An essay on the forces of nature observed by research biologists working on the treatment of waste highlights the dramatic challenges of sanitary engineering.

Observations and Speculation on Waste Treatment Research

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GILBERT WHITE, the great English naturalist of the 18th century, noted that dung dropped by cattle standing in ponds became food for aquatic insects, which in turn became food for fishes. He observed: "Thus nature, a great economist, converts the recreation of one animal to the support of another" (*1*).

The phenomenon of the food chain which so impressed itself on the good Reverend's attention is still of enormous interest to biologists, particularly to those working in the field of waste disposal. Certainly we have gained a great deal of sophistication in biology in the last few centuries. Our colleagues in the biological sciences speak now of ecologic systems, food chains, food webs, energy flow and balances, and material transformations. Our knowledge has been vastly extended in phenomena related to the interdependence of species and in all the subtle, delicate mechanisms by which micro-organisms react to their environments.

For approximately one-half century, our colleagues and our predecessors have been applying their best knowledge to creating optimum environments for the satisfactory disposal of human wastes. We now have at least a basic understanding of the biological communities on which we depend for waste stabilization. We have rational and intellectually satisfying procedures for designing trickling filters, activated sludge systems, and waste stabilization ponds for the intensive bio-oxidation of putrescible materials, and in a reasonably accurate fashion we may predict a stream's ability to assimilate such materials. Much has been accomplished, therefore, since the Reverend Gilbert White made his shrewd observation. However, it must be admitted we are still a long way from desirable control of the biological processes of interest, particularly with respect to new organics with which our microbial communities have had no experience.

In the course of our striving to understand and to control this biological action, many observations of interest have been made and we have speculated long as to their nature. In discussing some of these odd observations, what I have to say represents my own views and not necessarily those of the biologists with whom I have been associated. It is, perhaps, much easier for me, a sanitary engineer and in a sense an outsider, to speculate on this subject. This I shall proceed to do with a free hand.

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Although many of you have seen a trickling filter in a sewage treatment plant, probably few have ever run fingers through the slime covering on the filter rock, wondering what was in it and how it could be so effective and so reliable in treating highly putrescible human wastes. This organic film is obviously a filthy, slimy mess, but it holds great interest for the biologist. Dr. Bridge Cooke, our mycologist, recently completed an intensive study of the surface of trickling filters serving Dayton, Ohio, and what emerged from his report was an entrancing picture of a jungle in microcosm (*2*).

The slime was virtually a reproduction of the earth surface, especially with respect to the harsh competition for survival among organisms. The fungi in the slime held tightly to the underlying rock and gave the slime its structural strength. Filamentous algae likewise gave support to the slime layer. The fungi were akin to jungle trees, and the filamentous algae akin to green foliage. Broad savannahs were represented by extensive growths of surface algae. A variety of protozoa and higher animals browsed among the diverse plant life, and still others preyed indiscriminately on microscopic forms. Feeding on everything they contacted were insect larvae, worms, and snails. Here, in the slime, as in our familiar life, it is an eternal struggle for survival. Fortunately for us, the survivors represent a well-balanced energy and material flow, assuring good waste treatment.

The presence of the algae in the slime film was especially interesting. Obviously, they were thriving. Through their photosynthesis they were producing oxygen, much of which presumably remained dissolved in the film. Could this oxygen possibly be of any use in supporting the respiration of the micro-organisms feeding on the organic wastes? What gave point to this question was the common knowledge that the interface between the slime film and the atmosphere presents a barrier to oxygen movement. Here at the filter surface we have perhaps a built-in oxygen supply. Calculations based on the density of algal growth and on their normal rate of oxygen production indicated that if the entire slime film could be exposed to daylight or its equivalent, the total oxygen requirement for stabilization of the organics could be met by algal photosynthesis alone.

Is it possible to open up a trickling filter so that every part of its surface may be exposed to daylight? There actually seems to be no good reason why novel surfaces arranged in perhaps an unconventional manner may not be used. It may well be that we have here a possible scheme for deriving another benefit from our algae, that is, growing them in a form for easy harvesting so that the high protein concentrations in their cellular material may be recovered with economic benefit. This possibility is envisaged also in the work of the University of California on oxidation ponds, or waste stabilization ponds, as a means of sewage treatment. I believe it is correct to say that a major deterrent to the application of the oxidation pond is the lack of an economical way of capturing and removing in concentrated form the algae suspended in the pond mass.

We may go beyond secondary benefits and consider the possibility of tertiary values that could result from the removal of nitrates and phosphates in solution by our algae. This is really an enormously interesting prospect in view of the increasing troubles being experienced all over the country with dense, highly objectionable algal blooms in our streams and lakes. The use of algae in special ponds to remove these minerals from sewage treatment plant effluents is under intensive study by Dr. Rohlich and his colleagues at the University of Wisconsin in Madison, and by others.

Recently I re-read that series of papers on activated sludge studies conducted by Theriault, Butterfield, McNamee, Ruchhoft, and others engaged in research at the station now known as the Robert A. Taft Sanitary Engineering Center. This set of papers is, incidentally, a classic of sanitary engineering. These engineers and scientists attempted to come to grips with basic questions of activated sludge treatment, addressing themselves to such fundamental questions as: What is activated sludge? How does it form? What is its function in waste treatment? Much sewage has flowed over the weir since then, and it is not necessary now to go into the spirited debates of a generation ago, debates on chemical oxidation versus bio-oxidation, the significance of bioenzymatic action, the identification of slime-producing bacteria, and the general phenomenon of clarification. However, certain questions remain as stubbornly unresolved now as they were a generation ago.

One cannot but wonder what competitive advantage in the fight for life this jelly-like matrix called activated sludge confers on the organisms able to produce it. I put this question to our experts, physical chemists as well as biologists, and was assured that this slimy material is an excellent food-gathering mechanism. By processes of adsorption and absorption, nutrients are removed from the flowing waste and are brought within reach of the organism. This, I am sure, is true. But it is also, after all, a physical barrier to predators normally feeding on free-swimming bacteria. Does this film, therefore, provide a physical security to the organism as well as a more assured food supply? That the element of physical security is present is suggested by the work of many investigators. The following statement was made by Butterfield (*3*) in 1935: "Colpidium (a protozoan) added to a container of zooglea bacteria gave slightly better BOD removal and much clearer effluent than zooglea bacteria alone. Microscopic tests showed that the free-swimming bacteria had been eliminated."

The gelatinous matrix we also call "floc" definitely has survival value for the organisms. It protects them from their enemies while simultaneously bringing them food. In protected environments where food is plentiful, not only will the organisms survive, but they will grow fat. This is expressed in the polysaccharides composing the floc. So much is produced that, in waste treatment, we are forced to discard the great bulk of floc.

This matter of floc production and of the competitive advantages it provides recalls the discussions of about a generation ago on the physiological function of shells grown by marine animals on the ocean floor. In "Patterns of Survival" by John Hodgdon Bradley, the following is quoted from the address given in 1934 by the retiring president of the Paleontological Society. "All animals, and many plants, ingest with their water and food more calcium carbonate than they can profitably use. The problem of eliminating this surplus mineral material has always been most successfully solved by active living. The most energetic organisms possess light silicious or chitinous skeletons or no skeletons at all. The sluggards carry heavy shells." Competition forced the weak and lazy toward the ocean bottom where they are able no longer to cope with the involuntary accretion of calcium carbonate. "The more indolent began to grow shells."

Perhaps our friends, the slime-producing bacteria, are also indolent. This should not be surprising in view of the bountiful food supply they enjoy.

It is not really suggested that life in the slime is an easy one. The lurking predator sees to that. As a matter of fact, the predator is essential to a healthy system. Otherwise, our bacteria would grow fat, lazy, and inefficient.

Strange things sometimes happen in a treatment system, particularly when an intruder upsets the delicate balance. Last year when a maverick fungus got into the experimental activated sludge units (*4*), it succeeded in a very short time in exterminating a species of rotifer, minute bacteria-eating animals, important in the biological balance of the system. As a result, the stabilization process stopped dead.

It is well known that an effective biological treatment system depends on the voracity of microscopic animals. Their essential job is to keep the working microbial population fit and active. Recent discoveries suggest they play another important role by inhibiting the growth of *Sphaerotilus natans*, the so-called sewage fungus, which is associated with sludge bulking and fouls our streams. When Dr. Clarence Tarzwell was studying Lytle Creek, he found that profuse growths of this nuisance were accompanied by swarms of single-cell, microscopic animals, mostly ciliates and rhizopods (*5*). It appeared that the amebalike rhizopods, in particular, had keen appetite for this bacterial weed. A personal communication from Dr. H. Heukelekian of Rutgers University indicates that a rotifer with a similar function has been found.

When Butterfield was engaged in his study on the identification of organisms in the activated sludge floc, he recorded an observation which impresses me as being particularly odd. It occurred while he was trying to isolate bacteria from the gelatinous, zooglea matrix by washing it with distilled water. He reported as follows: "In carrying out this cleansing procedure, an unexpected phenomenon was encountered. During the course of the washing, the embedded bacterial cells would free themselves from the gelatinous matrix and move away with incredible speed, dispersing themselves throughout the dilution water long before a satisfactory washing had been accomplished" (*3*). Subsequent observations suggested that the bacteria had left the gelatinous matrix because no nutrient was being absorbed. The dispersing action was prevented by washing with water containing dissolved organic material. The interpretation of this occurrence is, in my opinion, simple. Bacteria have to feed continuously. When their food concentrating mechanism fails them, they are literally forced to leave their homes and go searching for food.

Butterfield's observation is of particular interest in the light of a recent report by Dr. Herman Amberg indicating that, in the laboratory, growth of *Sphaerotilus* could be prevented by substituting a schedule of intermittent discharges of pulp mill wastes for one of continuous discharge (*6*). Perhaps here too, the individual organisms composing the *Sphaerotilus* filaments were forced to leave their abode during the period of no discharge and seek their food by freely swimming about. Although I am not aware of any extensive observations on this point, the hypothesis is appealing.

We are slowly learning more of what goes on in the microworld of waste treatment. Eventually we may even be able to develop microbial systems to handle lignins and certain synthetic organics that presently pass through treatment practically unaltered in character or concentration. The success of geneticists in developing new strains of bacteria encourages use of their techniques in research. This goes much further than simply exposing the organisms to the organic chemicals, hoping they will, through hunger, learn how to use them as food. Bacteria can be trained. They have excellent capacity for adapting themselves, and they breed so fast that a mutant strain can quickly establish itself in the appropriate environment.

We are engaged in developing intelligent ways of getting micro-organisms to accept strange compounds as nutrients, shocking them if necessary into evolving the necessary appetite and digestive muscle to do the job efficiently. However, we have much to learn ourselves before we can, in scientific fashion, routinely mold microbial communities into smoothly functioning organizations for breaking down highly complex compounds.

Bacteria must feed on highly energized carbon and nitrogen compounds to satisfy growth and living requirements. They soon learn to relish most manmade compounds, even very complex ones. Others they reject, presumably because certain molecular structures are too hard to break up and digest. Perhaps, if research is sharp enough, we may be able to identify many of these barriers and learn how to overcome or avoid them. It is evident that we must learn more about bacterial enzyme systems, particularly how to stimulate the development of new ones.

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