CHAPTER 3

THE BIOLOGY OF SPHAEROTILUS NATANS KUTZING IN RELATION TO BULKING OF ACTIVATED SLUDGE

The term bulking, as applied to sewage disposal plants is usually definable only in terms of its user. It may mean rising of the sludge blanket until the sludge passes the weir or it may mean great increase in sludge volume accomplished by loss of density. In any case, it means the operator has lost control of the sludge. Its causes are likewise variable and according to the type of bulking; whether we are dealing with a given mass of sludge occupying several times the volume it normally should; or flotation of the floc by gas bubbles; or flotation of the floc by filamentous organisms; or other causes. In one sense, the end result is the same. The effluent is no longer clear but contains objectionable putrescible matter.

Sewage usually contains a variety of filamentous growths ranging from bacteria which develop in masses because of confluent gelatinous sheath-like secretions to ramifying branched mycelia of the true molds. A number of these filamentous organisms have been isolated from sewage and one, Sphaerotilus natans Kutzing, has long been associated with bulking. Sometimes itis regarded as the cause of bulking and again as merely being associated with the trouble. Its first description recorded it from 'factory water' and it is almost invariably found in

polluted waters.

Sphaerotilus is not normally an abundant growth in an activated sludge chamber, although a few filaments may usually be seen on microscopic examination. But a bulking sludge frequently contains enormous quantities of it.

3.1 Characteristics and Identification of the Organism (Lackey, 1940)

Kutzing first described the species as "an attached, colorless, thread-like filament, showing false branching." The strands are cylindrical with a thin firm sheath. The sheath is slimy and optically invisible but can be demonstrated in India ink mounts or by several staining methods. The cells, cylindrical or ovoid in longitudinal section, vary in diameter from 1.0 to 3.0 microns, and in length from 3.0 to 8.0 microns, according to age, source (for wild cultures), and culture medium.

The filaments are colorless, but in streams masses of the fungus may be light brown, even with little enmeshed foreign matter. This is probably due to age and dead cells. Single filaments may be several millimeters long and often many are entwined in a braided manner giving a cord-like appearance. A single strand ends abruntly if free; if attached, in a small disc. Flocs are often 1 cm. in diameter and 5 cm. in length if free floating and are very ragged in contour. When attached, they are long and plumose. Attachment in pure culture depends simply on the degree of agitation of the water by bubbles from the diffuser. Figures 2 and 3 show cultures in serum bottles

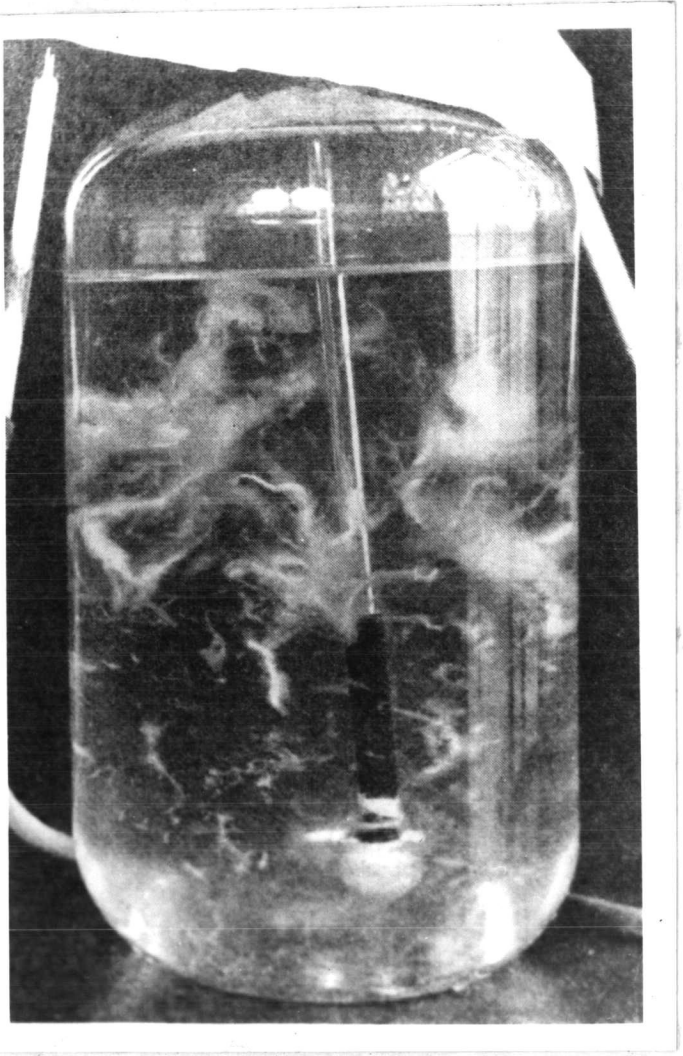

FIG. 2 FREE FLOCS DUE TO TURBULENT AERATION (Lackey, 1940)

FIG. 3 ATTACHED FLOCS DUE TO SLIGHT AERATION

demonstrating free flocs due to turbulent aeration and attached flocs due to slight aeration.

The small size precludes determination of much cellular detail. Young cells (Fig. 4) show a relatively homogenous cytoplasm with a few vacuoles. Often a few granules are seen. The vacuoles take up neutral red and are presumably sap vacuoles. Differential staining of granules with haematoxylin and acid stains has not been accomplished. Such chromatin as is present is in very small particles. In old cells the vacuoles become quite large and appear as white spaces after methylene blue, fuchsin or haematoxylin staining. The filament sheath stains faintly with haematoxylin after Schaudinn's fixative, but not after flemming with or without acetic acid. It does not stain with methylene blue or fuchsin. The reproductive cells (conidia) which are simply vegetative cells fragmented from a filament, have flagella which are very difficult to stain. They can not be demonstrated with cytological fixatives, but by using air drying fixation followed by Gray's flagella stain they can be shown, as in Fig. 5 . Such conidia are then seen to have one to have one to several long flagella, subapically inserted. Cases where there is apparently one flagelleum are probably due to the aggregation of several on air drying. According to Bergey's Manual motile conidia have a clump of flagella near one end.

These conidia are very active. They swim in only one direction and eventually attach themselves by one end whereupon a long filament grows out from the substrate. Attachment is by

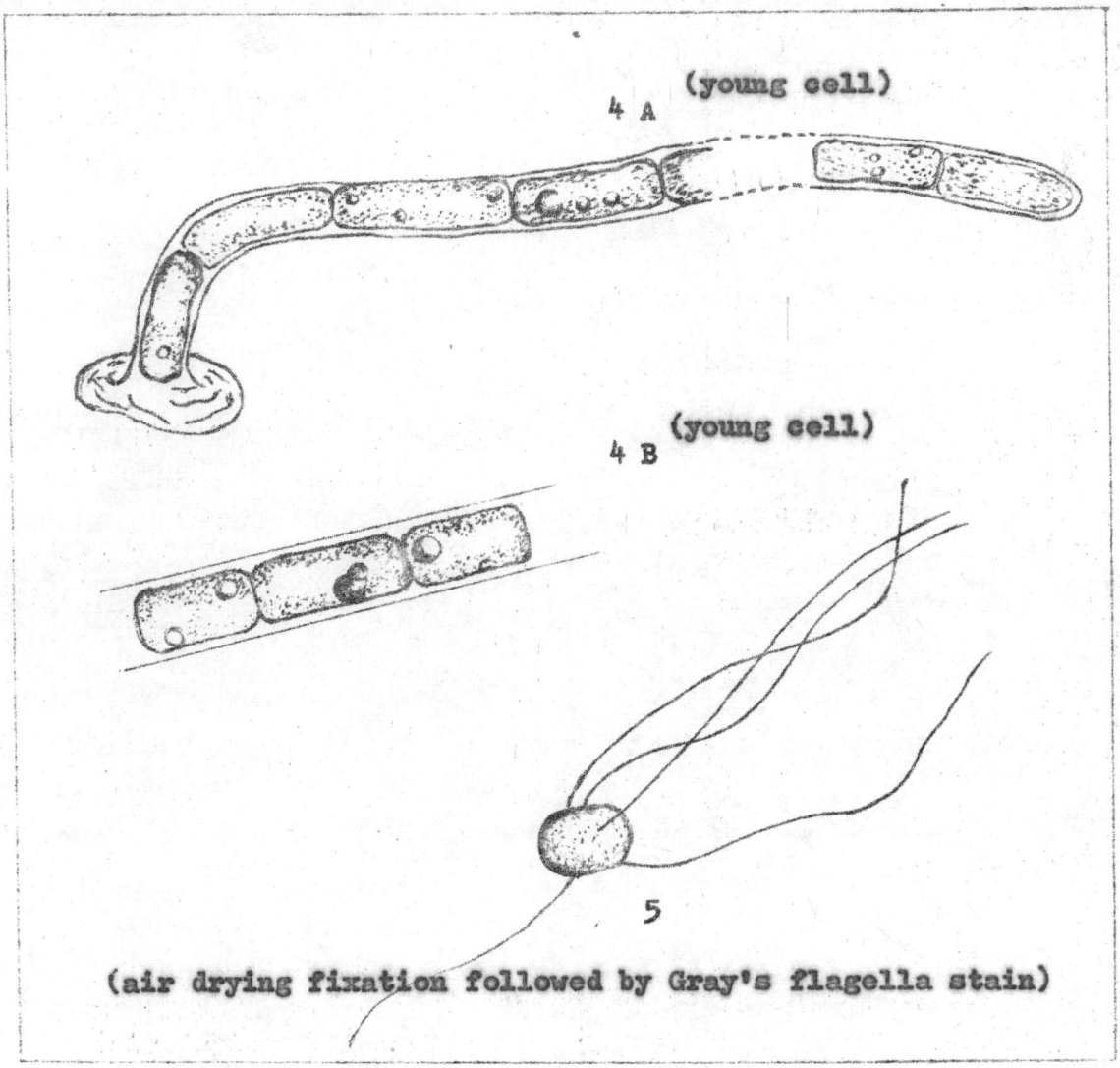

FIG. 4 AND 5 SPHAEROTILUS NATANS KUTZING (Lackey, 1940)

the non-flagellated end, for attached conidia still move their free ends for a time. Conidia inoculated into a petri dish of nutrient medium will grow into filaments several millimeters long overnight.

Colonies on sewage or nutrient agar are very delicate, irregular in contour and rarely thick enough for a definite color to be seen. Occasionally on agar slants a gray or white color is seen.

The question of attachment seems to depend, as stated above, merely on the degree of agitation in the culture flask. With slight turbulence most of the growth is on the walls of the containing vessel, but with an increase in turbulence most of the growth consists of unattached flocs of all sizes, very ragged and very light. If the air is shut off they settle slowly, but better than the flocs in bulking sludge. The slightest current keeps these flocs in suspension. This explains why Sphaerotilus is so objectionable in bulking. Growing out in all directions from sludge flocs, it greatly increases their buoyancy. The fungus also decreases the relative weight of the sludge flocs and assists in trapping air bubbles wherewith to produce a raising sludge.

3.2 Relative Settling Rates of Some Sludge Organisms

Several fungi and organisms have been found to interfere with sludge settling but have not been studied extensively. Under laboratory conditions sludges have developed at times excessive growths of various branching fungi, filamentous bacteria,

yeasts and colonial vorticellid ciliates belonging to the genera Epistylis and Opercularia. Table I gives the relativetime of settling of some of these in pure culture, using a synthetic sewage. Table 1 indicates that organisms other than Sphaerotilus will rarely be found associated with or causing bulking.

Table 1. Relative Time of Settling of Certain Sewage Organisms

	Time Required for:		
	10% settling	50% Settling	90% Settling
Sludge flocs alone	1 min.9	2 min.	3-5 min.
Sphaerotilus on sludge flocs..	30 min.	1-2 hours	2-5 hours
Sphaerotilus flocs	30 min.	1-3 hours	3-12 hours
Zoöglea flocs	1 min.	4 min.	7 min.
Penicilium colonies	30 seconds	1 min.	1 min.
Other phycomycete colonies	30 seconds	1 min.	1 min.
Yeast alone	30 min.	12 hours	Indefinite
Bacillus subtilis alone	No settling		
Bacillus mycoides alone	40 min.	6 hours	12 hours

The above rates of settling were determined by allowing the culture under investigation to stand in a 1 liter glass graduate.

3.3 Sphaerotilus and Bulking- Cause or Effect ?

Since bulking of activated sludge may occur in the absence of Sphaerotilus, it is not the cause of all bulking. But it is the most abundant organism in most cases of bulking. That it plays a part in this condition is quite evident from a comparison

of the settling rate of Sphaerotilus infested sludge flocs and sludge flocs with no Sphaerotilus. Many substances were tested to see if they would stimulate the organism to excessive growth. Both mixed cultures and pure cultures were thus tested. The results are shown in Table II.(Lackey,1940)

Table II. Effects of Various Substances on Growth of Sphaerotilus

Mixed or Pure Culture	Basic Medium	Substance Added	Range in P.p.m.	Growth of Sph.	Settling Quality	Condition of Culture at End
Mixed	Activated sludge	Sucrose	1000-5000 Twice daily	Good	Poor	Sphaerotilus dominant
Mixed	Act. sl.	Sucrose	1000- Four times daily	Good	Poor	Sphaerotilus replaced
Mixed	Act. sl.	Sucrose	5000 Four times daily	Good	Poor	Sphaerotilus replaced
Mixed	Act. sl.	Sucrose	20,000 Once only	Poor	Poor	Yeasts dominant
Mixed	Act. sl.	Glucose	1000-5000 Twice daily	Good	Poor	Sphaerotilus dominant
Mixed	Act. sl.	Starch	1000-5000 Twice daily	Poor	Good	Poor
Mixed	Act. sl.	Whole wheat	5000 Once only	Poor	Good	Sludge good
Mixed	Act. sl.	Peptone	100-1000 Once only	Slight	Good	Sphaerotilus replaced
Mixed	Act. sl.	Sulfite liquor	5% Once only	slight	Good	Sphaerotilus replaced
Pure	Sterile raw sewage			Poor	Good	Sphaerotilus present
Pure	Sterile raw Sewage	Sucrose	1000-10,000 Once only	Good	Poor	Sphaerotilus thriving
Pure	Sterile raw sewage	Sucrose	1000 Four times	Good	Poor	Sphaerotilus thriving

Table. II Continued

Mixed or Pure Culture	Basic Medium	Substance Added	Range in P.p.m.	Growth of Sph.	Settling Quality	Condition of Culture at End
Pure	Sterile raw sewage	Glucose	1000-10,000 Once only	Good	Poor	Sphaerotilus thriving
Pure	Sterile raw sewage	Glucose	1000 Four times daily	Good	Poor	Sphaerotilus thriving
Pure	Sterile raw sewage	Maltose	1000-10,000 Once only	Good	Poor	Sphaerotilus thriving
Pure	L 100 p.p.m. Peptone	Sucrose	50-100 Once only	Good	Fair	Sphaerotilus thriving
Pure	L 100 p.p.m. Peptone	Sucrose	10,000-60,000 Once only	Fair	No growth at 60,000	
Pure	L 100 p.p.m. Peptone	Glucose	50-1000 Once Only	Good	Fair	Sphaerotilus thriving
Pure	L 100 p.p.m. Peptone	Glucose	10,000-60,000 Once only	Fair	No growth at 60,000	
Pure	L 100 p.p.m. Peptone	Maltose	50-10,000 Once only	Good	Fair	Sphaerotilus thriving
Pure	L 100 p.p.m. Peptone	Lactose	50-10,000 Once only	Good	Fair	Sphaerotilus thriving
Pure	L 100 p.p.m. Peptone	Glycerol	50-1000 Once only	None		
Pure	L 100 p.p.m. Peptone	Glycerol	50.1000 Once only	None		
Pure	L 100 p.p.m. Peptone	Whole wheat	100-40,000 Once only	Poor		

Table II. —Continued

Mixed or Pure Culture	Basic Medium	Substance Added	Range in P.p.m.	Growth of Sph.	Settling Quality	Condition of Culture at End
Pure	L 100 p.p.m. Peptone	White flour	1000-5000 Once only	None	Fair	
Pure	L 100 p.p.m. Peptone	Laundry starch	100-5000 Once only	None		
Pure	L-6	Laundry Soap	50-200 Once ,Only	Good		Sphaerotilus Thriving
Pure	L	Sodium Oleate	50-1000 Once only	None		
Pure	L	Sodium Stearate	50-1000 Once only	None		
Pure	L	Oleic acid	50-1000 Once only	None		
Pure	L	Stearic acid	50-1000 Once only	None		
Pure	L	Peptone	50-1000 Once only	Fair	Fair	Sphaerotilus thriving
Pure	L	Peptone	1000-30,000 Once only	Poor to none		
Pure	L	Meat extract	5000-1000 Once only	Poor to none		
Pure	L-6	NaCl	5-200 Once only	Variable to poor		
Pure	L-6	Sulfite liquor	5% Once only	Good		
Pure	L	Ammonium glycero-phosphate	100-1000 Once only	Slight		

Table II. – Continued

Mixed or Pure Culture	Baxic Medium	Substance Added	Range in P.p.m.	Growth of Sph.	Settling Quality	Condition of Culture at End
Pure	L,dextrose	Urea	50-100 Once Only	Good		
Pure	Synthetic	Dextrose 500 p.p.m.	Peptone 100 p.p.m. Once only	Good	Fair	
Pure	Synthetic	Dextrose 500 p.p.m.		None		
Pure	Synthetic	Dextrose 500 p.p.m.	Urea 100 p.p.m Once only	Fair		
Pure	Synthetic	Dextrose 1000 p.p.m.	Glycerine 250 p.p.m. Once only	Slight		
Pure	Synthetic	Dextrose 1000 p.p.m.	$NaNo_3$ 112 p.p.m. Once only	Fair		
Pure	Synthetoc	Dextrose 500 p.p.m.	l-tyrosinee 100 p.p.m. Once only	Slight		
Pure	Synthetic	Dextrpse 500 p.p.m.	l-cystine 100 p.p.m. Once only	Slight		
Pure	Synthetic	Dextrose 500 p.p.m.	l-asparagine 100 p.p.m. Once only	Good		
Pure						
Pure	Synthetic	Dextrose 500 p.p.m.	l-leucine 50 p.p.m. Once only	Slight		

Table II Continued

Mixed or Pure Culture	Basic Meduim	Substance Added	Range in P.p.m.	Growth of Sph.	Settling Quality	Condition of Culture at End.
Pure	Synthetic	Dextrose 500 p.p.m.	Acetontrile 100 p.p.m. Once only	None		
Pure	Synthetic	Dextrose 500 p.p.m.	dl-alanin 100 p.p.m. Once only	None		
Pure	Synthetic	Dextroxe 500 p.p.m.	Na-gluconate 500 p.p.m. Once only	Fair		

Medium L is only mineral salts and Water.

Medium L-6 is the same, with dextrose and peptone added.

Medium synthetic is only organic salts and water, but contains no nitrate.

3.4 Control of Sphaerotilus

Inasmuch as the organism grows excessively because of some substance in sewage or some defect on plant operation, knowledge of the cause and control of growth is useful, both for prevention and cure of the disease. Smith and Purdy (1936) suggested chlorine for control of growth and it has since been extensively used with good results. Laboratory experiments were carried out by Lackey and Wattie (1940) to find possibly some specific killing agent for Sphaerotilus. The results are shown in Table III. None of the substances tried seem to be specific, although silver nitrate and some of the dyes are toxic at low concentrations and malachite green, 5 p.p.m., is not toxic to a number of other organisms of the activated sludge community. Present cost of this dye would make its use prohibitive, in comparison to chlorine; in fact, the relatively low cost of chlorine has discouraged any extensive search for killing agents. The method of using the chlorine depends largely on the individual plant, but it seems probable that chlorination of the returned sludge is most feasible.

Table III. Effects of Possible Toxic Agents on Growth of Sphaerotilus

Culture	Toxic Agent	Method of Application	Effect
Mixed act. sludge	Toluene vapor	Bubbled into normal sludge	None
Mixed act. sludge, bulking	Toluene vapor	Bubbled into bulking sludge	None
Mixed act. sludge, bulking	Chloroform	Bubbled into bulking sludge	Toxic
L-6, heavy culture	Chlorine, as H. T.H.	Dosed to a residual of 0.5 p.p.m.	Toxic
L-6, inoculated with Sphaerotilus	Chlorine added	0.5 to 3.0 p.p.m. before inoculation	No growth
L-6, heavy culture	$AgNO_3$	0.5 to 2.5 p.p.m..added to heavy culture	Toxic
L-6, inoculated with Sphaerotilus	$AgNO_3$	0.5 to 2.5 p.p.m. before inoculation	No growth
Mixed act. sludge, good culture	$AgNO_3$	0.5 to 2.5 p.p.m. to heavy culture	Toxic at 2 p.p.m.
Mixed act. sludge, good culture	Phenol	1.0 p.p.m. to heavy culture	No effect
Mixed act. sludge, good culture	Phenol	5.0 to 50 p.p.m. to heavy culture	Toxic, variable
L-6, heavy culture	Phenol	1.0 p.p.m. to heavy culture	No effect
L-6, heavy culture	Phenol	5.0 p.p.m. to heavy culture	Toxic
Mixed act. sludge, good culture	Acetic acid	50 p.p.m. to heavy culture	Toxic
Mixed act. sludge, good culture	Citric acid	100 to 1000 p.p.m. to heavy culture	Sphaerotilus replaced
Mixed act. sludge, good culture	Lactic acid	100 to 1000 p.p.m. to heavy culture	Sphaerotilus replaced
Mixed act. sludge, good culture	Brilliant green	5 to 20 p.p.m. to heavy culture	Toxic at 5 p.p.m.

Table III. Continued

Culture	Toxic Agent	Method of Application	Effect
Mixed act. sludge, good culture	Fast green	5 to 25 p.p.m. to heavy culture	Non-toxic
Mixed act. sludge, good culture	Malachite green	5 to 10 p.p.m. to heavy culture	Toxic at 5 p.p.m.
Mixed act. sludge, good culture	Manus green	5 to 20 p.p.m. to heavy culture	Toxic at 20 p.p.mn.
Mixed act. sludge, good culture	Eosin	5 to 50 p.p.m. to heavy culture	Variable
Mixed act. sludge, good culture	Methylene blue	5 to 20 p.p.m. to heavy culture	Toxic at 20 p.p.m.
Mixed act. sludge, good culture	Gentian violet	5 ot 15 p.p.m. to heavy culture	Toxic at 10 p.p.m.
Mixed act. sludge, good culture	Uranin	5 to 200 p.p.m. to heavy culture	Non-toxic

3.5 Sludge Bulking with Carbohydrates

There was no difficulty in producing bulking with carbohydrates when normal air was used for aeration , where as bulking could not be produced with sewage in the laboratory unless the oxygen supplied in the air was cut down. The concentration of sugar that produced an increased in sludge index with normal air was about 125 p.p.m. or more , this would have a 5-day B.O.D. of about 60 p.p.m. . A sewage with a similar BOD could not be made to bulk even when it was aerated with one cu.ft. of air per gallon therefore carbohydrates were specific stimulants in inducing bulking when they were present . In other words their concentration need not be high enough to creat an oxygen deficiency but their presence in appreciable quantities would stimulate the growth of Sphaerotilus to carbohydrates , an additive factor of oxygen deficiency might be created which would cause the further preponderance of Sphaerotilus over Zooglea. Zooglea was strictly an aerobic organism while Sphaerotilus function both under aerobic and anaerobic conditions. Sphaerotilus could utilize the sugar preferentially to the Zooglea under aerobic conditions, while under anaerobic conditions the Zooglea couldnot utilize the sugar.(Heukelekian*)

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* Heukelekian, H., and Ingols, R.S. ,"Studies on Activated Sludge Bulking," Sewage Work Journal, 12(1940), 709.