CHAPTER V

BIONONIC STUDIES ON THE VISCOUS SCUM FORMED AT THE SURFACE

Viscous scum

As already stated at the beginning of chapter 4, when settled sewage was stored in the glass aquarium, a viscous scum was gradually formed within 24-48 hours. The viscous scum was leather-like or rubber-like and could be easily removed from the surface by a spatula or even by a long needle. The viscous scum as a whole also appeared to resemble " the netted, lace-like masses of cells (flocs)" of crabtree, Boyle, McCoy and Rohlich (1965).

Biological

On microscopic examination of a small portion of the scum, it was found to consist of hundreds of zoogloeas of different shapes and sizes (Figs.5-1); very long thread-like bacteria, long wavy rods, stout comma bacillus, long thin spirilla, irregular egg-shaped cocci in twos and fours, short thin and stout rods, cocci and cocco-bacilli(Fig.5-2).

In dried, fixed and stained smears, the zoogloea colonies were found packed with rods. With Burdon's stain either all the cells or a part of the cells or all the colonies or only a few branches or portions of a few branches showed sudanophilic granules (Fig. 5-3). On many occasions cells escaping from the

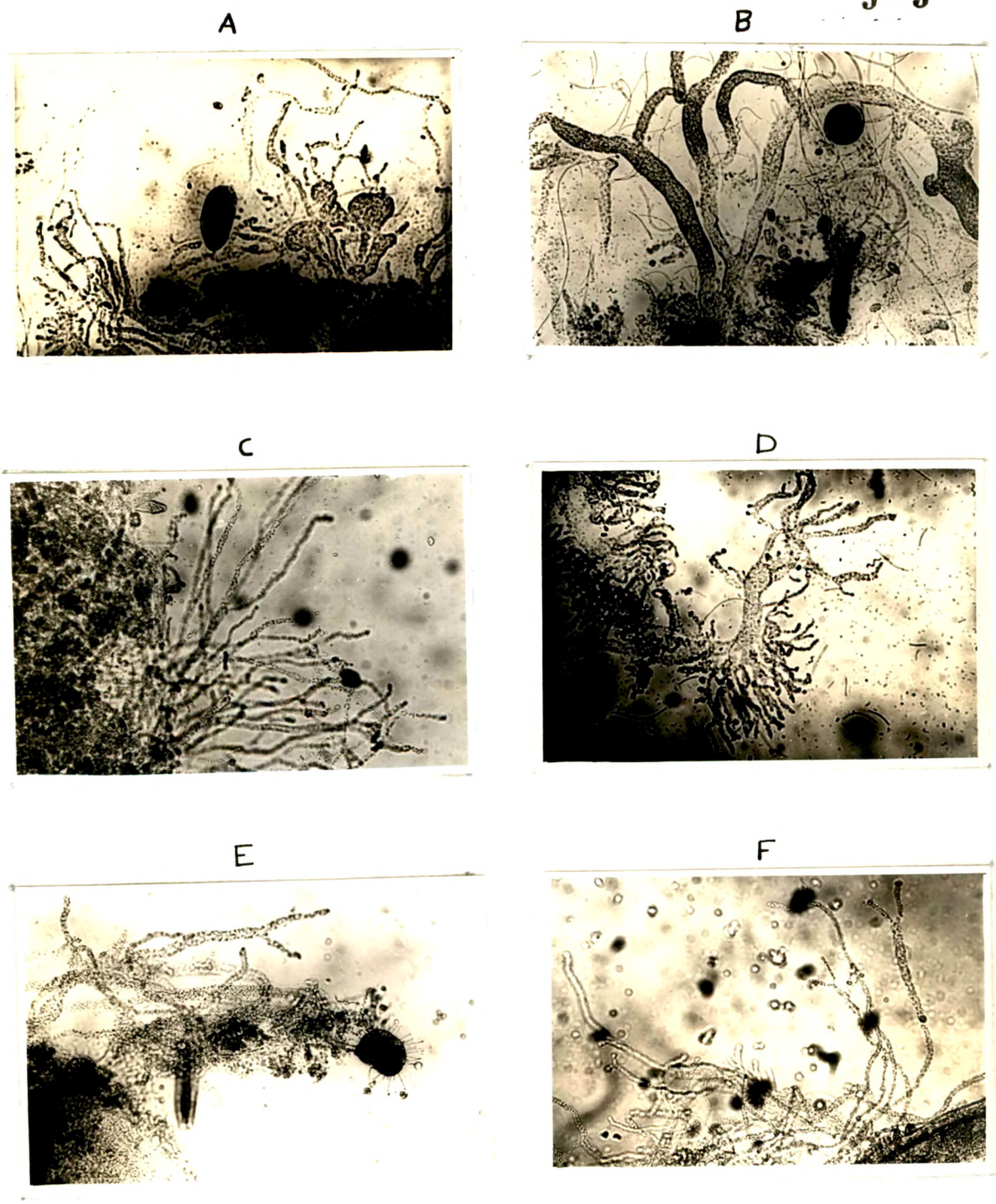


Fig. 5-1: Photomicrographs of Zoogloea Colonies showing various shapes and sizes. C, E and F shows cells of typical Zoogloea lying inside a tough hayline matrix.

finger-like projections (Fig. 5-3D); and disintegrating zoogloea colonies (Fig. 5-3E) were seen. The gelatinous matrix of each colony is clearly visible only with Indian ink wet moutns (Fig. 5-3C).

On staining dried and fixed smears with any of the basic dyes, very long thread-like bacteria, long wavy rods, some zoogloea colonies, spirilla and short rods were often found "vacuolated." With the Burdon's stain, all the organisms excepting the stout comma bacillus showed fat droplets.

Once the viscous scum was removed there was no formation of a similar secondary scum, but a fragile or brittle thin layer was formed where hundreds of big spirilla, long rods and Paramecium caudatum in fairly large numbers were noticed.

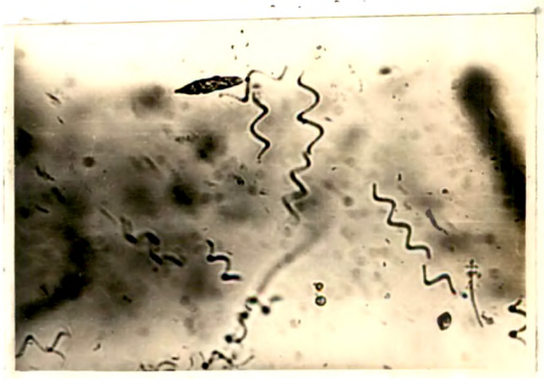
Biochemical

Enzymatic activity. The wet weight of the scum was 95.00 gm. and the acetone powder obtained was 1.85 gm. in a typical case.

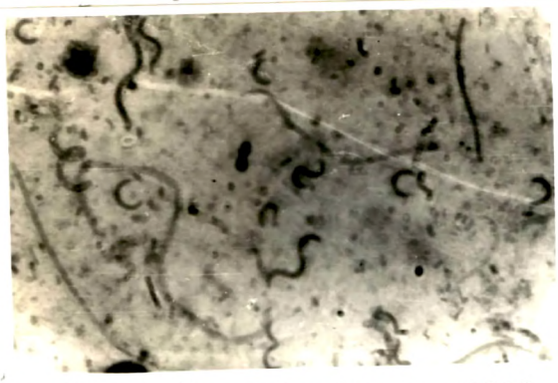
The acetone powder showed protease (0.266 µmoles of tyrosine liberated per mg of acetone powder per hour), lipase (0.47 µmoles of butyric acid liberated per mg of acetone powder per hour) and amylase activity (0.288 µmoles of maltose liberated per mg of acetone powder per hour).

Fig.5-2: Photomicrographs of Bacteria not isolated

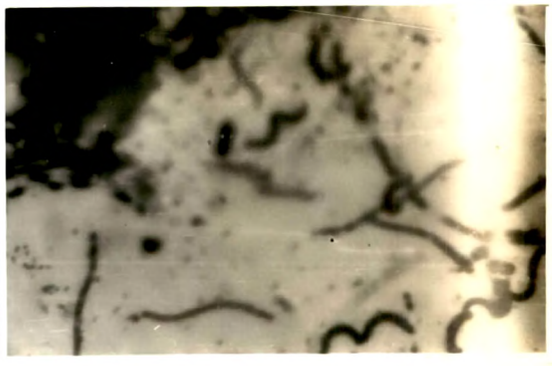
A: Thin Spirilla x 100



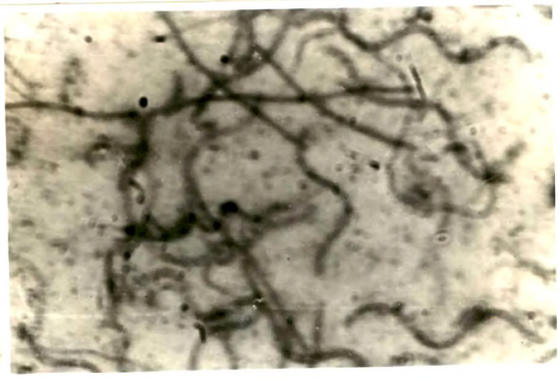
B: Thin Spirilla x 1000



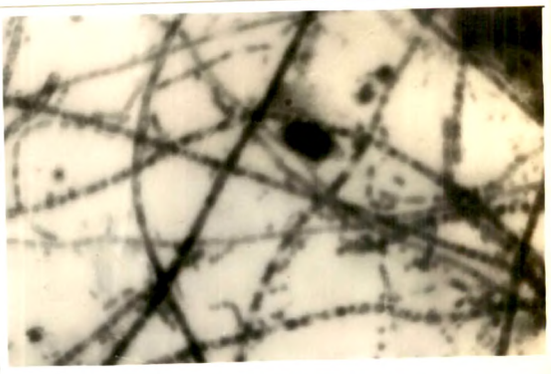
C: Stout Comma bacillus x1000



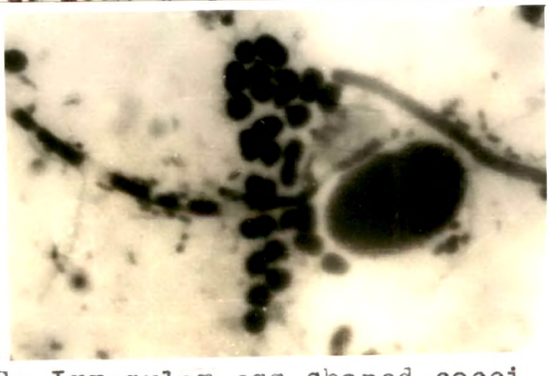
D: Long wavy rods x 1000



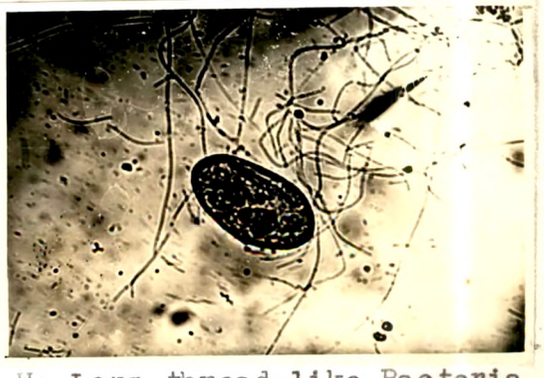
E: Long wavy rods x 1000



F: Long discontinuous rods



G: Irragular egg-shaped cocci in twos & fours



H: Long thread like Bacteria

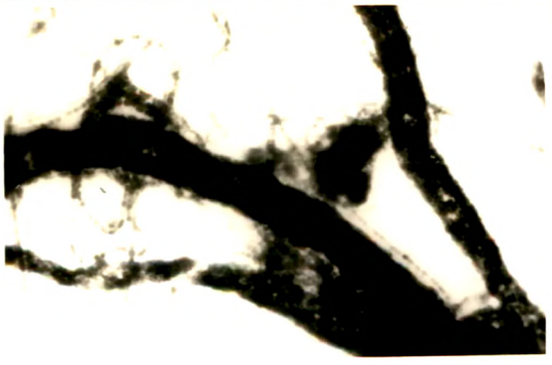
Organic Constituents

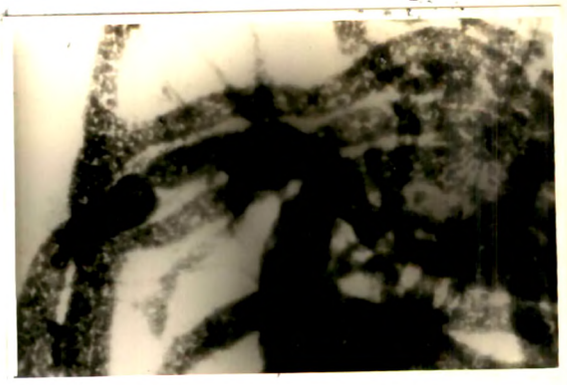
A typical analysis of the scum for its organic constituents is furnished below :

Description	Miligram per gm dry weight	Percentage
1. Free sugar	0.29	0.03
2. Total sugar	0.67	0.07
3. Protein	4.38	0.44
4. Amino acid nitrogen	0.30	0.03
5. Total fat	76.12	7.61
6. Poly-β-hydroxy butyric acid (PHB)	1.55	0.15

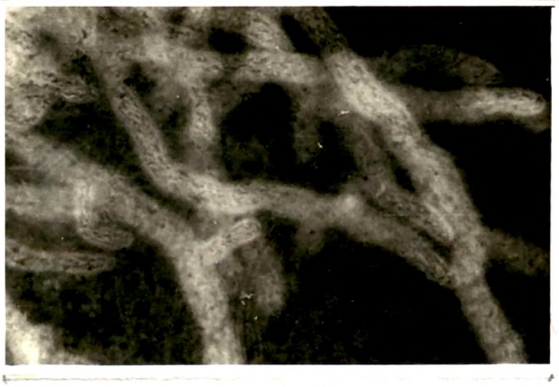
Vishwanathan, Meera Bai and Pillai (1962) found 5.8% of total fatty matter in activated sludge; and we have found 7.61% in the viscous scum. These results would seem to show that the fat content of activated sludge flocs and that of the viscous scum formed after four days contain nearly the same amount. But Dias (1964) estimated the chloform soluble lipid and expressed it as percentage of the dry weight of activated sludge flocs and the amount of PHB in activated sludge flocs. His results are given below :

Fig.5-3: Photomicrographs of typical Zoogloeas

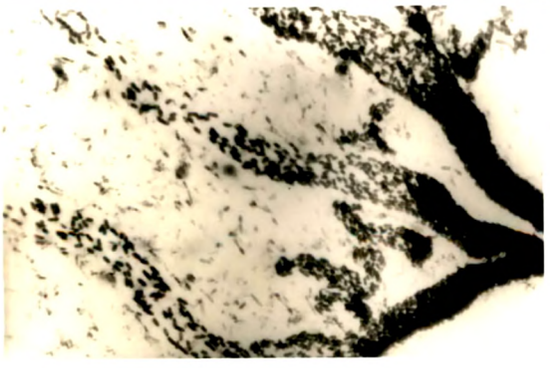




A & B: Branches with cells full of fat granules, thickly embeded in matrix, seen after Burdon's staining



C: Wet mount photograph of Zoogloea cells embeded in tough hayline matrix, visible after treatment with Indian ink



D: Naturally raptured branches of Zoogloea, escaping cells full of fat droplets are seen

E: Disintigrating Zoogloeal branches are seen

Synthesis and Break-down of Poly-β-hydroxy Butyric Acid (PHB) by activated Sludge

Experiment No.	Assimilating Sludge % of dry weight: Total Chloroform soluble lipid	Assimilating Sludge % of dry weight: PHB	Endogenous Sludge % of dry weight: Total Chloroform soluble lipid	Endogenous Sludge % of dry weight: PHB	% Reduction in Total chloroform soluble lipid in endogenous sludge	% Reduction in PHB in endogenous sludge
1	3.80	1.000	2.546	0.454	33.0	54.6
2	3.91	1.632	2.565	0.461	34.3	71.6
3	4.10	1.506	3.066	0.500	25.2	66.8
4	3.11	1.088	2.513	0.434	16.5	60.1

He found the assimilating activated sludge (after an hour's aeration) to contain more PHB than the endogenous sludge (the sludge after aeration). The polymer PHB has been detected in activated sludges formed from laboratory units and from the activated sludge plant at the Indian Institute of Science, Banglore. But the PHB content of the viscous scum formed during the bacterial phase I was only a tenth of the content in assimilating sludge and only a third of the content in the endogenous sludge.

Bacteriological

The total number of pure isolates from the viscous scum was 38; and from the raw sewage used for the purpose was 40.

All the 78 pure cultures were studied for comparison of their cultural and physiological characteristics. The results are discussed below under several heads :

Cultural characteristics of the isolates are shown below:

Table :5-1: Cultural characteristics of the Bacteria isolated from Raw Sewage and the Viscous scum (4th day) formed during Bacterial Phase I.

Source		Raw Sewage	Viscous scum
Dilution used		10^8	10^4
Total Number of Isolates		40	38
Sr.No.	Cultural Characteristics	Percentages	
A.	Chromogenesis		
	White	60	84.2
	Yellow	40	10.5
	Pink	Nil	5.2
B.	Staining reactions		
i)	Gram stain :		
	Gram-negative rods	10	89.5
	Gram-positive rods	20	10.5
	Gram-positive cocci	70	Nil
ii)	Flagella :		
	Polar	Nil	77.0
	Peritrichous	Nil	18.0
	Non-motile	100	5.0
iii)	Special		
	Sudanophilic inclusions	20	84.0
	Capsule	90	37.4
	Spores	Nil	Nil

Chromogenesis. Whitish colonies were found in greater numbers in the scum, while yellowish colonies were found in larger numbers in raw sewage. A very small percentage of pink colonies was also noticed in the scum.

Staining reactions. Gram-negative rods were found maximum (nearly 90%) in the scum while they were present only 10% in raw sewage. Gram positive cocci were found maximum(70%) in raw sewage while they were absent in the scum. Gram positive rods in sewage were nearly double the number found in the scum.

Polar and peritrichous flagella bearing organisms were found in largest number (nearly 96%) in the scum while they were not detected in raw sewage.

Organisms accumulating lipid reserves as indicated by Burdon's staining technique were found in maximum (84%) in the viscous scum while they were only 20% in raw sewage. But capsule bearing organisms were 90% in raw sewage and only 37% in the viscous scum. Spore-bearers were not detected in both the cases.

Physiological characteristics. The results are shown in Table 5-2. It will be seen from a study of the table (5-2) that the bacteria in raw sewage and in the viscous scum differ fundamentally in several respects. Gelatine liquefying, and nitrogen fixing bacteria were found in largest numbers in addition to bacteria producing a neutral reaction in litmus

Table :5-2: Biochemical reactions of the Bacteria Isolated from Raw sewage and the Viscous scum (4th day) formed during Bacterial Phase I.

Source		Raw Sewage	Viscous scum
Dilution used		10^8	10^4
Total Number of Isolates Studied		40	38
Sr.No.	Biochemical characteristics	Percentages	
1.	Starch hydrolysis	20.0	5.2
2.	Gelatine liquefaction	50	10.5
3.	Tributyrin hydrolysis	70.0	100.0
4.	H_2S production	20.0	Nil
5.	Indole production	Nil.	Nil
6.	Methyl red positive	10.0	Nil
7.	V.P. positive	10.0	Nil
8.	Litmus milk : acidic	10.0	Nil
	Alkaline	20.0	36.8
	Neutral	70.0	63.2
9.	Nitrate reduction	60.0	68.3
10.	Ammonia oxidation	20.0	63.2
11.	Citrate utilization	60.0	42.0
12.	Nitrogen fixation	90.0	42.0
13.	Acid from Glucose	30.0	36.8
14.	Acid from sucrose	20.0	26.3
15.	Acid from lactose	10.0	Nil

milk being found in greatest numbers (70%) in raw sewage. In the viscous scum, on the other hand, the most distinguishing feature was the 100% occurrence of tributyrin hydrolysers. However, bacteria producing acid from glucose, and sucrose and nitrate reducers were comparatively greater. Tests for Indole alone was negative in both cases. None of the isolates in both cases produced gas from glucose, sucrose and lactose.

Classification of the Bacteria

The results are shown in table 5-3 below :

Table :5-3: Bacterial types in the raw sewage and Viscous scum formed in the Bacterial Phase I of Laboratory Model Oxidation *Pond*

Source		Raw Sewage	Viscous scum
Dilution used		10^8	10^4
Total Number of Isolates studied		40	38
Sr.No. Bacterial Types		Percentage	
1. Alcaligenes	spp	Nil	10.5
2. Bacillus	"	Nil	Nil
3. Brevibacterium	"	20	10.5
4. Corynebacterium	"	Nil	Nil
5. Flavobacterium	"	10	Nil
6. Micrococcus	"	40	Nil
7. Pseudomonas	"	Nil	15.8
8. Sarcina	"	30	Nil
9. Xanthomonas	"	Nil	5.5
10. Zoogloea	"	Nil	58.0

From a comparative study of the types recorded for the raw sewage and the viscous scum, it will be seen that there

is a distinct difference between the two cases. In the case of raw sewage Micrococcus spp were found maximum constituting 40%, then came Sarcina spp forming 30%, Brevibacterium spp forming 20% and lastly came Flavobacterium spp forming only 10% of the total.

Brevibacterium spp was the only group which was found both in the raw sewage and the viscous scum. Flavobacterium spp, Micro-coccus spp and Sarcina spp which were found in fairly large numbers in raw sewage were not recorded in the viscous scum.

Bacteria not Isolated from the Scum

The several types of bacteria seen on microscopic examination of a wet or dried and stained smear of the viscous scum have been described at the beginning of this chapter. The bacteria actually isolated by the technique followed are shown in table 5-3, from these two statements it will be seen that the types isolated do not include several of those seen in the stained smears of the scum. It is likely that a few of the dominant bacteria in the viscous scum have not been isolated by the technique employed.

Characterisation and Identification of Zoogloeal Organisms

Microscopic examination of the scum revealed the presence of zoogloea colonies having varying shapes and sizes (Fig.5-1). They resembled either the leafless, dried-up branches of a tree or the long hanging roots of a banyan tree or the main and

branching hair-like roots of trees or the tentacles of an octopus, or the flashes of lightning seen before the stormy south-west monsoon rains. Very often, very large-sized and independent zoogloeas covering more than one entire field (of 100 times magnification) have been noted especially in samples within 18 hours of incubation at 37°C. The Zoogloeas, in the present case, not only resembled those from activated sludge(Taylor,1930; Butterfield, 1935; Flugge, 1896; Dugan and Lundgren 1960; Crabtree, McCoy, Boyle and Rohlich,1965) but also those found in trickling filters (Butterfield and Wattie 1941). They were also found in greater numbers and in considerably larger sizes. It was found very difficult to differentiate between the various types of zoogloea formations as the same sample gave rise to different odd shapes and sizes.

Regarding the cellular contents of the zoogloeas, they were similar to those of the activated sludge, and consisted of packed inactive rods each measuring 2 to 4 μ in length and from 1 to 2 μ in diameter with rounded ends. The bacillary nature of the cells was evident only after staining with the usual basic dyes. Similar rods were also seen just outside the zoogloeal masses in stained preparations. In short, the morphological studies showed that the zoogloeas resembled those found in activated sludge and in trickling filters.

The staining reactions showed that the zoogloea colonies in situ in the scum contained Gram-negative rods, which were

vacuolated and contained sudanophilic granules; and the colonies were surrounded by a polysaccharide material. Phosphate, carboxyl compounds and iron were absent.

To get a better picture of the zoogloeas, pure strains were isolated from the viscous scum as already stated and their morphological and biochemical characteristics were studied. It was possible to isolate 22 strains of zoogloea (58%) in pure cultures out of 38 isolates from the scum. The percentage which could be cultured in different media is shown below :

Table :5-4: Zoogloea formation seen in Different Synthetic Liquid Media

Medium	Total Isolates	No.of Positive Isolates	% of the Total
1. Autoclaved Sewage	38	14	37
2. Unz and Dondero's	38	22	58
3. Nutrient broth	38	20	52.6
4. Crabtree's	38	20	52.6

The morphological and biochemical characteristics of the 22 isolates showing finger-like branchings are given in Table 5-5.

Table :5-5: Morphological and Biochemical characteristics of the Isolates classified as Zoogloea spp.

	Tests	No.of Positive Isolates	% of the Total
i.	Chromogenic : White	22	100
ii.	Gram staining (Negative rods)	22	100
iii.	Flagella staining(Single Polar)	22	100
iv.	Staining for fat droplets	22	100
v.	Capsule staining	12	54
vi.	Starch hydrolysis	Nil	Nil
vii.	Gelatin liquefaction	4	11
viii.	Tributyrin hydrolysis	22	100
ix.	H_2S production	Nil	Nil
x.	Indole production	Nil	Nil
xi.	Methyl red	Nil	Nil
xii.	Acetyl-Methyl-Carbinol production	Nil	Nil
xiii.	Citrate utilization	8	36
ixx.	Acid from glucose	10	45
vx.	Acid from sucrose	8	36
xvi.	Acid from Lactose	Nil	Nil
xvii.	Nitrate reduction	10	45
xviii .	Ammonia oxidation	6	27

It was found that none of these organisms hydrolysed starch. Very few liquified gelatin, whereas all could hydrolyse tributyrin. These data along with the fact that the scum contained 8% of fat and 0.2% poly=β-hydroxy-butyric acid and the acetone powder of the scum showing lipolytic activity

suggested that fat metabolism might be predominent in zoogloea organisms.

The morphological and biochemical characteristics of the zoogloeas isolated from the scum are next compared with those isolated from activated sludges and trickling filters in table 5-6. From a study of this table it will be seen that our pure zoogloeal strains resemble morphologically those isolated by Butterfield (1935), Butterfield and Wattie (1941), Winogradsky (1935), Dugan and Lundgren(1960), Dias and Bhat (1964), Crabtree, Boyle, McCoy and Rohlich(1965) and Unz and Dondero (1964, 1967). Biochemically, like others, our strains did not hydrolyse starch, did not produce H_2S, indole and acetyl-methyl-carbinol and were methyl red negative. They resemble the strains of Dias and Bhat (1964) in their capacity to reduce nitrates while resembling the strains of Winogradsky (1935) in their capacity to oxidise ammonia salts. In contrast to all others, some of our strains are not only found to be citrate utilizers but also to produce acid from glucose and sucrose. While the strains of Dias and Bhat (1964) are found to be iodophilic, ours are not.

Another point of interest is that Butterfield (1935), Butterfield and Wattie (1941), Buck and Keefer (1959), Dugan and Lundgren (1960) and Unz and Dondero (1967) have stated that the zoogloeal strains are capsulated, while Crabtree, Boyle, McCoy and Rohlich (1965, 1966) have found these organisms

Table :5-6: Comparative Characteristics of Isolates from Activated Sludge, Trickling Filter and Stored Raw Sewage

Tests	Butter-field & Wattie	Buck & Keefer	Crabtree et al	Dias & Bhat	Dugan & Lundgren	Unz & Dondero	Winograd-sky	Ganapati et al
Year	1935,1942	1959	1966	1964	1960	1964	1935,1937	1967
Source *	A.S.	A.S.	A.S.	A.S.	A.S.	T.F.	A.S.	S.R.S.
Morphological								
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Ellep. to rod	Rod(22)
Size (μ)	1.5x2.4	1.0x5.0	0.5x1.0 by 1.0x3.0	..	1x2.4	1 to 2.5	0.5 to 1.5	1x2.4(22)
Flagella, single Polar	P	N	P	P	P	P	P	P
Staining Reactions								
Gram	N	N	N	N	N	N	N	N(22)
Capsule	P	P	N	N.R.	N.R.	P	N.R.	P(10)
Burdon	N.R.	N.R.	P	P	N.R.	P	N.R.	P(22)
Growth in Synthetic Broths								
Nutrient broth	Floccu-lent	Good growth	N.R.	Floccu-lent	Floccu-lent	N.R.	N.R.	Flocculent(20)
Casitone Glycerol Unz & Dondero	N.R.	N.R.	N.R.	N.R.	N.R.	Floccu-lent	N.R.	Flocculent(22)
Casein Hydrolysate (Crabtree)	N.R.	Tyrod's	Floccu-lent	N.R.	N.R.	N.R.	N.R.	Flocculent(20)

* A.S. = Activated Sludge ; T.F. = Trickling filter ; S.R.S. = Stored Raw Sewage

(Table :5-6:) contd:

Tests	Butter-field & Wattie	Buck & Keefer	Crabtree et al	Dias & Bhat	Dugan & Lundgren	Unz & Dondero	Winograd-sky	Ganapati et al
Autoclaved Sewage.	Luxuriant after aeration	..	N.R.	N.R.	N.R.	N.R.	N.R.	Flocculent(14)
pH.	8.2-8.4	N.R.	7.0-7.5	N.R.	N.R.	6.5-8.0	N.R.	8.2-8.4
Cultural Characteristics								
Agar Colony formation	N.R.	Pin pointed	Entire raised, glistening, cream to straw colour after 72 hrs. then undulate, tough and leathery	N.R.	Pin pointed to4-5mm in 72 hrs, Pink to straw glistening moist, tough, leathery.	Colourless punctiform, dry, tough 3 days old colonies	Silicagel; pale yellow	Whitish, round, pin pointed to 1.0mm diameter and glistening in 48 hrs.(22)
Biochemical Reactions								
Starch hydrolysis	N.R.	N.R.	N	N	N	N.R.	N.R.	N(22)
Gelatinn liquefaction	N.R.	N.R.	N	N	N	Variable	N.R.	P(4)
Tributyrin hydrolysis	N.R.	N.R.	N.R.	N.R.	N.R.	P	N.R.	P(22)
H_2S production	N	N	N	N	N.R.	N	N.R.	N(22)
Indole production	N	N	N	N	N	N	N.R.	N(22)
Methyl Red	N.R.	N	N	N.R.	N	N	N.R.	N(22)
Voges and Proskauer	N.R.	N	N	N.R.	N	N	N.R.	N(22)
Nitrate reduction	N.R.	N.R.	N	35%	N.R.	P	N.R.	P(10)
Ammonia oxidation	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	P	P(16)
Citrate utilization	N.R.	N	N	N.R.	N.R.	N	N.R.	P(8)
Acid from glucose	N	N.R.	Oxidatively used	N	N	N	N.R.	P(10)
Acid from sucrose	N	N.R.	"	N	N	N	N.R.	P(8)
Acid from lactose	N	N.R.	"	N	N	N	N.R.	N(22)
Reaction to litmus milk	N.R.	P(alk)	P(alk)	N.R.	N.R.	Variable	N.R.	Slightly alk(22)
Iodophilic material stored.	N.R.	N.R.	N.R.	P	N.R.	N.R.	N.R.	N(22)

Number in parenthesis shows the number of zoogloeas showing positive characteristics.
N.R. = Not reported ; N - Negative ; P - Positive

to be non-capsulated. In our case, 54% of the isolates were capsulated. As regards the test for gelatine liquefaction, Crabtree and McCoy (1967), Dias and Bhat (1964), Dugan and Lundgren (1960) have stated that these organisms are non-gelatin liquefiers. On the other hand, Unz and Dondero (1964) have found them variable, while only 11% of our isolates were gelatinolytic. The above observations would appear to confirm the conclusions of Unz and Dondero (1964) that there might exist in nature strains of Zoogloea ramigera which are physically and/or biochemically different from the neo-type recently described by Crabtree and McCoy (1967). More of this later.

In short the zoogloeas discovered by us in the viscous scum of stored raw sewage have characteristics almost similar to those isolated from activated sludge and trickling filter eco-systems. The pure strains isolated from the scum resembled also the pure culture isolates in their morphological traits and staining reactions of other workers but disagree with them in their relation to oxidise ammonia and to reduce nitrates to nitrites.

Therefore we are inclined to classify tentatively our pure zoogloeal strains as variants of the classical Zoogloea ramigera with the characteristics as shown in table 5-7 and 5-8 below:

Table :5-7: Sub-Grouping of the Zoogloea spp. on the basis of Nitrate Reduction and Ammonia Oxidation

Sub-Groups	Reaction	No.of Positive Isolates	% of the Total
A	Nitrate Reduction only	4	18.5
B	Ammonia Oxidation only	4	18.5
C	Nitrate Reduction and ammonia oxidation both Positive	8	36.0
D	Nitrate reduction and ammonia oxidation both negative	6	27.0

So, the zoogloeas isolated by us can be divided into four sub-groups as shown in Table 5-7. The important characteristics of these four sub-groups are shown in the next table 5-8 below.

Table :5-8: Important Biochemical Characteristics of the Zoogloea spp.

Tests	Sub-groups			
	A	B	C	D
No.of Isolates	4	4	8	6
1. Sudanophilic	4	4	8	6
2. Capsule staining	0	2	4	6
3. Citrate utilization	0	2	4	2
4. Acid from glucose	4	4	0	2
5. Acid from sucrose	4	4	0	0
6. Gelatin liquefaction	0	2	2	0

On page 5-3 , it has been stated that the viscous scum showed high lipolytic activity. In order to find out whether the above four typical strains isolated from the viscous scum were responsible

for the high lipolytic activity, they were tested for the same using Mcdonald and Lefave (1962) method. It was found that none of these organisms showed any activity, indicating thereby that some other organisms were involved. So, further work is necessary to find out the organisms responsible for the high lipolytic activity.

Pure Zoogloea spp Strains Isolated from the Viscous scum compared with the Pure Zoogloeal Strains obtained from Dr.Barry Friedman of Ohio State University and Dr. Koby Crabtree of the University of Wisconsin, U.S.A.

Four typical strains, (Sc-8,55,58 and 62) of the four sub-groups A, B, C and D (vide Table 5-8) were compared with the two strains (19173 and C-3) obtained from Dr.Barry Friedman of Ohio State University, and one strain (1-16-M) obtained from Dr.Koby Crabtree of the University of Wisconsin. The seven strains were grown on sewage agar slants, and the growths were suspended individually in sterile distilled water as already described under methods in Chapter 3. Five ml of the suspensions were inoculated into 100 ml of sterile sewage taken in flasks which were incubated at room temperature for 4 days along with one uninoculated control. The filtrates were analysed for the several physico-chemical changes as already described in Chapter 3. This experiment was repeated thrice and the average results are shown in table 5-9.

Table :5-9: Comparative Study of the Zoogloeal Strains Isolated from the Viscous scum and those obtained from Dr.Friedman and Dr. Crabtree of U.S.A.

5-23 Samples	Uninoculated Sterile Sewage	Viscous Scum				Dr.Barry Friedman		Dr.Coby Cabtree
Strain No.		Sc-38	Sc-55	Sc-58	Sc-62	19173	C-3	1-16-M
Tests				% Reduction after 4-days				
1. Turbidity(klett units at 660mμ)	37 (30-45)	28.1 (25.0-30.4)	+81.2* (+74.0-+85.2)	47.0 (42.0-50.4)	40.6 (37.5-43.8)	68.7 (64.0-71.4)	84.3 (80.1-89.4)	43.7 (40.5-47.0)
2. pH	7.3 (6.1-7.5)	7.3 (7.0-7.6)	7.8 (7.5-8.0)	7.7 (7.5-8.0)	7.4 (7.0-7.6)	7.8 (7.45-7.9)	7.7 (7.4-7.9)	7.6 (7.4-7.9)
3. Acid $KMnO_4$ Value (4 hrs.) mg/l	7.7 (6.5-8.3)	21.9 (17.4-27.8)	+4.8* (+1.3-+9.0)	46.4 (40.0-51.4)	25.6 (21.3-29.4)	40.3 (35.6-42.8)	62.4 (59.4-67.0)	22.4 (17.6-28.0)
4. Orthophosphate (mg/l)	16.4 (13.5-20.8)	+62.3* (+59.4-+66.8)	+27.2* (+24.8-+31.4)	+157.0* (+130 + 190)	+105* (+97 - +115)	+92.2* (+89.6-+98.4)	+105* (+100-+120	+202* (+198-+208)
5. Ammonia nitrogen (mg/l)	14.0 (11.5-18.0)	0.6 (0.0-1.4)	+6.5* (+2.8-11.4)	19.5 (13.4-22.6)	22.0 (19.7-27.3)	4.5 (1.8-8.3)	28.5 (26.4-32.8)	2.6 (0.8-4.3)
6. Relative stability (%)	21.0 (18-24)	37 (30-40)	21 (18-24)	80 (74-84)	37 (30-40)	60 (54-68)	60 (54-68)	60 (54-68)

* + indicate % increase over 0 day value

The results are discussed below :

Turbidity. Excepting the strain Sc-55, all others showed varying degrees of reduction. Both the strains of Dr.Friedman gave the highest percentages; and the strain Sc-38 gave the lowest. The other strains showed about 45% reduction.

pH. None of the strains showed significant increases.

Acid $KMnO_4$ value (4 Hours). Excepting Sc-55 all other strains showed varying percentages of reduction. The strain C-3 showed the highest (62%) reduction; the strains (Sc-38, Sc-62 and 1-16-M) showed the lowest (21-25%); and the remaining two strains (Sc-58 and 19173) showed 40-46% reduction. The strain Sc-55 showed a slight increase of about 5%.

Ortho-phosphates (PO_4). All the strains were found to show a high increase (27 to 200%). The maximum was shown by the strain 1-16-M and the lowest by the strain Sc-55.

Ammonia-nitrogen. Excepting the strain Sc-55, all others showed only a small amount of reduction (0.5-29%). The strain Sc-55 showed an increase of about 7%.

*Relative Stability. Excepting the strain Sc-55, all others showed varying degrees of relative stability. The strain Sc-58 gave the maximum stability of 80% ; the strains 19173, C-3 and

*Relative stability test is a rough measure of the quality of the supernatant liquor in the cultures. It is quite a simple test involving little equipment and time. Its result is the percent ratio of the available oxygen present in the sample to its total oxygen demand. This percentage or ratio is indicated by determining the number of days required to exhaust the available oxygen in the sample, using methylene blue as indicator.

1-16-M showed about 60%, and the strains Sc-38 and Sc-62 showed about 40%. As for the strain Sc-55, there was no change.

DISCUSSION

Zoogloea ramigera - The present position

In chapters 5,6 and 7, it is shown that the tree-like or branch-bearing colonies of zoogloea ramigera as originally described by Itzigsohn (Breed, Murray and Smith 1957) are often seen in habitats containing excessive organic matter in different stages of decomposition. Their presence in such situations would seem to indicate either that they played an important role in purification of these organic wastes or that conditions most favourable for them existed.

Their presence in activated sludge flocs and in trickling filter slimes was first recognized in the early half of this century by Butterfield (1935); Butterfield, Ruchhoft and McNamee (1937); Heukelekian and Littman (1939); and Butterfield and Wattie (1941) who assumed that they consisted of only one bacterial species of the same name, which was embeded in the zoogloeal or jelly-like matrix. These workers also stated that they were the chief agents of purification in the two systems of sewage treatment.

The zoogloeal colonies are surrounded by a halo of sticky gelatinous material which is optically homogenous with water and therefore cannot be readily seen ordinarily under the microscope unless it is specially stained with Indian ink or alcian blue capsular staining technique of McKinney. The Jelly stuff surrounding the zoogloeal colonies seem to have high adsorptive properties, and that the adsorption of specific substrats is accomplished in the surrounding capsular stuff and that a stock of nutrient is established here for the eventual metabolism by the cellular contents. The number of individual cells on a zoogloeal colony may run into thousands and may constitute the major portion of the solid matter. It is to these highly developed zoogloeal growths which are seen floating as scums in oxidation ponds that we have to look for an answer to the modus operandi of purification in oxidation ponds in addition to other factors. May be that the bacterial cells within the zoogloeal colonies consisting of Zoogloea ramigera and other bacterial species act directly on soluble materials in sewage or become operative through their enzymes secreted into the liquid phase or by some other agency i.e. the jelly-like capsular material surrounding the colony. The insoluble materials are first adsorbed on the surface of Zoogloeal colonies so that they may be made available for the embeded bacteria later after hydrolysis and oxidation (Heukelekian 1941).

Criteria used by the Earlier Investigators

Butterfield (1935) described the dominant organisms in these zoogloea colonies as of rods 2 to 4 μ long, and 1.5 μ in diameter and with rounded ends. They showed a marked tendency to grow in flocs or zoogloeal masses or as fingered tree-like flocs in liquid media. Capsules were always observed but no spores. They were found having a single, polar flagellum and Gram-negative. Good growth was found between 20° and 37°C with optimum at 28° to 30°C. The optimum pH was about 7.0 to 7.4. No pigment, no acid from sugars, no indole, and no H_2S were formed. They were not killed under anaerobic conditions for 7 days. The peculiar tree-like form of zoogloeal growth in liquid media was considered almost sufficient for identification. So, Butterfield considered this organism to be a variety of Zoogloea ramigera.

Within a decade or so, other types of flocs forming and non-floc forming bacteria were reported by Allen (1940,1944) McKinney and Horwood(1952), and by McKinney and Weichlein(1953) in the activated sludge flocs with the result that the dominant idea which was prevailing in the earlier half of this century about the undisputed claimants of sewage purification was shattered. Also, Hawkes (1963) added further to the confusion by stating that Zoogloea ramigera might not be a true bacterial species but probably a colonial manifestation of several types of organisms.

Criteria used by the Latest Investigators

Crabtree and McCoy (1967) recently added a neo-type of Zoogloea ramigera with its important characteristics (Table 5-10). Unz and Dondero (1967) have also described the general taxonomical characteristics of "the etiological cells of the branching zoogloeas" isolated directly by "single cell isolations of bacteria, primarily from, natural, branching, waste water zoogloeas made by the micro-manipulation. Therefore they claim to the correctness of their bacteria having originated only from the zoogloeas on account of the method of isolation employed by them and not from "the more rapidly growing extraneous bacteria," seen outside the zoogloea colonies. They implied thereby that the isolations of zoogloeal bacteria made by others, though from natural flocs and slimes, by the conventional methods, did not warrant the conclusion that the isolates came only from the zoogloea colonies and not from extraneous sources. Also, some uncertainty seemed to exist as to whether the gelatinous matrix of the zoogloeal masses had been synthetised by the bacteria or whether the bacteria were simply embeded in a chemically preformed matrix(Butterfield 1935). The fact that the bacteria found within the zoogloeal colonies also might contain extraneous bacteria having got in by mere accident was also forgotten. The above presumption is amply supported by Unz and Dondero (1967) who have also obtained pigmented and non-pigmented rods from the same natural zoogloeas

Table :5-10: Taxonomical Characteristics of Zoogloea ramigera

Authors	Crabtree & Mecoy	Unz and Dondero	Friedman & Dugan			Ours			
Year	1967	1967	1968			1968			
Culture No.	1-16-M	Group I	115	C-1	C-3	A Sc-38	B Sc-55	C Sc-58	D Sc-62
Taxonomical Attributes									
Gram Stain	-	-	-	-	-	-	-	-	-
Flagella, single polar	+	+	+	+	+	+	+	+	+
Capsule	-	+	+	-	+	-	+	+	+
Burdon	+	-	+	-	+	+	+	+	+
Gelatin liquefaction	-	+	+	-	+	+	-	-	-
Starch hydrolysis	-	-	+	-	+	-	-	-	-
Litmus milk	Alk	Alk	Sl.Alk	Alk	Acid	Alk	Alk	Alk	Alk
Indole	-	-	-	-	+	-	-	-	-
V.P.	-	-	..	..	..	-	-	-	-
M.R.	-	-	-	-	+	-	-	-	-
Koser's citrate	-	-	-	-	+	+	-	-	+
Nitrate reduction	-	+	+	+	-	+	-	+	-
H_2S	-	-	-	-	-	-	-	-	-
Carbohydrates no gas no acid	Actively oxidised	-	-	-	-	Acid	-	-	-
Tributyrin	..	..	..	..	..	+	+	+	+
Zoogloea colony with matrix	Neg.	+	+	-	-	-	-	-	+
Ammonia Oxidation	..	..	..	..	..	..	+	+	-

from which they obtained their Group I true zoogloeal organisms. Hence, there appears to be no case for doubting the correctness of the so-called conventional method of isolation practised during the first half of this century. In our own case we could always get not one or two but hundreds of the classical type of zoogloeal colonies in one ml; and each colony was containing not less than several million cells. So, even in the conventional method of isolation one cannot but get "the etiological cells" of the branch-bearing zoogloeas in as much as they are found in preponderance.

Unz and Dondero (1967) isolated two groups of bacteria from the natural zoogloea colonies and considered the following taxonomical characteristics as being important for considering the bacteria designated as Zoogloea spp by them.

They found the group I zoogloea isolates to possess the characteristics of (a) zoogloea formation (b) denitrification, (c) urease, (d) hydrolysis of gelatin (e) catalase and oxidase. They did not (a) utilise Koser's citrate, (b) produce H_2S, indole, acetylmethyl carbinol and (c) did not hydrolyse starch.

Very recently Friedman and Dugan (1968) have made a study not only of their zoogloeal isolates but also a "thorough study of the taxonomic status of zoogloea in general" with special reference to "capsule or zoogloeal matrix formation;"

those zoogloeal-forming bacteria which are not generally recognized as belonging to the genus zoogloea," and the relationship of zoogloea to the geletinous matrix-producing genera of the Sidero-capsaceae. They also add that the formation of a zoogloeal matrix by a bacterial strain is not to be equated with floc formation for McKinney (1956b) has recorded several bacterial species forming flocs without producing gelatinous matrix. So, they consider zoogloeal formation as the production of a high polymerised exocellular material similar to a capsule, cell size and shape and gelatinous material production being dependent upon culture media, incubation temperature and oxygen tension; and therefore are not to be considered as adequate criteria for characterising zoogloea species. This is confirmed by comparisons of their zoogloea isolates with those of Crabtree's. They conclude that " the presence of a zoogloeal matrix appears to be the only valid criteria for distinguishing between a Zoogloea sp and a non-fluorescing Pseudomonas sp. However, production of zoogloeal matrix is related to growth conditions. Therefore, the absence of matrix is not an adequate criteria for identification, particularly when culture conditions are not accurately described. This is the situation in much of the older literature, and it probably contributes to the confusion which exists with regard to the classification to these bacteria."

Now, the four typical zoogloeal strains isolated by us from viscous scums are compared taxonomically and biochemically with those described recently by Crabtree and McCoy(1967) Unz and Dondero (1967) and Friedman and Dugan (1968) in Table 5-10.

In Bergey's Manual (Breed, Murray and Smith 1957), an important distinction is made between organisms with polar flagella and those with peritrichous flagella, the former being placed in the order Pseudomonadales and latter in Eubacteriales. The genera Pseudomonas and Xanthomonas attack carbohydrates oxidatively with the formation of acid; the genera Aeromonas and Zymomonas attack sugars fermentatively with the formation of acid and gas and the genus Zoogloea shows no action on carbohydrates. All the five genera have single polar flagella. But Galarneault and Leifson (1956) have created a new genus called Lophomonas for lophotrichously flagellated bacteria which do not utilise Carbohydrates. So, the characteristics of Lophomonas are very much like those of Zoogloea except that the latter do have the ability to form flocs. Dias and Bhat (1964) differentiated Zoogloea spp. from Comamonas on account of zoogloea formation.

Comparison with Crabtree and McCoy's Zoogloeal Strain 1-16-M

Excepting capsule formation, citrate utilization, nitrate reduction and carbohydrate fermentation, all other traits of our cultures agree with those of the strain 1-16-M. Our

strain Sc-38 alone resembles 1-16-M in not having a capsule while all the other three strains are capsulated. In the case of the Koser's citrate utilization test, our two strains Sc-55 and Sc-58 resemble 1-16-M in not utilizing citrate; while the other two strains of ours utilized citrate as the sole carbon source. Our strains Sc-55 and Sc-62 and Crabtree's 1-16-M do not reduce nitrate as against the other two strains Sc-38 and Sc-58 which reduce nitrates. Lastly while Crabtree's 1-16-M is reported to oxidize glucose actively, only one of our four strains viz. Sc-38 produces acid from glucose, the other three do not do so, though found to be growing in it.

Comparison with the Group I Zoogloeal Isolates of Unz and Dondero (1967)

In respect of the test for gelatin liquefaction, the group I isolates were active gelatin liquefiers while all our four strains are non-gelatinolytic. Two of our strains Sc-55 and Sc-58 like the group I isolates do not utilize citrates while the other two Sc-38 and Sc-62 do. Again, two of our zoogloeal strains Sc-38 and Sc-58 like those of Group I isolates are able to reduce nitrate, while the remaining two strains do not. Our strains Sc-55, Sc-58 and Sc-62 resemble the Group I isolates of Unz and Dondero in their inability to ferment glucose while our strain Sc-38 does produce acid from glucose. Finally, two of our strains Sc-38 and Sc-62 show definite matrix formation like the Group I isolates of Unz and Dondero while our two other strains do not.

Comparison with the Zoogloeal Strains Isolated by Friedman and Dugan (1968)

Our strain Sc-38 alone resembles in almost all respects with the C-1 strain of Friedman and Dugan excepting the matrix formation, Burdon's staining reaction, Koser's citrate test and glucose test. Our strain Sc-55 resembles the strain No. 115 in all respects excepting the following, gelatin liquefaction, starch hydrolysis, nitrate reduction and zoogloea colony with matrix formation. The isolate Sc-58 also resembles the strain C-3 of Friedman and Dugan in seven tests and differs in respect of gelatin liquefaction, starch hydrolysis, litmus milk, indole, methyl red, Koser's citrate and nitrate reduction. Our fourth organism Sc-62 resembles the strain No. 155 of Friedman and Dugan in eight tests and differs from it in 6 other tests.

Summing up, it will be seen from the above that different workers have described Zoogloea ramigera as having several different distinguishing taxonomical and biochemical characteristics. For example, Crabtree and McCoy (1967), Dugan and Lundgren (1960), MicKinney and Horwood (1957) and Wattie (1943) have reported it as not denitrifying and not ureolytic while Dias and Bhat (1964); Unz and Dondero(1967) found it giving positive reactions for the above two tests. Again Butterfield (1935), Crabtree and McCoy (1967), Dugan and Lundgren (1960), Heukelekian and Littman (1939), McKinney

and Horwood (1952), and Wattie (1943) found it non-gelatinolytic while Dias and Bhat (1964) found that only a few strains could hydrolyse gelatin but Unz and Dondero (1967) found the whole group I organisms gelatinolytic. Butterfield (1935) has stated that its peculiar tree-like form of zoogloeal growth in liquid media was sufficient for its identification while Unz and Dondero (1967) have stated that the Zoogloea formation in the branching form alone may be unreliable for identification. Still further Unz and Dondero (1967) and Friedman and Dugan(1968) stated that Zoogloea organisms formed a gelatinous matrix in which the cells were embeded while the Zoogloea organisms of Crabtree and McCoy did not form a gelatinous matrix though the cells were arranged in finger-like projections. Also, crystal violet is not decolourised by the gelatinous matrix forming Zoogloea while the other type produced violet colonies. So, it would seem that several varieties of zoogloea ramigera exist in nature as in the case of other microscopic organisms. Further detailed work is, therefore, necessary to reconcile the important differences noted by individual workers and by us in the field.

Comparison between the typical microflora in seven different activated sludge samples (Dias 1964) and in the viscous scum formed during bacterial phase I.

As there are several good references pertaining to the dominant bacterial flora of activated sludge, an attempt was made to compare the bacterial types found in them with those of the viscous scum. Dias (1964), recorded the dominant bacterial

flora of seven different activated sludge samples of Bangalore. An attempt is made below to compare the types of bacterial flora isolated from his activated sludge samples with those isolated from the viscous scum formed during bacterial phase I of the oxidation pond. The results are compared in tables 5-11, 5-12 and 5-13 under several heads.

From a study of the results shown in table 5-11 it will be seen that the dominant bacteria in the viscous scum resemble those isolated from the seven activated sludge samples of Bangalore in (a) the very high percentage of Gram-negative rods; (b) in the low percentage of Gram-positive rods and cocci; (c) in the high percentage of bacteria showing sudanophilic inclusions and (d) in the low percentage of yellow and orange pigmented bacteria.

In table 5-12, the distribution of some physiological characteristics of the dominant activated sludge bacteria are compared with that found in the viscous scum formed during the bacterial phase II of oxidation pond. Here again, our isolates resemble those found in the several activated sludges in : (a) the fairly high percentage of bacteria showing reduction of nitrate, (b) the low percentage of bacteria showing gelatin liquefaction, (c) starch hydrolysis; and (d) the highest percentage of bacteria showing tributyrin hydrolysis.

Table :5-11: Distribution of certain Morphological and Cultural Types among the Dominant activated Sludge Bacteria from Dias (1964) compared with those found in the Viscous scum formed during the Bacterial Phase I

Sludge Sample No.	1	2	3	4	5	6	7	Baroda viscous scum
Total No. of Isolates	41	45	43	39	42	57	52	38
Types of Bacteria	Positive Isolates (Percentages)							
A. Morphological								
1. Rods: Gram negative	88	93	93	96	93	58	96	89.5
2. Rods and Cocci Gram positive	10	7	2	4	7	42	4	10.5
3. Non-Motile Gram negative	15	9	2	8	19	4	8	-
4. Polar flagella: Gram negative	76	82	93	83	74	54	85	58.4
5. Sudanophilic inclusions	69	60	49	46	43	32	59	84.0
B. Cultural								
Flocculent growth in PPYE broth or PYE broth	54	53	19	40	19	22	50	52.6
C. Pigmentation								
1. Yellow and orange	15	7	14	6	5	39	0	10.5
2. Melanin	2	2	26	6	7	0	4	-
3. Green	0	0	0	0	10	0	0	-
D. Growth at 36°C	94	98	95	91	97	97	97	100.0

Table :5-12: Distribution of some Physiological Characteristics among the Dominant activated Sludge Bacteria compared with those found in the Viscous Scum formed during the Bacterial Phase I

Sludge Sample No.	From Dias (1964) : 1	: 2	: 3	: 4	: 5	: 6	: 7 :	Baroda Viscous scum
Total number of isolates	41	45	43	39	42	57	52	38
Types of Bacteria	Positive Isolates Percentages							
Acid from glucose *	7	2	14	10	12	5	6	36.8
Gas from glucose	0	0	0	0	0	0	0	0
Nitrate reduced								
a) to nitrite	41	33	12	28	45	49	25	68.4
b) beyond nitrite	10	4	51	5	10	6	13	68.4
Growth in milk resulted in								
a) acid reaction	5	0	5	3	7	2	0	0
b) alkaline reaction	22	13	7	9	17	18	8	36.8
H_2S produced	15	20	26	19	19	28	17	0
Indole produced	7	9	44	13	12	0	8	0
Urea hydrolysed	46	51	24	36	58	56	26	-
Uric acid decomposed	16	27	21	13	19	14	8	-
Arginine hydrolysed	5	0	9	13	12	3	6	-
Gelatine hydrolysed	15	18	26	19	31	46	12	10.5
Starch hydrolysed	10	11	5	8	19	14	8	5.2
Iodophilic polysaccharide accumulated	37	51	16	37	29	16	44	0
Pectin hydrolysed	3	2	0	0	5	4	8	-
Cellulose and alginate decomposed	0	0	0	0	0	0	0	-
Tributyrin hydrolysed	61	79	53	62	38	41	53	100

But in certain other physiological traits, the bacteria found in the viscous scum differ from those found in the activated sludge samples such as in (a) the higher percentage of bacteria showing acid from glucose; and (b) the production of alkaline reaction to litmus milk.

In table 5-13, a comparison between the bacterial types found in the seven different sludge samples (Dias and Bhat 1964 and Dias 1964) with those in the viscous scum formed during the bacterial phase I of oxidation pond is made. Dias (1964) stated that all the sludge samples showed organisms similar to Zoogloea ramigera and that the percentages in individual samples varied between 19 and 61%. In six out of seven samples the bacterial population was made up chiefly of Zoogloeas and Comamonas; in sample No.6, there were 35% of Corynebacterium besides 23% of Zoogloea and 30% Comamonas suggesting the possibility that under certain ecological conditions, the otherwise predominanently Gram-negative flora of activated sludge could turn out to be one in which both Gram-negative and Gram-positive forms coexist. Coryneforms were normally present in sludge though to a small extent. Sludge sample No.3 which did not show a typical microscopic appearance was the only sample which gave rise to growth of yeasts and molds on sewage agar plates. He has also added that the two forms identified as Comamonas fowleri nov. sp. and Comamonas Butterfieldii nov. spp were to be considered as representative

forms characteristic of activated sludge.

Table :5-13: A Comparison between the Bacterial types in the Seven Different Sludge Samples (Dias 1964), and the Viscous scum formed during Bacterial Phase I

Sludge Sample No.	Bangalore Sewage : 1	2	3	4	5	6	7	Baroda Sewage : Viscous Scum
Total No.of Isolates	41	45	43	39	42	57	52	38
Organisms Types	Percentage of the total							
Achorombacter sp	0	0	2	3	5	2	0	0
Aerobacter "	0	0	0	3	0	0	0	0
Alcaligenes "	0	2	0	3	2	0	0	10.5
Bacillus "	0	5	0	0	2	0	0	0
Brevibacterium "	5	0	0	0	0	0	0	10.5
Corynebacterium "	0	0	0	0	0	35	4	0
Comamonas "	24	33	65	41	52	30	35	0
Flavobacterium "	2	2	2	0	0	0	4	0
Micrococcus "	2	2	2	5	2	7	0	0
Pseudomonas "	2	0	2	5	12	12	4	15.8
Spirillum "	2	0	0	0	0	0	0	0
Zoogloea "	61	56	19	41	19	23	54	58.0
Yeasts "	0	0	0	0	0	0	0	0
Xanthomonas "	0	0	0	0	0	0	0	5.0

In the case of the viscous scum, the zoogloeal group of organisms formed the maximum percentage of 58 as in the cases of sludge samples No. 1,2 and 7 of Dias and Bhat (1964). The Pseudomonas group which constituted 15.8% in our scum was found

in 6 out of 7 samples of Dias and Bhat (1964) and the group percentages varied between 2 and 12. In sludge No.2 this group was not represented. Brevibacterium group was 10.5% in the scum and 5% in sludge No.1 only, and in all other sludge samples, it was not represented. Alcaligenes spp were the last group of organisms in 10.5% of the total isolates in the scum and this group was represented only in three out of seven sludge samples (2 to 3%) in samples numbering 2,4 and 5.

The viscous scum formed at the surface during bacterial phase I of lagooned sewage has been studied from the physical, chemical and microbial aspects. Physically, the viscous scum is brownish, leather like or rubber like. It is found to contain hundreds of zoogloeas of varying shapes and sizes, protozoans and other types of bacteria. The scum contains not only 8% fat on a dry weight basis but also 0.2% of poly-β-hydroxy butyric acid (PHB). The acetone powder prepared from it shows lipolytic activity towards tributyrin.

Bacteriologically, the scum differs from raw sewage but resembles activated sludge flocs. Ninty percent of the scum isolates are Gram-negative rods, 84% of them show sudanophilic inclusions and nearly 70% are flagellated, polar or peritrichous. None of them is spore-forming; and none accumulate and iodophilic substance. The dominant group of organisms is zoogloeal, constituting 58% of the total isolates, and is accompanied by Pseudomonas spp., Alcaligenes spp. Brevibacterium spp, and

Xanthomonas spp playing a sub-dominant role.

Again, the scum isolates resemble the activated sludge samples of Dias (1964) in the dominant group of microorganisms being Zoogloeas, and the Pseudomonas, Brevibacterium, and Alcaligenes groups playing a sub-dominant role. Poly-β-hydroxy butyric acid is present in both the cases; but nearly double the number of microflora is able to hydrolyse tributyrin-[illegible] than in the activated sludge samples; and the fat content is also found to be greater than in activated sludge samples.

Other points of similarity are the high percentage of Gram-negative rods, low percentage of Gram positive rods and cocci; the high percentage of bacteria showing sudanophilic granules; and the low percentage of yellow and orange pigmented cells.

The only point of difference is that none of the strains isolated from the viscous scum accumulates an iodophilic substance in [illegible]its cells as in the activated sludge samples (Ganapati, Amin and Parikh, 1967).

So, it would appear that the viscous scum formed at the surface during bacterial phase I of lagooned sewage is similar to activated sludge flocs in several important respects. The implications of this finding seem to be of considerable interest, and need further elucidation.

SUMMARY

1. The microscopic, bacteriological and biochemical characteristic of the viscous scum formed at the surface of stored sewage,

and of the zoogloea colonies found in *situ* in the viscous scum are given.

2. A comparison between the four typical zoogloeal strains isolated from the viscous scum is made with similar strains described by other workers during the last 5 years.

3. The similarities existing between the viscous scum and activated sludge flocs are discussed.
