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Microbial Ecology of Activated Sludge

I. Dominant Bacteria

F. F. DIAS AND J. V. BHAT

Fermentation Technology Laboratory, Indian Institute of Science, Bangalore, India

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ABSTRACT

DIAS, F. F. (Indian Institute of Science, Bangalore, India), AND J. V. BHAT. Microbial ecology of activated sludge. I. Dominant bacteria. Appl. Microbiol. **12**:412-417. 1964.—Over 300 bacterial strains were isolated from seven samples of activated sludge by plating on sewage agar. Gram-negative bacteria of the genera *Zoogloea* and *Comamonas* predominated. Many isolates (51%) showed sudanophilic inclusions of poly-β-hydroxybutyric acid, whereas 34% accumulated iodophilic material on media containing starch. A large number required either vitamins or amino acids, or both, for growth. None of the isolates tested for their ability to bring about changes in autoclaved sewage produced an effluent comparable in quality to the activated sludge control, although the *Zoogloea* did produce activated sludgelike flocs. A study of 150 bacterial strains isolated from raw sewage revealed that they differed from the sludge isolates in several respects. Coliforms, which constitute nearly a quarter of the sewage isolates, were rarely encountered in sludge.

Although the activated sludge method of sewage stabilization is an outcome of microbial activity, very little information exists on the microflora of this ecosystem. After the pioneering work of Butterfield (1935), it was generally believed that *Zoogloea ramigera* was the only bacterium of any consequence in sludge from the point of view of sewage purification. McKinney and Horwood (1952) and McKinney and Weichlein (1953), however, focused attention on other bacteria concerned in activated sludge formation, but provided no information on their relative distribution in sludge. Allen (1943), Jasewicz and Porges (1956), and Rogovskaya and Lazareva (1959) also studied the bacterial flora of sludge, but their investigations were far from comprehensive and did not lead to the isolation of *Z. ramigera*. A detailed investigation on the bacterial flora of sludge was therefore undertaken, and in this paper the dominant bacterial flora is described. When this investigation was nearing completion, an abstract of a paper (Anderson and McCoy, 1963) appeared in which the flora of sludge was shown to be dominated by *Pseudomonas* species.

MATERIALS AND METHODS

Seven sludge samples were examined. Samples 1 and 2 were collected from the final settling tank of the Institute's activated sludge plant. Samples 3 and 4 were drawn from units built up in the Biochemistry Department by the fill-and-draw technique. Microscopic examination of sample 3 revealed the presence of several filamentous forms in addition to the usual flora and fauna. The effluents obtained from both units were reported to be satisfactory. Samples 5, 6, and 7 were built up in this laboratory by aerating sewage on a rotary shaker (250 rev/min; 5-cm eccentric throw), and differed from samples 3 and 4 in that the latter were prepared by bubbling air through sewage. Sludge developed on the shaker was comparable to a "healthy" conventional sludge in every respect. All laboratory samples, except sample 4 which was drawn after 6 hr, were collected 24 hr after feeding sewage. All samples were serially diluted in sterile distilled water and dispersed by shaking vigorously on a reciprocal shaker.

For culturing the dominant bacteria, a nonselective medium was desired. Sewage agar (sewage solidified with 2% agar and neutralized, after autoclaving, with dilute phosphoric acid) was chosen. The plates inoculated with samples of diluted sludge were incubated for 10 to 15 days at room temperature (varied from 16 to 27 C). All colonies in a sector of a plate having a total of 100 to 200 colonies were chosen (in preference to selecting a few that appeared dissimilar visually or microscopically), and cultures were made in PPYE broth [proteose peptone, 0.5%; yeast extract (Difco), 0.1%]. The survival of isolates in PPYE broth was 80%. Other media tested, including sterile sewage, were less suitable. Bacteria from raw sewage, on the other hand, gave 91% recovery. The purification of the isolates was achieved by single-colony isolations on PPYE agar (PPYE broth + agar).

The isolates were studied by methods based on those described in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957). Flagella were stained by Bailey's method; lipid inclusions were examined by Burdon's technique as well as that of Widra (1959). Sugar tests were done in PPYE broth containing 1% sugar + 0.004% bromocresol purple and in the oxidative-fermentative medium of Hugh and Leifson (1953). Gelatin hydrolysis was tested on PPYE agar plates containing 0.4% gelatin with Frazier's reagent, and starch hydrolysis was tested on PPYE agar containing 0.2%

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starch. If the bacterial colonies stained a dark blue with iodine, we interpreted it as indicating accumulation of an iodophilic substance, whereas a clear zone around the colonies indicated starch hydrolysis. Uric acid utilization was tested on PPYE agar containing 0.4% uric acid. A clear zone around the colonies after flooding the plates with 1 N HCl indicated degradation. Nitrate reduction was followed in PPYE broth plus 0.1% $NaNO_3$; the ability to produce H_2S was tested by use of PPYE broth and lead acetate paper strips. Indole formation was studied with Kovac's reagent in a Tryptone-yeast extract medium. The utilization of cellulose, alginate, and pectin was screened in PPYE broth containing 0.5% of the test substrate. Pectinolysis and alginolysis were judged by noting loss of alcohol precipitability (Dias, Bilimoria, and Bhat, 1962).

Nutritional studies were done in the salt solution of Stokes and Gunness (1945) containing 0.5% each of glycerol and sodium succinate and 0.1% NH_4NO_3. Media of varying nutritional complexities were obtained by making various additions as follows: medium 1, no addition; medium 2, medium 1 plus 0.5 μg/ml each of calcium pantothenate, pyridoxine, nicotinic acid, thiamine, riboflavine, *p*-aminobenzoic acid, pteroylglutamic acid, 0.001 μg/ml of biotin, and 0.002 μg/ml of vitamin B_{12}; medium 3, medium 1 plus 0.5% vitamin-free acid-hydrolyzed casein: medium 4, medium 2 plus 0.5% vitamin-free acid-hydrolyzed casein; medium 5, medium 4 plus 0.1% yeast extract.

The pH of the media was about 7 after autoclaving, and the tests were done in test tubes (1.9 by 15 cm) each containing 6 ml of medium. Growth from each tube was serially transferred twice to obviate any response due to carryover of nutrient with the inoculum. Growth was assessed by visual observation.

Poly-β-hydroxybutyric acid (PHB) was estimated gravimetrically (Dias and Bhat, 1963). The cells were derived from shake cultures of the test organism in PPYE broth plus 0.5% each of glycerol and sodium succinate.

The ability of some isolates to bring about changes in sterile sewage was tested by inoculating the cultures into sterile sewage (autoclaved at 15 psi for 30 min and reneutralized with phosphoric acid before use) and incubating for 6 days on a rotary shaker. The sewage was then filtered through cotton, and the turbidity of the effluent was measured on a Bausch & Lomb Spectronic-20 colorimeter at 540 mμ with water as blank. The "permanganate value (4 hr)" was measured as described in *Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents* (Ministry of Housing and Local Government, 1956). The strains tested included bacteria isolated from sludge and sewage as well as a *Loxophyllum* species, a protozoan isolated from sewage in axenic culture (*unpublished data*).

RESULTS

The distribution of some attributes among the dominant sludge bacteria is recorded in Table 1. The results revealed that, with the exception of sludge sample 5, which contained as many as 42% gram-positive strains, the rest were comprised predominantly of gram-negative, polar-flagellated bacteria. Sample 3, which had an unusual microscopic appearance, was the only sample to result in the isolation of yeasts and molds.

Culturally, the gram-negative strains could be broadly grouped into two types, i.e., (i) those which produced flocculent growth in PPYE broth, and (ii) those which did not. The former types, most of which had a single polar flagellum, initially produced a general turbidity and subsequently flocculated in a typical manner, leaving the medium clear. This pattern was also observed in other aggregating bacteria (Stove and Stainer, 1962). The flocs formed, when examined microscopically, were similar to the "zoogloea" illustrated in the literature (Butterfield, 1935; Dugan and Lundgren, 1960).

Many isolates (51%) had inclusions which failed to take the usual stains. In some isolates a greater portion of the cell remained unstained. The spore-staining techniques were ineffective for staining the inclusions. Burdon's lipid stain stained the inclusions of a few isolates; Widra's (1958) stain revealed them to be sudanophilic in all cases.

Most of the flocculating bacteria accumulated an iodophilic substance (only four nonflocculating types did so) when grown on media containing starch. Colonies on PPYE agar containing starch stained a dark blue with iodine, whereas those on glucose, sucrose, lactose, maltose, dextrin, glycogen, glycerol, sodium acetate, sodium succinate, or glucose-1-phosphate (filter-sterilized) failed to do so. Corynebacteria which accumulate iodophilic material from starch only were reported by Carrier and McCleskey (1962). When the sludge strains were grown for about 1 week in PPYE broth containing 0.1% starch,

TABLE 1. *Distribution of some attributes among the dominant activated sludge bacteria*

Type	Sludge sample no.*						
	1	2	3	4	5	6	7
Rods; gram-negative	88	93	93	96	93	58	96
Rods and cocci; gram-positive	10	7	2	4	7	42	4
Polar flagella; gram-negative	76	82	93	83	74	54	85
Sudanophilic inclusions	69	60	49	46	43	32	59
Flocculent growth in PPYE† broth	54	53	19	40	19	22	50
Accumulate iodophilic substance	37	51	16	37	29	16	44
Acid from glucose‡	7	2	14	10	12	5	6

* Results are expressed as percentage of positive isolates. Total number of isolates for each sludge sample was as follows: sample 1, 41; sample 2, 45; sample 3, 43; sample 4, 39; sample 5, 42; sample 6, 57; sample 7, 52.

† Proteose peptone, 0.5%; yeast extract, 0.1%.

‡ Cultures that did not produce acid from glucose did not do so from arabinose, mannose, sucrose, lactose, maltose, inositol, or mannitol.

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TABLE 2. *Dominant bacteria in activated sludge*

Organism	Sludge sample no.*						
	1	2	3	4	5	6	7
Achromobacter	0	0	2	3	5	2	0
Aerobacter	0	0	0	3	0	0	0
Alcaligenes	0	2	0	3	2	0	0
Bacillus	0	5	0	0	2	0	0
Brevibacterium	5	0	0	0	0	0	0
Corynebacterium	0	0	0	0	5	35	4
Comamonas	24	33	65	41	52	30	35
Flavobacterium	2	2	2	0	0	0	4
Micrococcus	2	2	2	5	2	7	0
Pseudomonas	2	0	2	5	12	2	4
Spirillum	2	0	0	0	0	0	0
Zoogloea	61	56	19	41	19	23	54
Yeasts	0	0	5	0	0	0	0

* Results are expressed as percentage of positive isolates. Total number of isolates for each sludge sample was as follows: sample 1, 41; sample 2, 45; sample 3, 43; sample 4, 39; sample 5, 42; sample 6, 57; sample 7, 52.

TABLE 3. *Characteristics of activated sludge bacteria classed as Zoogloea**

Characteristic	No. of strains				
	92	24	3	5	2
Flagella	Single polar	Single polar	Single polar	None	None
Sudanophilic inclusions	Present	Present	Present	Absent	Absent
Flocculent growth in proteose peptone-yeast extract broth	92†	24	0	5	2
Nitrate reduced	32	7	1	1	0
Urea hydrolyzed	50	12	2	4	2
Gelatin hydrolyzed	3	0	0	1	0
Starch hydrolyzed	0	1	0	0	0
Accumulate iodophilic material	92	0	3	4	2

* All strains were gram-negative and grew at 36 C. None produced a pigment, acidity from glucose, H_2S, indole, or degraded uric acid, pectin, alginate, or cellulose.

† Figures indicate the number of strains positive.

the medium no longer stained blue with iodine; it stained violet instead.

Most of the polar-flagellated, gram-negative bacteria did not produce acidity in the medium of Hugh and Leifson (1953), thus placing them in the genera *Zoogloea* (Breed, Murray, and Smith, 1957) and *Comamonas* (Davis and Park, 1962), the main attribute distinguishing these two being the ability of the former to form zoogloea. The identities of the sludge bacteria are shown in Table 2. A few nonflocculating isolates which were otherwise similar to the flocculating forms have also been classed as *Zoogloea*. The present description of the genus *Zoogloea* (Breed et al., 1957) is far from satisfactory. The physiological and other attributes of the *Zoogloea* and *Comamonas* are detailed in Tables 3 and 4.

Fifty sludge isolates were tested for their ability to produce antibiotics against *Escherichia coli*, *Aerobacter aerogenes*, *P. fluorescens*, *Staphylococcus aureus*, and four strains isolated from sewage. None did so.

A total of 150 sludge isolates were examined with respect to their nutrition (Table 5). Most of the flocculating bacteria grew poorly, but this did not prevent the assessment of their nutritional requirements. Most of the sludge bacteria required either vitamins or amino acids, or both. The nutritional requirements of the *Zoogloea* strains varied (Table 5), but these differences could not be correlated with the physiological attributes. Most demanded amino acids. Because of the meager growth in the above medium, it was considered desirable to test the salt solution of Dugan and Lundgren (1960). Twenty strains were tested, and the growth response in all cases was luxuriant. However, the nutritional requirements noted in the two media were identical.

The bacteria isolated from raw sewage on sewage agar contained neither sudanophilic inclusions nor accumulated iodophilic material. Of the 150 isolates tested, 123 strains produced acid from glucose and, of these, 36 produced gas in addition, thus differing vastly from the sludge isolates. Nutritionally, the 110 strains examined fell into groups shown in Table 5. Most strains grew well in media based on Stokes and Gunness' (1945) salt solution. Except for the gas producers (coliforms), the sewage strains generally demanded both vitamins and amino acids, as did their sludge counterparts, although there were clear-cut differences between the two groups.

The ability of some sludge isolates to synthesize PHB is recorded in Table 6. The variation in melting points (156 to 171 C) of different PHB preparations is in accord with past records (Sierra and Gibbons, 1962). The material isolated when heated beyond the melting point gave copious fumes with a crotonic acid smell. The preparations from two cultures (*Zoogloea* and *Comamonas*) were subjected to the following additional tests. The isolated substances on destructive distillation gave crystalline products melting at 68 to 71 C, which corresponds to the melting point of *trans*-crotonic acid (Levine and Wolochow, 1960). The isolated material was soluble in chloroform, glacial acetic acid, and 1 N sodium hydroxide, but was insoluble in water, acetone, ether, ethanol, "alkaline hypochlorite reagent" (Williamson and Wilkinson, 1958), and dilute hydrochloric acid; this is consistent with previous records (Williamson and Wilkinson, 1958; Levine and Wolochow, 1960). Finally, a mixture of the isolated material from the two strains was purified by several precipitations of chloroform solutions with ether. Elemental analysis data (C, 55.9; H, 6.9; N, absent) tallied with theoretical expectations (C, 55.8; H, 7.0). The above results, together with the presence of sudanophilic inclusions, leave little doubt that the latter are composed of PHB.

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TABLE 4. *Characteristics of activated sludge bacteria classed as Comamonas*[a]

Characteristic	No. of strains															
	20	12	17	6	4	1	8	1	3	5	7	16	3	16	1	1
Flagella	Polar[b]	Polar	Polar	Polar	Polar	Polar	Polar	Polar	None	None	Polar	Polar	Polar	Polar	Polar	Polar
Sudanophilic inclusions	−[c]	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
Pigment	0[d]	0	0	0	0	0	Yellow	Melanin	0	0	0	0	Yellow	Melanin	Melanin	Melanin
Grow at 36 C	20	12	17	4	4	1	8	1	3	5	7	16	2	16	1	0
Nitrate reduced	19	12	0	0	0	0	4	1	1	4	7	0	3	16	0	0
H_2S produced	15	2	0	6	0	0	2	0	0	1	1	4	0	0	0	0
Indole produced	20	0	0	0	0	0	0	1	1	0	0	0	0	16	0	0
Urea hydrolyzed	0	3	6	1	4	0	0	0	0	3	2	3	0	0	0	0
Uric acid hydrolyzed	1	3	1	0	0	0	5	0	0	3	2	3	1	0	1	0
Arginine hydrolyzed	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Gelatin hydrolyzed	19	2	1	4	0	1	1	0	1	0	0	3	0	0	1	0
Starch hydrolyzed	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
Accumulate iodophilic material	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0

[a] All strains were gram-negative. None produced acidity from glucose or degraded pectin, alginate, or cellulose.
[b] One to three flagella per cell; majority of strains had a single flagellum.
[c] Minus sign indicates sudanophilic inclusions were absent; plus sign indicates they were present.
[d] Figures indicate number of strains that were positive.

The changes brought about in autoclaved sewage by some microorganisms are recorded in Table 7. The protozoan grew well but did not flocculate. Neither individually nor in combination with certain bacteria was it able to produce a "pure effluent." The *Zoogloea* isolates usually produced a flocculent growth resembling activated sludge. The other bacteria grew either as a diffuse growth or in the form of a granular sludge similar to the "bioprecipitate" of Tischer, Brown, and Cook (1962). In no case did any bacterium produce an effluent comparable in clarity and permanganate value (4 hr) to that obtained with the sludge-seeded control.

It is important to record that the supernatant fluid of homogenized (Waring Blendor) sludge, when examined microscopically after Gram staining, always revealed the presence of gram-negative bacteria, with unstained areas similar to the isolates obtained.

DISCUSSION

The isolation of a large number of *Zoogloea* strains from sludges is evidence for the long-held view that this genus is important in the aerobic biological methods of wastewater treatment. It is even likely that the number isolated is an underestimate of the actual numbers present, because of their flocculent growth. At any rate, *Zoogloea* and *Comamonas* species constitute a greater part of the sludge bacterial population. From the data recorded for sample 6, it is clear that other bacteria can occasionally come to the fore without adversely affecting the system. It is not unlikely, at the same time, that the less prevalent species (not isolated by the method employed in this study) could also have a significant role in sewage treatment. A consideration of these species could form the subject of future reports. However, it is reasonable to assume that the species present in superabundance are the most active in the ecosystem (Hungate, 1962).

The question as to how bacteria that do not flocculate

TABLE 5. *Nutritional status of the dominant bacteria in raw sewage and activated sludge*

Nutritional requirement	Activated sludge bacteria		Raw sewage bacteria	
	Per cent total*	Per cent *Zoogloea*†	Per cent total‡	Per cent non-gas producers
Neither vitamins nor amino acids essential	8	0	24	0
Vitamins but not amino acids essential	37	14	3	4
Vitamins and amino acids essential	26	33	48	64
Amino acids but not vitamins essential	24	44	0	0
Vitamins stimulatory but not essential; amino acids essential	20	44	0	0
Yeast extract essential	5	9	15	20
Do not grow in any of the five media	0	0	9	12

* Total, 150 strains.
† Of 64 of the 150 strains.
‡ Total, 110 strains.

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occur in sludge can be answered only by speculation. In all probability they live in association with the floc-formers. It may even be that, under the exigencies of survival in the natural biotic complex, the organism behaves in a manner different from that observed in the highly artificial conditions of the laboratory. The ability to flocculate, besides favoring their selection, may also act as a protective mechanism against the sludge fauna.

TABLE 6. *Production of poly-β-hydroxybutyric acid (PHB) by activated sludge bacteria**

Organism and strain no.	PHB content (% of dry weight)	Melting point
		C
Zoogloea		
2	37	156
12	23	170
32	6	160
56	38	169
72	40	169
99	18	161
107	13	163
154	51	160
213	16	158
Comamonas		
101	33	161
122	28	171
124	36	168

* The following bacteria which did not contain sudanophilic inclusions were devoid of polyester: *Comamonas* (strain 3), *Flavobacterium* (strain 5), and *Corynebacterium* (strain 217).

TABLE 7. *Changes in permanganate value (4 hr) and turbidity of sewage due to growth of various microorganisms*

Organism	No. of strains examined	Per cent reduction*	
		Permanganate value (4 hr)	Turbidity
Achromobacter	1	16	44
Alcaligenes	1	35	16
Brevibacterium	1	0	10
Comamonas	18	−10 to 51	−18 to 48
Flavobacterium	1	36	73
Micrococcus	2	−5 to 10	−13 to 18
Pseudomonas	1	26	18
Spirillum	1	16	15
Zoogloea	20	16 to 63	18 to 49
Corynebacterium ureovorans (UR 13)†	1	30	46
Corynebacterium laevaniformans (1)‡	1	26	22
Loxophyllum	1	−6	18
Loxophyllum + *C. ureovorans* (UR 13)†	1	38	48
Loxophyllum + *Zoogloea* (10)	1	51	48
Zoogloea (10)	1	40	59
Activated sludge control	3	81 to 91	90 to 95

* Minus sign indicates an increase.
† Dias and Bhat, Indian J. Microbiol. *in press.*
‡ Dias and Bhat (1962).

The finding that many sludge bacteria have reserves of PHB suggests a possible means by which the organic matter in sewage is rapidly removed during the initial stages of activated sludge treatment and is subsequently metabolized. PHB is known to act as a reserve substance in several species (Williamson and Wilkinson, 1958; Doudoroff and Stanier, 1959; Rouf and Stokes, 1962; Sierra and Gibbons, 1962). It is possible, therefore, that the so-called "adsorption" occurring at the earlier phase of sewage treatment is due, at least in part, to conversion of organic pollution into bacterial reserve material, PHB, which, during subsequent aeration, is metabolized. The observation that a large number of isolates accumulate iodophilic material is also of interest in this connection, but the specificity of the reaction would appear to render it of minor importance. From the point of view of ecology, the ability to store food probably acts as a factor which helps bacteria to dominate in a system where food is in short supply.

None of the cultures was able to produce an effluent as "pure" as that produced by activated sludge. Notwithstanding past claims (Butterfield, 1935; Buck and Keefer, 1959), it seems doubtful that a single bacterial species could utilize all the organic substances that occur in sewage and bring about its complete stabilization. The negative results obtained with the protozoan are in no way a reflection of the inactivity of the sludge fauna, because the organism tested, a free-swimming ciliate, is not a typical sludge species.

In conclusion, it may be mentioned that the selection of a flora as characteristic of activated sludge shows clearly that the species so selected has a vital role in the process. It would seem that the flora is responsible in the metabolism of soluble substrates, whereas the protozoa, by and large, have the ability to remove the particulate fraction, including the bacteria that come in with the sewage. The bacterium, with its large surface-to-weight ratio and its mode of feeding, has an immense advantage over the protozoan in the metabolism of soluble substrates, not to mention the improbability of exocellular enzymes functioning effectively in the activated sludge system. However, the mechanical entrapment of the particulate matter by the sludge, the adsorption of enzymes on the sludge surface, and the occurrence of surface-bound enzymes (Pollock, 1962) are some of the factors that have to be considered before a definite role can be assigned to the bacteria with respect to the protozoa and the adsorption phenomenon.

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LITERATURE CITED

ALLEN, L. A. 1943. The bacteriology of activated sludge. J. Hyg. **43**:424–431.

ANDERSON, R., AND E. MCCOY. 1963. Floc-forming bacteria from activated sludge. Bacteriol. Proc., p. 162.

BREED, R. S., E. G. D. MURRAY, AND N. R. SMITH. 1957. Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore.

BUCK, T. C., AND C. E. KEEFER. 1959. Studies of a zoogloeal forming organism found in activated sludge. Sewage Ind. Wastes **31**:1267–1274.

BUTTERFIELD, C. T. 1935. Studies of sewage purification. II. A zoogloea forming bacterium isolated from activated sludge. Public Health Rept. U.S. **50**:671–684.

CARRIER, E. B., AND C. S. MCCLESKEY. 1962. Intracellular starch formation in corynebacteria. J. Bacteriol. **83**:1029–1036.

DAVIS, G. H. G., AND R. W. A. PARK. 1962. A taxonomic study of certain bacteria currently classified as *Vibrio* species. J. Gen. Microbiol. **27**:101–119.

DIAS, F. F., M. H. BILIMORIA, AND J. V. BHAT. 1962. *Corynebacterium barkeri*, nov. spec., a pectinolytic bacterium exhibiting a biotin-folic acid inter-relationship. J. Indian Inst. Sci. **44**:59–67.

DIAS, F. AND J. V. BHAT. 1962. A new levan producing bacterium *Corynebacterium laevaniformans* nov. spec. Antonie van Leeuwenhoek J. Microbiol. Serol. **28**:63–72.

DIAS, F. F., AND J. V. BHAT. 1963. Accumulation of poly-β-hydroxybutyric acid and iodophilic material by the dominant activated sludge bacteria. Current Sci. India **32**:501–502.

DOUDOROFF, M., AND R. Y. STANIER. 1959. Role of poly-β-hydroxybutyric acid in the assimilation of organic carbon by bacteria. Nature **183**:1440–1442.

DUGAN, P. R., AND D. G. LUNDGREN. 1960. Isolation of the floc-forming organism *Zoogloea ramigera* and its culture in complex and synthetic media. Appl. Microbiol. **8**:357–361.

HUGH, R., AND E. LEIFSON. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. **66**:24–26.

HUNGATE, R. E. 1962. Ecology of bacteria, p. 95–119. *In* I. G. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 4. Academic Press, Inc., New York.

JASEWICZ, L., AND N. PORGES. 1956. Biochemical oxidation of dairy wastes. VI. Isolation and study of sludge microorganisms. Sewage Ind. Wastes **28**:1130–1136.

LEVINE, H. B., AND H. WOLOCHOW. 1960. Occurrence of poly-β-hydroxybutyrate in *Pseudomonas pseudomallei*. J. Bacteriol. **79**:305–306.

MCKINNEY, R. E., AND M. P. HORWOOD. 1952. Fundamental approach to the activated sludge process. I. Floc-producing bacteria. Sewage Ind. Wastes **24**:117–123.

MCKINNEY, R. E., AND R. G. WEICHLEIN. 1953. Isolation of floc-producing bacteria from activated sludge. Appl. Microbiol. **1**:259–261.

MINISTRY OF HOUSING AND LOCAL GOVERNMENT. 1956. Methods of chemical analysis as applied to sewage and sewage effluents. Her Majesty's Stationery Office, London.

POLLOCK, M. R. 1962. Exoenzymes, p. 121–178. *In* I. G. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 4. Academic Press, Inc., New York.

ROGOVSKAYA, Z. I., AND M. F. LAZAREVA. 1959. Intensification of biochemical purification of industrial sewage. I. A microbiological specification of activated sludges purifying various industrial sewages. Mikrobiologiya **28**:565–573.

ROUF, M. A., AND J. L. STOKES. 1962. Isolation and identification of the sudanophilic granules of *Sphaerotilus natans*. J. Bacteriol. **83**:343–347.

SIERRA, G., AND N. E. GIBBONS. 1962. Role and oxidation pathway of poly-β-hydroxybutyric acid in *Micrococcus halodenitrificans*. Can. J. Microbiol. **8**:255–269.

SOCIETY OF AMERICAN BACTERIOLOGISTS. 1957. Manual of microbiological methods. McGraw-Hill Book Co., Inc., New York.

STOKES, J. L., AND M. GUNNESS. 1945. Microbiological methods for the determination of amino acids. I. Aspartic acid and serine. J. Biol. Chem. **157**:651–659.

STOVE, J. L., AND R. Y. STANIER. 1962. Cellular differentiation in stalked bacteria. Nature **196**:1189–1192.

TISCHER, R. G., L. R. BROWN, AND D. W. COOK. 1962. Decomposition of wastewater by thermophilic microorganisms. J. Water Pollution Control Federation **34**:1244–1255.

WIDRA, A. 1959. Metachromatic granules of microorganisms. J. Bacteriol. **78**:664–670.

WILLIAMSON, D. H., AND J. F. WILKINSON. 1958. The isolation and estimation of poly-β-hydroxybutyrate inclusions of *Bacillus* species. J. Gen. Microbiol. **19**:198–209.

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