alcohols (methyl and ethyl alcohols, ethylene glycol and glycerine); formaldeyde, ammonium acetate, calcium gluconate, organic acids (formic, acetic, tartaric, citric, lactic and oxalic acids); amino acids (glycine, alanine, glutamic acid, tyrosine, cystine etc.); proteins (peptone, gelatin) and miscellaneous compounds (such as olive oil, soap, minetal oil, acetonitrite- thio-acetamide and thio-glycolic acid).

In Baroda, the following organic constituents have been found in settled sewage :

Free sugar (mg/litre)	-	0.73
Total " (mg/litre)	-	2.10
Amino acid nitrogen(mg/litre)	-	0.25
Protein (mg/litre)	-	10.00
Total fat (mg/litre)	-	18.40

The Stevenage raw sewage in England has the following composition (Water Pollution Research Annual Report for 1959).

Constituents	Concentration in Candle Filtrate(ppm carbon) Fresh	Aerated
Carbohydrates - Total	56.0	3.8
Amino acids - free	6.3	0.9
- bound	7.4	1.4
Acids - Voltile	4.6	0.8
- Non-volatile	6.0	2.8
Anionic surface-active agents	7.3	1.6
Creatinine	3.0	1.9
Total organic carbon - by analysis	118.0	32.0
- by addition	90.6	13.2

So, compared to the English sewage, Baroda sewage seems to contain more fat and protein.

Changes taking place in the liquid medium

During the entire period of 28 days when sewage was lagooned definite changes were taking place in the electrolytes and non-electrolytes in the two phases. The bacterial phase was limited to 0-7 days and the algal phase 7-28 days. The two phases are compared in table 4-8. It will be observed from a study of the table that there are more points of difference than similarity between the two due to the metabolic processes taking place in the two phases which are also different.

From the physical aspect the colour is brownish to greenish yellow in the bacterial phase I as against greenish yellow to yellowish in the algal phase II. The hydrogen ion concentration expressed in terms of pH is more on the alkaline side in the algal phase II.

From the chemical point of view, the values for phenolphthalein alkalinity, nitrite nitrogen and dissolved oxygen content, percentage reductions in BOD and phosphate are more in the algal phase II than in the bacterial phase I. But the percentage reductions in acid $KMnO_4$ values and ammonia nitrogen are comparatively lower in the algal phase II.

The several constituents of the soluble organic matter content show considerable increase during the bacterial phase I and reduction during the algal phase II while the fat content registers an increase in both the phases.

Table :4-8: Comparison between Bacterial Phase I and Algal Phase II

Period in Days	Bacterial Phase I 0 to 7	Algal Phase II 7 to 28	Over all 0 to 28
A. Physical			
Colour	Brown to Gr.Yellow	Greenish Yellowish Yellow to Green	Brown to Green
Turbidity (% Reduction)	50.9	59.2	80
pH.	6.7 - 7.6	7.6 - 8.6	6.7 to 8.6
Temperature (^{0}c)	26.6 -27.3	27.3 -28.5	26.6 - 28.5
Sludge deposits	Very little	Very little	Very little
B. Chemical			
Phenol. alk. (mg/l)	Nil	Nil - 10.0	Nil - 10.0
Dissolved oxygen (mg/l)	Nil	Nil - 0.6	Nil - 0.6
5-day BOD at 20^{o}C. (% Reduction)	74.8	86.3	97.0
$KMnO_4$ value 4 hrs. (% Reduction)	52.0	43.9	73.0
Ortho-phosphate (% Reduction)	10.5	30.8	40.2
Ammonia nitrogen (% Reduction)	64.4	9.3	67.7
Nitrite nitrogen (mg/l)	Nil	Nil - 0.24	Nil - 0.24
C. Biochemical			
Free Sugar (% Reduction)	+27.4 *	90.3	87.6
Total sugar (% Reduction)	+ 66.6 *	82.8	71.4

(Table :4-8:) (Contd.)

Period in Days	Bacterial Phase I 0 to 7	Algal Phase II 7 to 28	Over all 0 to 28
Amino nitrogen (% Reduction)	+64.4*	46.3	12.0
Protein (% Reduction)	+30.0*	13.5	+12.5 *
Fat (% Reduction)	+69.5 *	+41.8 *	+140 *
Chlorophyll-a. (mg/l)	Nil to 46:6	46.6 to 11.9	Nil to 11.9
D. Bacteriological			
Total colonies count (% Reduction)	66.9	39.5	86.6
Coliforms at 37°C(% Reduction)	99.7	99.9	99.9
E.Coli Type I at 44°C(% Reduction)	97.4 @	100.0 @	100
Faecal streptococci at 45°C(% Reduction)	98.9	100	100
Citrate utilisers at 37°C (% Reduction)	62.2	85.8	95
E. Biological			
Rhizopoda	rrr to Nil	Nil	rrr to Nil
Flagellata	Nil to rr	rr to Nil	Nil
Free swimming ciliates	Nil to C	C to Nil	Nil
Stalked ciliates	Nil to rrr	rrr to Nil	Nil
Rotatoria	Nil	Nil to rr	Nil - rr
Algae	Nil to rr	rr to r	Nil - r

* Percent increase over the initial values. : @ Calculated from the 4th day instead of 7th day
rrr = 1 ; rr = 1-50 ; C = 100 - 200.

The values for coliform group density show, generally, reductions in both the phases. But the percentage reduction in the total colonies count is greater during the bacterial phase I. In other respects the percentage reductions are almost the same in both the phases excepting citrate utilisers where the percentage reduction is lower in the bacterial phase I.

Biologically, the bacterial phase contains more of rhizopods, flagellates, free-swimming and stalked ciliates and less of algae. A few rotifers are seen only in the algal phase II.

So, the important points noted are : absence of acidity in the two phases; absence of dissolved oxygen which is so vital for aerobic metabolic processes in the bacterial phase I and its presence in large amounts during the algal phase I; very large reductions in coliform group density; fairly large increases and decreases in biochemical constituents (such as total sugar; free sugar and amino acid) during bacterial phase I and algal phase II respectively; increase in fat content throughout; gradual increase in chlorophyll content; comparatively large protozoan population in the bacterial phase I and large algal flora in the algal phase II and no appreciable amount of sludge seen in both the phases. There appears to be an intimate correlation among the several factors mentioned above, which needs explanation.

Absence of Acidity in the two Phases

The values for pH and phenolphthalein alkalinity go to show that the ecosystem during both the phases was on the alkaline side. During the bacterial phase I bacteria were comparatively more active. If any soluble organic acid had resulted from carbohydrate metabolism they would be indicated by a drop in pH. As significant drops in pH were not noted at any time, it must be concluded that such products were either not formed at all or were not formed to any significant amount. One may also infer from the aforestated results that the bacteria in the system either did not attack the carbohydrates to produce acid end products or produced alkaline substances from proteins which might have neutralised the acids as rapidly as they were formed as suggested by Heukelekian and Littman(1939). Also, Watkins(1925) isolated a number of bacterial strains from activated sludge and trickling filter slimes, which attacked glucose without acid formation. But it can be seen from table 4-6 that we have isolated a fairly good percentage of acid producers from glucose, sucrose and lactose in our ecosystem and still they did not effect any change in alkalinity. Placak and Ruchhoft (1947) have also stated that it would be erroneous to think that lack of acid or gas production is an indication of biochemical inactivity as in the case of carbohydrates. They add that these soluble substances are frequently capable of being utilized in relatively large quantities and /

at high rates without acid production. Theycall this process as "oxidative assimilation" a terminology used by Clifton and Logan (1938). A similar reaction may be taking place in our system as well.

Again any pH value above 8.1 shows that photosynthesis exceeds respiratory activities and higher pH values often accompany supersaturation of Oxygen (Atkins and Haris,1924), and this is also the case during the algal phase II.

Also any value for phenolphthalein alkalinity is an indication of increasing pH. The increase in pH and phenolphthalein alkalinity especially during the algal phase II is due to photosynthesis of the algae present. During this period of rapid growth, algae utilize the CO_2 from the bicarbonate alkalinity making the system more alkaline due to the formation of carbonates. It is also well known that at higher pH values, CO_2 from the atmosphere combines with the carbonates forming bicarbonates, which are, again, available to algae for extracting CO_2.

During photosynthesis oxygen is liberated into the liquid medium making it more oxygenated. So, the higher the phenolphthalein alkalinity, the greater is the production of of oxygen due to photosynthesis. This is evident during the algal phase II in our case. Also, any organic acid produced in the eco-system will also, be neutralised.

Absence of dissolved oxygen in the bacterial phase I and its presence in large amounts in the algal phase II

Dissolved oxygen is vital for all aerobic metabolic processes in order to break down the highly complex organic matter to its final degradation products such as CO_2, NH_3 and H_2O, involving several oxidation steps for which O_2 is essential as an ultimate H^+ acceptor. During the bacterial phase there is an increase in protozoan population, considerable reduction in BOD, phosphate, and ammonia-nitrogen and also increase in the several constituents making up the soluble organic matter. These facts show that there is an intense metabolic activity in the eco-system. But there is also considerable reduction in the total colonies count and coliform group density, and these facts may appear to be contradictory, which is more apparent than real, and so requiring an explanation.

During the bacterial phase I, the dissolved oxygen required for the aerobic metabolic processes appears to be obtained by reaeration of the surface layer by atmospheric oxygen drawn into the system on account of temperature gradient between eco-system and the ambient air. The quality of oxygen received by a water surface from the atmosphere is stated by Imhoff and Fair (1956) to follow approximately the equation :

$R = 0.271 \times (a) \times (d) \times (D_o)$ where R is the reaeration in pounds of oxygen per acre per day, d, is the depth of the water basin in feet and D_o is the mean daily saturation deficit.

The factor (a) was given an arbitrary value of 20 by Oswald (1960). That is, in a water basin with an average depth of three feet and having a mean deficit of 6 ppm, 6 lbs. of oxygen will be absorbed per day. The rate of reaeration will also be increased by wind or wave action under natural conditions.Also, generally atmospheric oxygen diffuses into the liquid at a slower rate than the rate of consumption of oxygen by the microorganisms. Under these conditions there will not be a surplus.

But during the algal phase II the main source of oxygen is photosynthesis. According to Oswald (1960) the rate of oxygen production seems to follow the equation :

$$NH^{+} + 7.6CO_2 + 17H_2O = 5.2H_2O + H \pm 886KKCal/g +$$

$$\underset{\text{algae}}{C_7H_{8.1}O_{2.5}N} + 7.6O_2$$

In other words, about 3.7 calories are fixed for each milligram of oxygen liberated as a result of photosynthesis or 1.67 milligrams of oxygen is liberated for each milligram of algae synthetised. So, sufficient oxygen appears to be available for the metabolic processes once algal synthesis is started.

Gradual increase in Chlorophyll-a Content : Ammonia-nitrogen

The figures for ammonia nitrogen are found to decrease gradually in both the phases indicating synthesis of cell

growth or cytoplasm. The algae developing in the system also seem to be using this substance as the principal source of nitrogen to build up the protein which is the basis of their cellular protoplasm. So, during the algal phase II most of the ammonia nitrogen (as much as two-thirds) is taken up by the algal organisms. Caldwell (1946) stated that ammonia nitrogen might disappear from the oxidation pond effluent in summer due to its utilization by bacteria and algae. Cooley and Jennings (1960) found an overall average reduction of 80% in oxidation ponds. Fitzgerald and Roehlich (1958) have stated that 87 to 95% of ammonia nitrogen is used up by algae during metabolic processes.

Another fact which has to be noted is that there is not a considerable reduction of ammonia nitrogen as stated by the above authors; and this would seem to show that some production of the same by protein hydrolysis in the system. Endogenous metabolism also may have released some nutrients back into the eco-system. In this way some more ammonia nitrogen could have been added so that the ultimate reduction is not very much.

There is also reduction in ortho phosphate, but the amount is not as large as in the case of ammonia nitrogen. Fitzgerald and Rochlich (1958) stated that a reduction of 96% is effected chiefly due to increased pH rather than due to its utilization by the algae. Bogan, Albertron, and Plunze (1960) found that the

removal of phosphates by algae was much less than by a chemical purification. Bush, Isherwood and Rodgi (1961) found that 96% of the phosphates and 100% of the nitrogen were removed by treating organic wastes with a continuous supply of CO_2 for increased algal growth and to maintain the pH between 7.0 and 8.5.

Large protozoan population seen in the bacterial phase

An attempt was made to find out if there was any relationship between the important physico-chemical variables and the protozoan populations. The results are shown in Fig. 4-5, from which it will be seen that there is no correlation; and this inference is supported by Cutler and Bal (1926), Meikle-John (1932) and Butterfield and Wattie(1941) who have shown that the predatory activity of ciliates enhances the activity of certain bacteria. Jenkins (1942), and Barker (1946) considered that flocculation was not dependent on protozoa. Reynoldson (1942) concluded that " any active part which Vorticella and other protozoa take in the purification of sewage remains to be determined," Baines, Hawkes, Hewitt, and Jenkins (1953) were unable to produce any evidence that purification was enhanced when the ciliate population was high. The ciliate population in the sludge was largely determined by the different conditions prevailing in the plant. Thus the protozoan fauna indicated only the quality of the effluent which is affected by the conditions of the sludge.

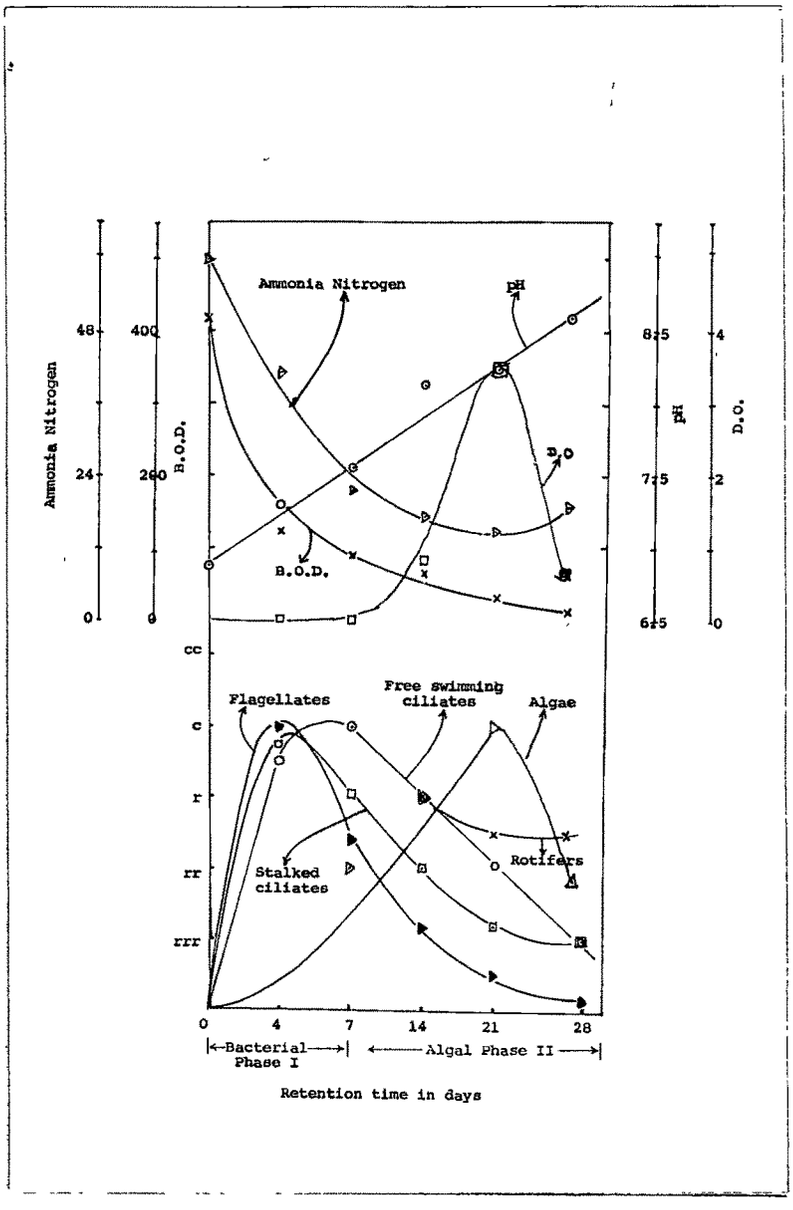

Fig.4-5: Relation between the important Physico-chemical variables and the Protozoan population

In Fig. 4-6, the relationship between the biochemical constituents and the protozoan population is shown. There seems to be some similarity between the two graphs. When the biochemical constituents (excepting fat) increase or decrease, there is a corresponding increase or decrease in the several constituents of the protozoan population, thereby making it appear that there is a close relationship. When organisms are found in dominance in a particular situation, it may mean either that they are actively participating or that they are found only because favourable conditions have been created by other agencies for them to live comfortably. Very often the presence of a certain protozoan species has been reported to indicate a good or a bad activated sludge (see page 2-27). That does not necessarily mean that protozoans play a chief role in the metabolic processes as some workers have stated. The protozoans simply reflect the conditions prevailing in the system (Hawkes 1960). In the same manner it is idle to correlate the organic constituents to the relative abundance of the several types of protozoan populations in this case also.

Coliform Group Density

Bacteriologically, there were also considerable reductions in Coliforms, E.Coli Type I and faecal strepto-cocci. The reduction in citrate utilizers was not so high as in the former case as will be seen from the tabular statement below :

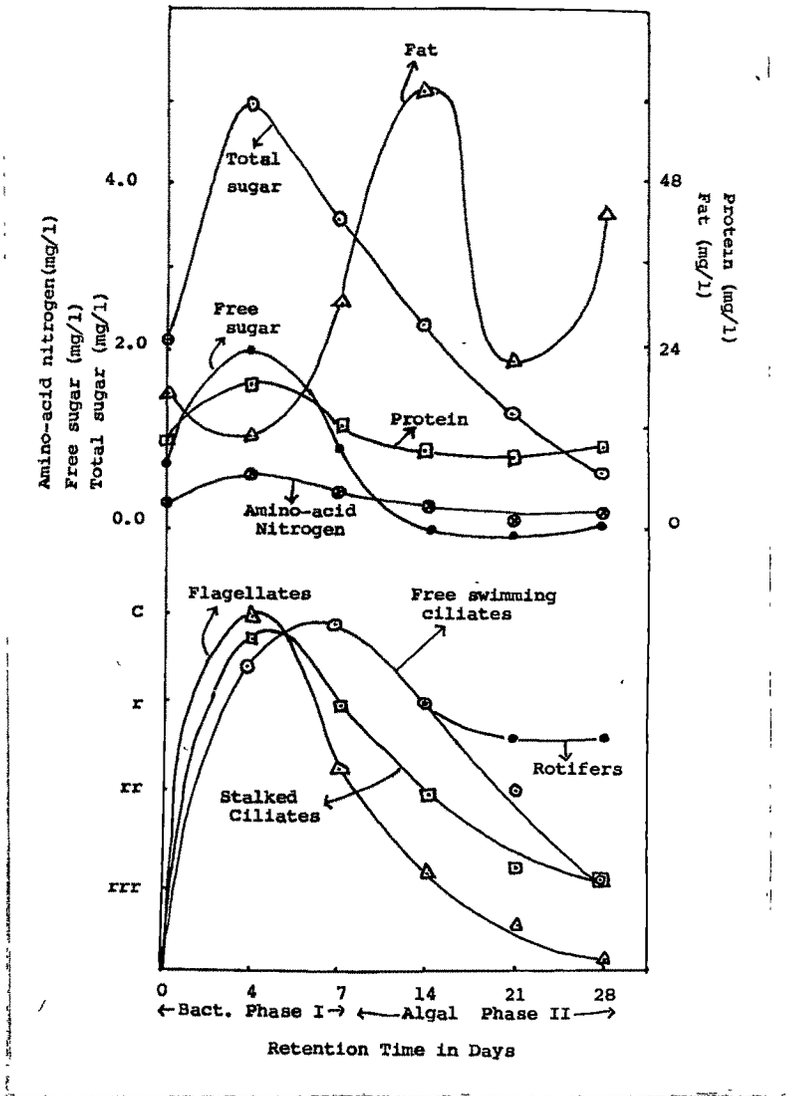

Fig.4-6: Relation between the Biochemical Constituents and the Protozoan population

Microorganisms	Retention Time in Days					
	Bactl. Phase I			Algal Phase II		
	0 *	4	7	14	21	28
A. Coliform Group		Percentage Reduction				
1.Coliforms at 37°C	$76x10^6$	99.5	99.7	99.9	99.9	99.9
2.E.Coli Type I at 44°C	$79x10^5$	97.1	97.1	-	99.9	100.0
3.Faecal Streptococci	$4.5x10^5$	-	98.8	99.9	99.5	100.0
4.Citrate Utilisers	$4.5x10^5$	+29.0	-62.2	-96.2	-99.0	94.7
B. Total Colonies Count	$39x10^9$	+44.4	-66.9	-97.0	-99.9	-80.0

* Actual Numbers

The greatest reduction was found during the bacterial phase I in coliforms. However, in the case of citrate utilisers and total colonies count, there is an increase amounting to 29% in the former case and about 44% in the latter case, on the 4th day, and reductions amounting to 60-67% on 7th day.

"The enteric organisms die more readily in the presence of other bacteria and higher forms of microscopic life "according to Taylor (1958). So, the death and decay of the coliform group of organisms are to be expected under the conditions prevailing during storage.

But in the case of the other type viz. citrate utilizers, the reduction is not so great as in the case of coliforms. As for the total colonies count representing all types of

microorganisms, first, there is an increase of nearly 44% on the fourth day and then a reduction of nearly 67% on the 7th day. During the algal phase II the percentage reduction is about 30% only.

The graphs (Fig. 4-3) representing the coliform group density seem to run almost parallel to each other.

Keiler (1960) has used Koser's citrate medium along with similar other tests for estimating the coliform group density in his studies on the bacteriological aspects of pollution in the Jukskei-crocodile river system in Transvaal, South Africa. He stated that theoretically the number of citrate utilizers and that for E.Coli Types should be equal to the number of organisms of the coliform group, in view of the fact that Koser's citrate medium is of similar specificity for Aero-bacter aerogenes as MacConkey broth is for E.Coli Type I. But this simple addition does not work out, for MacConkey broth may show the presence of coliforms of certain intermediate group also and the citrate test definitely shows the presence of certain pigment producing organisms, probably Pseudomonas and Chromobacter spp. both of which are, not of faecal origin.

He adds that Koser's citrate medium is not exclusively specific for non-faecal organisms, for E.Coli Type II and the irregular strains are incapable of using citrate, whereas a large number of bacteria which are not coliform at all, do

utilise citrate. "This latter group includes Pseudomonas and Chromobacter genera which are usually abundantly represented in soil, vegetation and natural waters and which, may, therefore be expected to have a substantial effect on the results where MPN procedures are employed."

Jayangoudar (1967) found citrate utilizers to be 1.0 to 7.4 times as numerous as the coliforms in the final effluent of the Ahmedabad oxidation ponds suggesting that the majority of the organisms were not coliforms at all, but probably Pseudomonas and Chromobacter spp. Neel and Hopkins(1956), Neel, Dermott and Monday (Jr)(1961), Parker, John and Taylor (1950), Towne, Bartsch and Davis (1957) have examined the bactericidal effect of the various waste water treatment systems on coliforms, and found complete reduction of the Salmonella group along with 99.9% reduction of coliforms.

Many theories and mechanisms have been proposed for bacterial reduction such as (i) the production of materials toxic to bacteria, (ii) germicidal effect of sunlight and (iii) competition for nutrients. Allen (1955), Oswald, Gotaas, Golueke and Kellen (1957) and Silva and Papenfuss (1953) state that the environment in the pond is antagonistic to coliform. Wachs, Rabhun, Meron, Kott, and Sless (1961) and Neel, Dermott and Monday (1961) have reported that the number of coliform bacteria and the total number of microorganisms are reduced during the long detention period provided by conventional

oxidation ponds. Parker (1962) has stated that in 30 to 40 days the coliform count will be reduced to drinking water standards in multiple cell oxidation ponds. Yousef (1962, cited by Gloyna 1965) stated that the rate of destruction of coliform bacteria is greater than the rate of dieaway of the total bacterial population. Others like Towne, Bartsch and Davis(1957) state that extreme competition for the limited supply of nutrients is responsible for the destruction of coliforms. But, Caldwell (1946), Pratt, Daniels and Eiler (1944) state that Chlorellin, an anti-bacterial substance liberated from Chlorella is responsible for the reduction. Spoehr, Smith, Strain, Milner and Hardin(1949) have determined the chemical nature of the antibiotic. They stated that Chlorella has been found to liberate fatty acids with a marked anti-bacterial activity. But Malina and Yousef(1964) state that the specific reason for the rapid destruction of coliform in oxidation ponds is not yet clearly understood.

Coliform group density reduction and its relation to soluble organic substrates

There is a rapid decline in numbers of coliforms, E.Coli Type I and Faecal-streptococci. As a result of their death and disintegration, proteins and other cellular products are released into the system. This is indicated by the large increase on the 4th day in protein, amino acid nitrogen, fat, free and total sugar and their decrease on subsequent days on account of their utilization for several metabolic processes. This is, again,

revealed by the increase and decrease in numbers of the several physiological groups of bacteria such as starch hydrolysers, tributyrin hydrolysers, ammonia oxidisers, gelatin liquefiers, citrate utilisers, capsulated and sudanophilic organisms(tables 4-5 and 4-6 and fig. 4-4). The products formed by the growth of one type will help in promoting the growth and development of other bacterial groups. One group of organisms oxidises one substance and another group another. Thus a heterogenous population will multiply until all the available nutrient substrates are used up. Some of the physiological groups of bacteria, will reach a stage in their development when death and lysis will take place-as a result of auto-digestion or endogenous respiration, when, proteins and other cellular products will be, again, released into the medium, resulting in a secondary predominance. The cycle continues until such time that almost all the nutrient substances are considerably reduced. This cycle of changes is indicated by the dominance of the several bacterial genera on different days in table 4-6.

Relation between total sugar, starch hydrolysers and capsulated organisms

The Fig. 4-7 shows the inter-relationship between the values for the total sugar on the one hand and those for starch hydrolysers and capsulated microorganisms on the other. The graph for starch hydrolysers which breakdown complex Carbohydrates

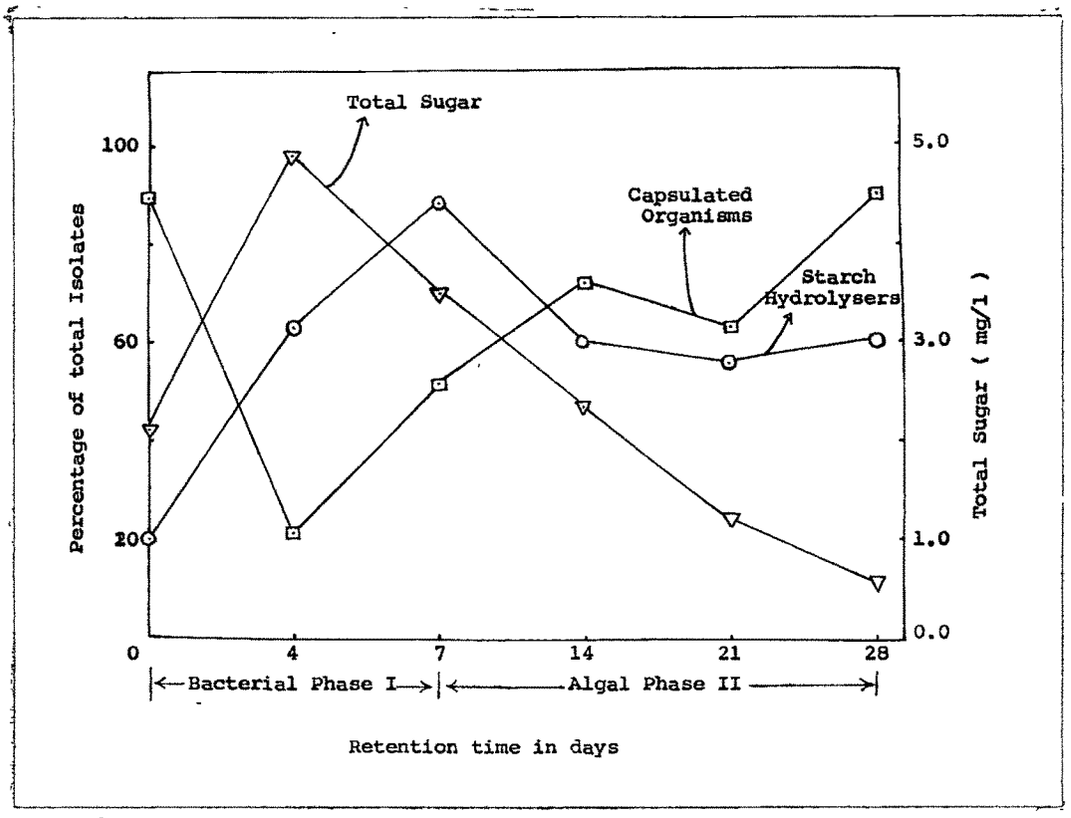

Fig.4-7: Relation between Total Sugar; and Starch Hydrolysers and Capsulated Microorganisms

runs almost parallel to that for the total sugar. This would seem to show that the starch hydrolysers are acting upon the carbohydrates present in the sewage to break them to simpler sugars, which in turn, may be utilised by capsule forming organisms, whose graph runs counter to the first two graphs. The value for the total sugar increases on the 4th day and thereafter there is a gradual decrease until the 28th day. The graphs for the starch hydrolysers and that for the total sugar run almost parallel to one another indicating that the greater the sugar content, the greater the number of starch hydrolysers and vice versa. The presence of a comparatively higher percentages (50-88%) of starch-hydrolysers would show that the amount of carbohydrates utilised was comparatively higher.

It is stated that many bacteria produce from sugar media a thick, transparent, gelatinous or slimy material forming a covering layer or envelope called capsule, which, in most cases, is largely made up of the polysaccharide. This material which is formed during different stages of growth may diffuse out and may impart a gummy or slimy quality to the liquid medium. Several types are synthetised. Some may contain mucoprotein with polysaccharide, while others polypeptide.

So, the inverse correlation existing between the values for the total sugar estimated on different days and the number of capsulated bacteria is perfectly understandable. During the bacterial phase I when the total sugar increases on the 4th day

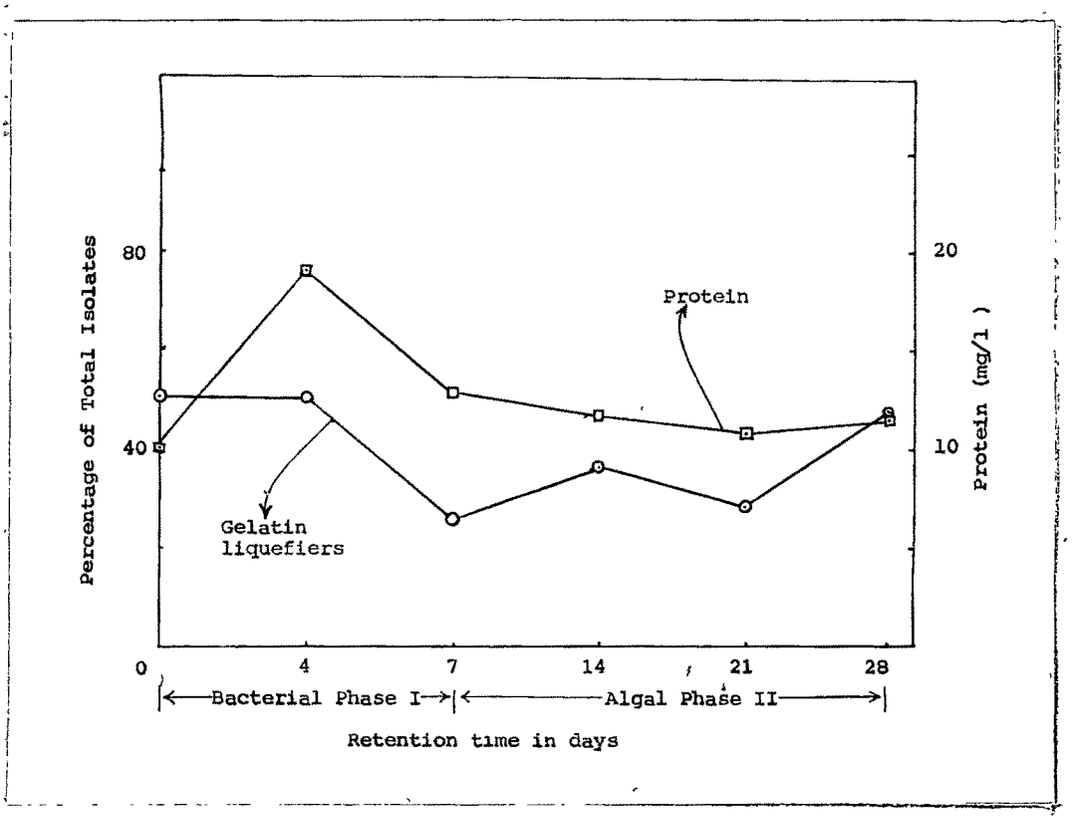

Fig.4-8: Relation between Protein Content and the Gelatin Liquefiers

and then begins to decrease gradually, the capsulated organisms are found to be the least on the fourth day, then found to increase until the 28th day. During the algal phase II capsulated organisms are found to be comparatively more.

Relation between the Protein content and the Gelatin Liquefiers

The relation between the values for protein and the number of gelatin liquefiers is shown in Fig. 4-8. The protein value increases on the fourth day and then gradually decreases until the 28th day. The graph for gelatin liquefiers is running almost parallel to the protein values indicating that these organisms vary in numbers according to the protein content in the media. The low percentages (26-50%) of gelatinolytic organisms in both the phases would seem to show that the quantity of protein utilised was extremely low.

Relation between Ammoniacal Nitrogen Content, Ammonia Oxidising and Nitrate Reducing Bacteria

It will be seen from a study of Fig. 4-9 that the graph for ammonia nitrogen gradually decreases until the 21st day and then there is a slight increase on the 28th day. The other two graphs for ammonia oxidisers and nitrate reducers seem to run in the opposite direction to that for ammonia nitrogen and parallel to each other. This would seem to show that ammonia is being oxidised by ammonia oxidisers and the nitrate thus formed is being acted upon by nitrate reducing bacteria. So this may, perhaps, account for the absence of nitrites and

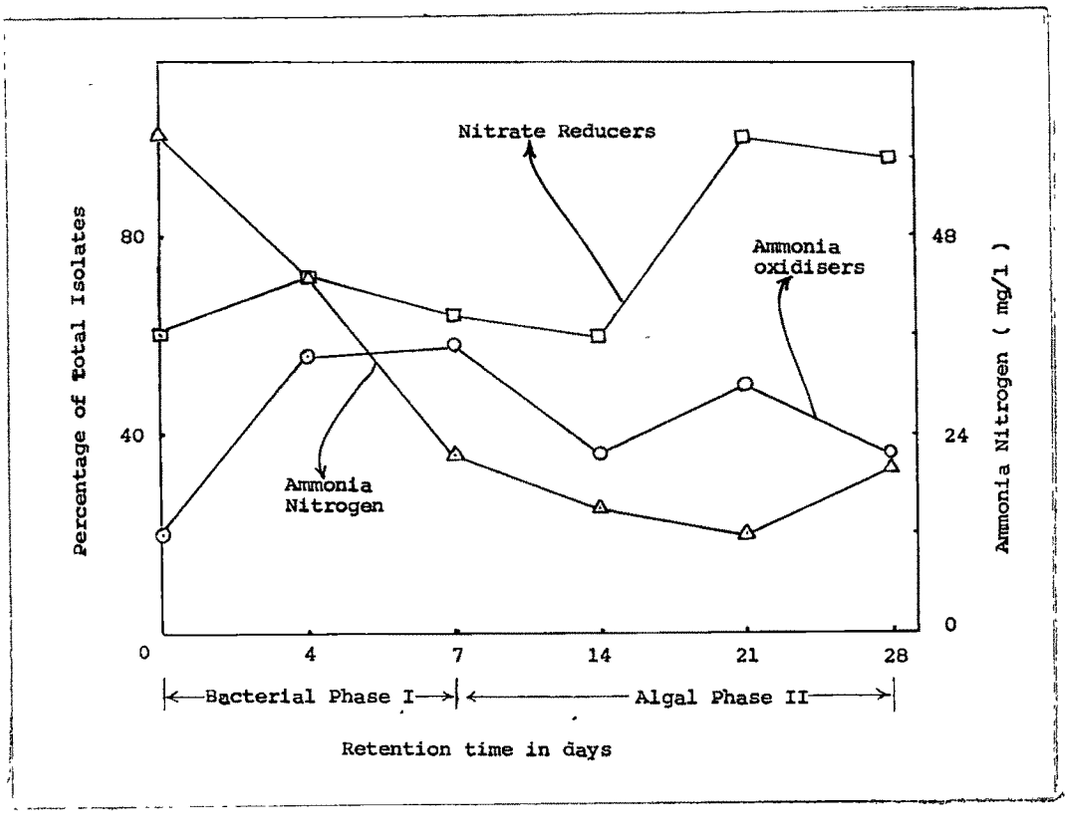

Fig.4-9: Relation between Ammonia Nitrogen Content, and Ammonia Oxidizing and Nitrate-Reducing Bacteria

nitrates in oxidation ponds generally.

Relation between Fat Content, Tributyrin Hydrolysers and Bacteria Storing Fat droplets

The relation between the fat content and the tributyrin hydrolysers and the microorganisms storing fat droplets in their bodies as indicated by Burdon's staining technique is shown in Fig. 4-10. The fat content decreases on the 4th day and increases upto the 14th day, then decreases and again increases on the 28th day. The graphs for tributyrin hydrolysers and for the number of bacteria with sudanophilic granules run almost parallel to one another but in the opposite direction to that of the fat content. This appears to be an intriguing situation for which an explanation may be found as follows. The first alga which began to appear in the medium was Chlorella which reached its dominance by the 14th day. This alga has been reported to liberate extra-cellular fatty acids, which seem to have a marked anti-bacterial activity according to Pratt, Daniels, and Eiler (1944), and Spoehr, Smith, Strain, Milner and Hardin (1949). The four-fold increase in the total fat content on the 14th day was perhaps due to the liberation of fatty acids by the alga Chlorella vulgaris during this period.

In order to verify the statements of the above authors, the following series of experiments were carried out. Fresh, settled sewage filtered through a layer of cotton was taken and distributed in 500 ml quantities into one litre capacity

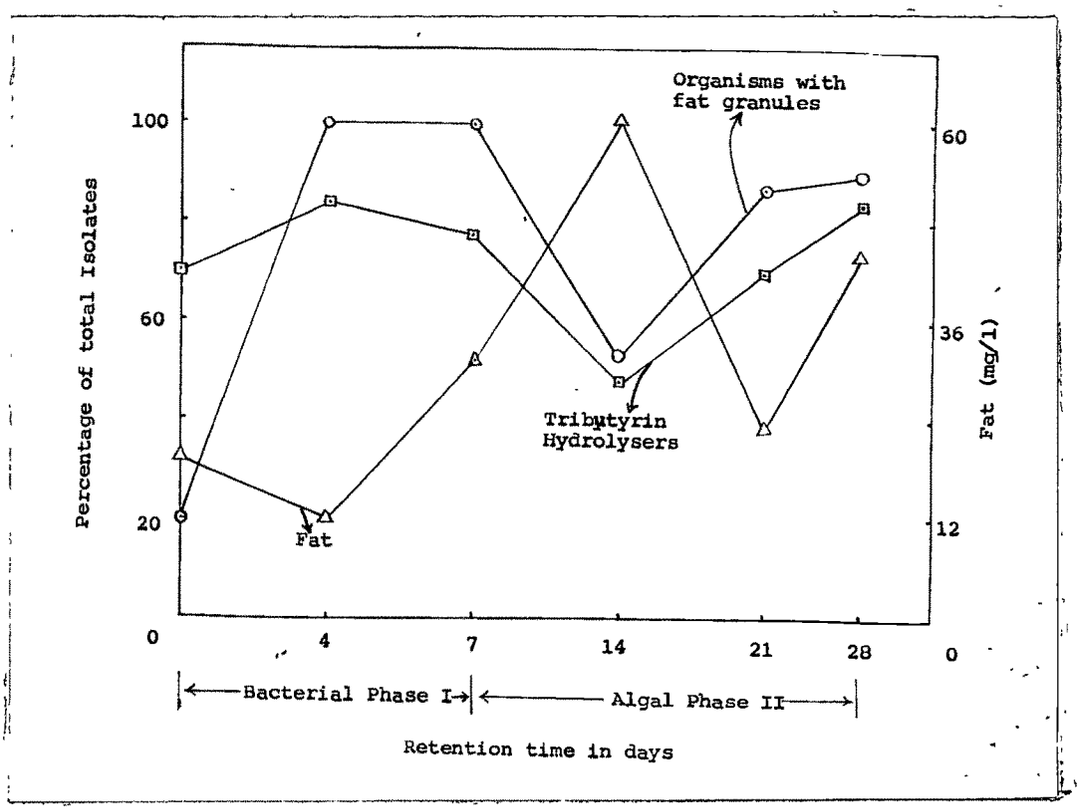

Fig.4-10: Relation between the Fat content, Tributyrin hydrolysers and Bacteria storing Fat droplets

flasks. Three flasks were inoculated with 50 ml quantities of Chlorella vulgaris culture grown in sterile sewage. These were kept in a continuously illuminated room maintained at 20°C. Three more flasks were kept in the same room, containing raw sewage only. A third set of three uninoculated flasks were kept at the laboratory temperature of 28-30°C and were exposed to continuous light.

On o, 7th and 14th days one flask from each set was removed and examined for total colonies count and fat content as already described in Chapter 3. The experiment was repeated thrice and the average results are given below :

Source	Temperature °C	0 Day		7th Day		14th Day	
		Fat mg/l	Total colonies count/ml	Fat mg/l	Total colonies count/ml/l	Fat mg/l	Total colonies count/ml/l
Raw Sewage	28-30°C	14.45	62×10^9	22.30	40.10^6	28.20	40×10^4
Raw Sewage	20°C	14.45	60×10^9	26.50	30.10^6	28.00	60×10^4
Raw Sewage+ Chlorella	20°C	14.00	20.4×10^9	64.00	31×10^5	21.00	60×10^3

It will be seen from the above that there is not only an increase in the content of fat on the 7th and 14th days but also considerable reduction in the bacterial content. It has also been found that the samples of raw sewage alone incubated

at 20°C and at 28-30°C became pale green on the 7th and 14th days due to Chlorella growth. The increased fat content and the bacterial reduction, thereafter is likely to be due to the growth of Chlorella in the cultures.

Relation between the Fat Content, Chlorophyll-a and the Total Colonies Count

In fig. 4-11, the relationships existing among the three factors - the fat content, the amount of chlorophyll-a and the total colonies count are shown. It will be seen from a study of the graphs that there is a direct correlation between the amount of chlorophyll-a and the fat content, and an inverse correlation between the above two factors on the one hand and the total colonies count on the other upto 21st day. In other words, the fat content of the eco-system is found to increase along with the quantity of chlorophyll-a, while the graph for the total colonies count runs counter to the first two graphs till the 21st day.

After the 21st day, there is a rapid decrease in the amount of chlorophyll-a due to the death and disintegration of the alga chlorella but the quantity of fat and the total colonies count increase indicating that there are bacteria which are unaffected by the fatty materials released with the death and disintegration of the alga.

Increased Fat Content in the medium

A study of the table 4-2 will show that the fat content in the liquid medium is far greater than in raw sewage both

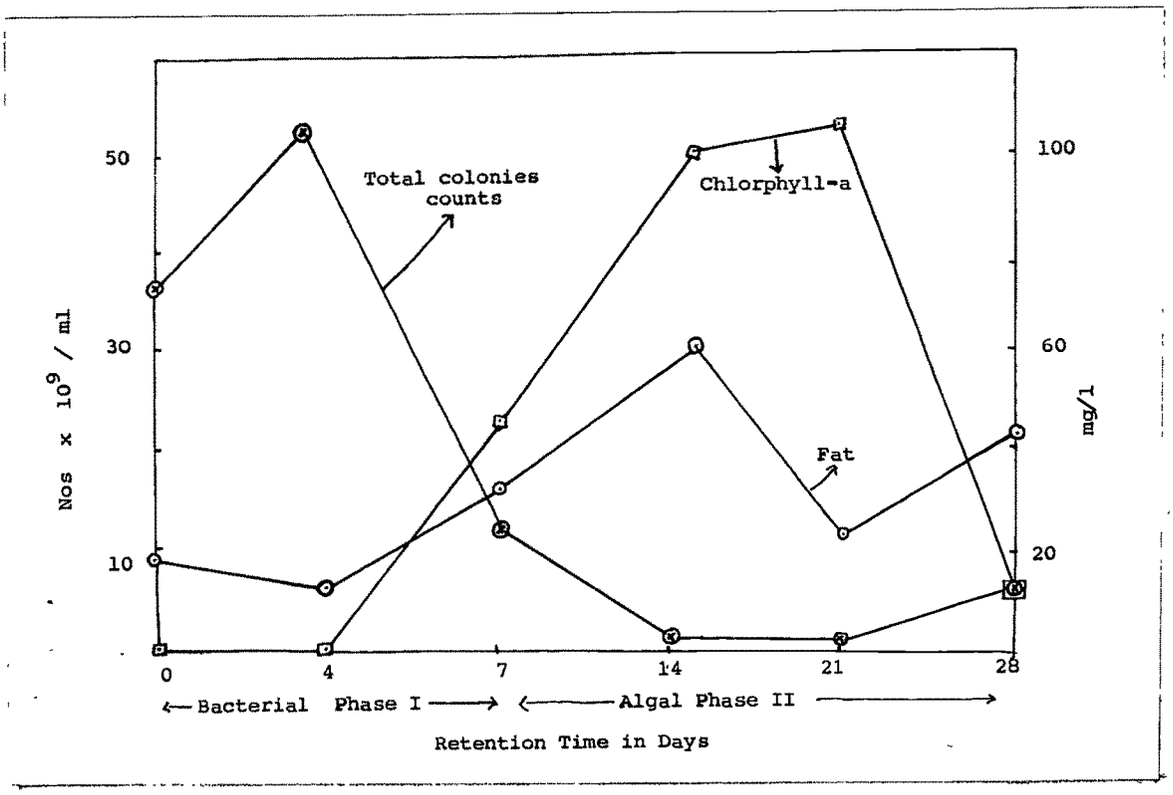

Fig. 4-11: Relation between the Total Colonies Count, Chlorophyll-a, and the Fat Content

during the bacterial phase I and algal phase II being nearly 70% and 42% more respectively. How this increase has originated needs explanation. It would appear that the increase is most probably due to carbohydrate metabolism, being different from that in the case of the activated sludge process.

Ruchhoft, Kachmar and Moore (1940), Ruchhoft, Kachmar and Placak (1940); Placak and Ruchhoft (1947) experimented with activated sludge and Crabtree, Boyle, McCoy and Rohlich (1965, 1966) with pure cultures of Zoogloea ramigera which is considered to be the most dominant organism in activated sludges. The results of the studies of the former who used glucose as a representative of the large fraction of the organic material present in true solution in sewage showed that (a) in the metabolism of glucose by activated sludge, only a small portion was consumed in the respritory process and a large part of the glucose was "quickly transferred in the bacterial cell to other materials possibly higher carbohydrates or fat.," (b) glucose was metabolised in a way different from that of peptone or sewage; (c) products other than CO_2 would not be demonstrated by drops in pH during the reaction; (d) over 70% of the glucose removed could be accounted for by the increase in sludge solids even after 24 hours of aeration; and (e) an increase in fats or fatty acids could not be demonstrated in sludge which had assimilated glucose for a 30-day period.

They have also stated that carbohydrate produced the largest yield of activated sludge.

Crabtree, Boyle, McCoy and Rohlich (1965,1966) showed that glucose was the precursor for the formation of the polymer PHB an endogenous metabolite inside the cells of Zoogloea ramigera but on which capsules or gums polysaccharides were not demonstrable. So, it will be readily seen from the above that in our case why we do not find the largest yield of sludge, but increased amounts of fat throughout. The increase in fat content appears to have originated from two sources. During the bacterial phase I, the increase appears to be primarily due to a different carbohydrate metabolism from that in the activated sludge process and during the algal phase due to the extracellular liberation of the same by the alga Chlorella in addition to the former process. This increase has taken place despite the removal of 8% of the original fat in the viscous scum. (See page 5-5).

Carbohydrate metabolism, endogenous respiration and sludge formation

Gloyna (1965) states : "For domestic wastes that have undergone primary treatment the amount of sludge accumulated in a pond is almost negligible. Even where ponds are used to treat excess sludge the accumulation is almost unnoticeable." He ascribes the absence of sludge formation in oxidation ponds to endogenous respiration. Eckenfelder and O'Connor(1961) state

that this phenomenon is taking place continuously along with the synthesis of new cells but is generally marked by the rapid growth of microorganisms.

It has been pointed out previously that the carbohydrate metabolism in the case of the oxidation pond, appears to be different from that of the activated sludge process. Viswanathan and Pillai (1959a, 1959b) found that the activated sludge process with 20% sludge, removed all the fatty constituents of sewage leaving practically nothing in the effluent. Crabtree, Boyle, McCoy and Rohlich (1966) found that carbohydrates are used for the synthesis of new cells and/or for the formation of endogenous metabolites such as PHB inside the cells. But in the case of the oxidation pond, carbohydrates appear to be used mostly for the synthesis of fatty substances (or fatty acids) and much less for the accumulation of the polymers inside the bacterial cells. The result is that there is more fat in the clear stabilized liquid. Other substances like protein and amino acids etc. also do not contribute much to be formation of sludge according to Placak and Ruchhof (1947).

It is also well known that the removal of the soluble organic matter in sewage by many bacteria results in the accumulation of the polymers (PHB, Glycogen, Starch, lipids etc.) inside their cells. We have shown in table 4-5 that cent per cent of the bacteria isolated during the bacterial phase showed the presence of sudanophilic granules, but they decreased considerably in

numbers during the algal phase II. This is an indirect evidence to show that during the algal phase II on the 14th day nearly 50% of the bacteria which had accumulated fat droplets as an endogenous metabolite (PHB) inside their cells had disappeared because of the endogenous respiration. "The digestion of organisms by their own respiration has definite practical importance. If endogenous respiration proceeds at a great enough rate, microorganisms oxidize their own tissue (auto-digestion of sludge) rapidly enough to keep the system in balance. Under such conditions, sludge does not accumulate. If auto-digestion is not sufficient, sludge accumulates making sludge disposal necessary "(Porges, Jasewicz, and Hoover, 1953). O'brein (1959) did not find any increase in soluble inorganic nitrogen compounds during the endogenous respiration in the liquid and so he concluded that amino acids did not act as energy source but stored polysaccharides were used. In our case also there is no increase in inorganic nitrogen compounds but only consistent reduction confirming the fact that there was endogenous respiration of stored metabolites like PHB and not of amino acids. So, there was no formation of sludge as in the activated sludge process, but there were algal denizens some of which settledown on death and decay.

We should remember that we are dealing with living micro-organisms. Though we can formulate certain rules and be assured

that they will hold true in most cases, we cannot always be sure of that strict and complete adherence that we find in a distinctly chemical reaction. (Placak and Ruchhoft,1947).

SUMMARY

1. The changes taking place in the physico-chemical, biochemical, biological and bacteriological conditions of stored sewage on different days during a period of 28 days are described.

2. The inter-relationships existing among the various factors are also shown.

3. The absence of sludge formation in the eco-system is explained.