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# True Short Stories in Microbiology with Surprise Endings*

ROBERT L. STARKEY

*Department of Biochemistry and Microbiology, Cook College, Rutgers University, New Brunswick, New Jersey*

President Stapley, members of the Society for Industrial Microbiology, ladies and gentlemen. I appreciate and treasure the honor of this Thom award. Needless to say, it was unexpected, principally because my studies in the field of industrial microbiology have been scattered and peripheral. I have been characterized throughout the years as a soil microbiologist, but in the current vernacular, some might call me an ecologist. Whatever the designation, most of my studies have been directed toward contributing to soil science. At times, the nature of the research problems has led me into industrial fields or to microbial biochemistry. This could be explained by the fact that I have been concerned generally with microorganisms as converters of materials, both organic and inorganic.

This award has particular significance to me because it bears the name of Charles Thom. I knew Dr. Thom for many years, first through contacts provided by Dr. Waksman and subsequently through our common interest in soil microbiology. I remember that Dr. Thom provided Dr. Waksman encouragement and helpful advice in his early studies of the actinomycetes. Although Dr. Thom had little knowledge of these organisms, as was the case with most microbiologists at that time, he made the investigation seem significant when aid and interest were very much needed. I clearly remember my surprise when Dr. Thom rose to present a paper at a symposium on soil microbiology and stated that he had chosen to discuss "Dick and Polly." This levity seemed out of character for one with an austere and solemn countenance, but I learned that he had many ways to enliven interest and attain attention. His subject proved to be the importance of the slime molds *Dictyostelium* and *Polyspondilium* on the decomposition of organic materials.

It is probable that, since I have been away from the laboratory for several years, you would not expect me to make an erudite presentation on any association with recent important developments in industrial microbiology. I have chosen to consider two items. First, I wish to remark briefly on the value of training and competence in the field of soil microbiology for an understanding of industrial microbiology and for solution of some of its problems. Microorganisms have a wide range of biochemical and physiological properties, some of which are truly bizarre and unlikely to be detected without experimentation and keen observation. Concerning this, I wish to note what seems to me to have been an unusually large number of such phenomena that I have encountered. These provided me interest and stimulation in what sometimes might have been routine and prosaic studies.

*This paper is the Charles Thom Award Address.

*Soil Microbiology, an Introduction to Industrial Microbiology*

Soil microbiology embraces the study of the microbial population of soils and composts, factors affecting the development of these organisms, and their conversion of a multitude of soil constituents both organic and inorganic. As has been said in the case of industrial microbiology, there are both useful and harmful microbial activities in soil. Some years ago, it was popular to investigate the effects of soil factors such as hydrogen-ion concentration, oxidation-reduction potential, temperature, moisture, and other physical soil factors on the numbers of microorganisms in soil. Such collections of census data have proved to be of little value. Subsequently, there has been more emphasis on metabolic activity of soil microorganisms and their role as converters and transformers of organic and inorganic substances in soils and composts. It is conceivable that practically all of the organic materials in plants, animals, and soil organisms, as well as the contents of domestic and industrial wastes, become incorporated with soil eventually. Since none of these constituents persists, with the possible exception of a few industrial compounds, it can be concluded that all are susceptible to breakdown by elements of the microbial population of soils. Therefore, in studies of the conversion of any of these substances, it is logical to search soil for microorganisms able to effect the transformations. I would call your attention to the studies concerned with the control of pneumonia by Dr. Avery, located at what was then named the Rockefeller Institute. He searched culture collections for microorganisms able to break down the polysaccharide capsules of the pneumococcus without success. Then he engaged Dr. Dubos who had just concluded his graduate studies in soil microbiology. Dubos sought such microorganisms in soil and was successful.

The almost limitless types of transformations brought about by soil microorganisms make soil a useful reservoir of microorganisms for various industrial uses. One of the best known was the search of soils for cultures able to produce effective chemotherapeutic antibiotics.

The science of soil microbiology has many ramifications. Whereas some studies definitely are applied others might be called basic, which brings to mind the following words of past SIM president, Saul Rich. He said, "Many of us have been led to believe that applied and basic research are antonyms. Actually the two are different aspects of the same activity . . . It is the extrapolation and general scientific usefulness of a study which decides whether it is basic. Conversely, research that cannot be applied is not necessarily basic. It may just be pointless." In this connection, one might take exception to the increasing emphasis by grant-making agencies that research projects should be mission-oriented. I interpret this to mean that they should have obvious practical significance. Continuation of this policy might be expected to cause us to miss important practical benefits that might result from what appeared to be a strictly basic study. Soil microbiology has increasing importance in its own subject area because of its significance in the important processes of nitrogen fixation, denitrification, disposal of plant and animal waste and rubbish, and decomposition of pesticides.

The relationship of soil microbiology to industry is further attested to by the fact that students trained in soil microbiology in our department and university as well as others have become industrial microbiologists in industries concerned with foods, paints, sanitation, pharmaceuticals, metal corrosion, production of chemicals for control of plant parasites, and others. My concern that there is decreasing emphasis and interest in the profession of soil microbiology can be understood.

*Strange Encounters*

It can be appreciated that one who has been away from the laboratory for several years has a

tendency to reminisce. During such diversions, it occurred to me that several of my research experiences led to bizarre and surprising results. I have assembled a few of these hoping they would interest you. Some of the results might qualify as Serendipity. Let me here acknowledge the collaboration of many diligent graduate students and other associates in the following investigations.

1. My first experience when I was a graduate student was to study the sulfur bacterium, *Thiobacillus thiooxidans,* the discovery of which had been reported the same year that I started my research (1921). It was indeed a remarkable bacterium; it grew in media devoid of organic matter, thrived only under strongly acid conditions, and could obtain all of its energy from the oxidation of elemental sulfur. Not only did it require high hydrogen-ion concentrations for growth, I found that it could produce and tolerate 10% sulfuric acid in the cultural medium. This was an astounding introduction to microbiology and I was conditioned to expect unusual behavior of other microorganisms as well. As you know, sulfur bacteria of the group to which *T. thiooxidans* belongs have been used industrially in the recovery of copper and other heavy metals from mine-waste dumps. At one time I studied the corrosion of cast iron pipes caused by this bacterium, oxidation of the sulfur used to seal the bell joints, and how to control this effect.

2. Upon completion of my graduate studies, and while I was at the University of Minnesota, I became involved in investigations of microbial transformation of iron. The work was initiated to obtain information on the formation of bog iron ore which occurred in the extensive iron deposits of the Mesabi Range in the northern part of the state. The information obtained led to the conclusion that microorganisms played important roles in both solution and precipation of iron and that the result was determined generally by the microbial effects on the hydrogen-ion concentration and oxidation-reduction potential. Increase in the acidity and development of anaerobic conditions promoted solution and vice versa. Also, other factors were involved such as the formation and decomposition of organic iron compounds. It was our conclusion that the prevailing opinion that iron bacteria were responsible for most of the iron precipitation was exaggerated. We concluded that the major factor responsible for the deposition of iron in the Mesabi Range was the chemical oxidation of ferrous iron contained in subterranean waters when they were discharged into oxygen-containing surface water.

3. At another time during the investigation of the occurrence and prevalence of nitrogen-fixing *Azotobacter* cells in soil, I encountered a large number of moist colonies of an unusual yeast. The oval cells were almost completely filled with large bodies of highly refractive material that proved to be lipid. On the dry-weight basis, the lipid content of the cells grown on media deficient in nitrogen was more than 60%. In studies made elsewhere, the lipid was found to be acceptable feed for experimental animals, but since the composition was similar to that of some plant lipids, it was not produced commercially. One other remarkable property of the yeast was the accumulation of many spores in each ascus. Whereas most sporogenous yeasts produce four spores in each ascus, this one produced up to 16 or more and even greater numbers were reported by others. It was a new yeast and became the type species of the new genus *Lipomyces.*

4. I was led to study the fate of the vitamins riboflavin, pantothenic acid, and nicotinic acid in soil because I questioned the assumption of Schopfer that they persisted and therefore had important influence on the development of higher plants. To quote Schopfer, "The presence of growth factors in natural manures must contribute to their superiority as compared with artificial fertilizers . . . It can readily be expected that soil acts on plants not only through its plastic foods and minerals, but also through its growth factors . . . One may

attribute to the rhizophere a certain richness in vitamin growth factors." The sources of these vitamins in soils were stated to be the microorganisms, roots of higher plants, the processes of decomposition of plant materials, and animal manures. Upon investigation, we found that the vitamins decomposed rapidly and disappeared more rapidly than the plant material as a whole. Although the vitamin content increased during the first few days of decomposition of plant materials, apparently due to their production by the developing microorganisms, they disappeared almost entirely in a week or 10 days. Even when straw, which contained an exceedingly small amount of vitamins, was added to soil, the residual vitamin content was even less in a few days than it was initially. This subject is mentioned not only because we found that vitamins were decomposed rapidly but because we encountered an unusual situation when we attempted to make determinations of the vitamins in soil. The vitamins became adsorbed by the soil colloids and resisted extraction. It surprised us that, if the total soil material was dispersed in water and if aliquots of the suspension were added to the test media, the assay bacteria grew the same as if the total amount of pure vitamins had been tested in the absence of soil.

5. A similar condition was encountered in experiments to determine the decomposition of the antibiotic streptomycin. This study was undertaken also because of skepticism about the statement, "Streptomycin is not destroyed by microorganisms" (Waksman and Schatz). As mentioned previously, it is conceivable that all natural products are decomposable under suitable environmental conditions, some more rapidly than others. Otherwise, one would expect them to accumulate in quantity over long periods of time. Petroleum, natural gas, coal, peat, sulfur, and other substances fortunately do accumulate under anaerobic conditions but they are decomposed in an aerobic environment. Consequently, we believed that streptomycin was decomposable. In studies of its transformation in soil, we observed that streptomycin was adsorbed by the soil colloids and no means was found to extract it completely. Nevertheless, when dilutions of the thoroughly dispersed soil to which streptomycin had been added were placed in the assay medium, the test cultures developed the same as if an aqueous solution of the antibiotic had been used. The outcome of the study, as you probably know, was that streptomycin decomposed readily in soil and culture media. Furthermore, a bacterial culture was isolated from streptomycin-treated soil that could grow on streptomycin as the only organic constituent of the medium.

6. Among other microorganisms in which I developed interest were the sulfate-reducing bacteria. This interest started when I was studying under Prof. Kluyver at the technical university of Holland. These bacteria have been particularly significant in Holland because there are so many canals, the bottom muds of which provide a favorable environment for the anaerobic bacteria and for evolution of hydrogen sulfide. Many years ago, they were discovered by and the first culture was named by the Dutch bacteriologist Beijerinck. These bacteria are interesting biochemically for many reasons, one of the most fundamental of which is their ability to grow from the energy liberated by the oxidation of organic nutrients by sulfate which is reduced to sulfide. The bacteria acquire special importance in industry because they are responsible for the corrosion of iron and steel under anaerobic conditions. In the electrochemical corrosion cells, hydrogen is produced at the cathodic areas. Under aerobic conditions, the system is depolarized by free oxygen which oxidizes the hydrogen to water. However, under anaerobic conditions, the system becomes polarized unless the cathodic hydrogen is removed. The role ascribed to the sulfate-reducing bacteria was their removal of the cathodic hydrogen which then was oxidized by sulfate, which itself was reduced to sulfide. I will not go into detail about our investigations of anaerobic corrosion because this

was the subject of the address by Dr. Warren Iverson at the time he received the most recent Charles Thom award. In particular, I want to mention one of the strange biochemical properties of many of the sulfate-reducing bacteria. This is that not only do they oxidize hydrogen as is indicated in the course of anaerobic corrosion but that these bacteria can obtain almost all of their energy for development through this oxidation. Had it been that the bacteria required no preformed organic matter, it would have been a truly remarkable biochemical process. We found that the bacteria grew anaerobically in a mineral salts medium containing only a steel strip. This latter served as a source of energy through the chemical release of gaseous hydrogen which was the actual energy source. Therefore, the bacterium appeared to be an autotroph that could grow on metallic iron. This proved not to be the case because others demonstrated that the sulfate-reducing bacteria required small amounts of organic compounds such as isobutanol or choline as supplementary sources of energy and obtained the carbon for assimilation from something in yeast extract. Assimilation of carbon dioxide was marginal.

7. Subsequently, I became involved in studies of anaerobic corrosion through inquiries by groups desirous of obtaining information on corrosion of pipe lines. The long pipelines which carried petroleum products and consumer gas corroded unpredictably. Since it was imperative that breaks in the lines should be avoided, portions of the lines were excavated periodically for inspection and to make necessary repairs. The company hoped to find some means for locating the "hot spots" where the pipe lines were particularly susceptible to corrosion. Given this information, most attention could be confined to those areas where the pipes could be exposed periodically for examination and repaired where necessary to avoid breaks. Our solution to the problem proved to be a nonbiological one. It was based on the fact that where there was anaerobic corrosion, the oxidation-reduction potential was low and the hydrogen-ion concentration was in the range suitable for growth of the sulfate-reducing bacteria. An appliance called a redox probe was developed whereby the $E_h$ and pH of the soil at pipe depth could be determined in the field without excavating the pipe. Almost immediately, information could be provided on the probable corrosiveness of the soil area. During World War II, we became further involved in anaerobic corrosion of naval ships and harbor installations.

One of the principal conclusions resulting from the studies of anaerobic corrosion was that the mechanism of the process differed from that commonly accepted. We found evidence that oxygen-concentration cells were important and probably the most important factor in this type of corrosion. Our conclusion was supported by the following observations. It was noted that anaerobic corrosion was relatively insignificant under strict anaerobic conditions even where there was active sulfate reduction. However, it was found that when the metal specimen was buried partly in mud where there was active sulfate reduction and partly exposed to aerobic conditions in the overlying water, corrosion was rapid on the portion of the metal located in the region of sulfate reduction. We proposed that strong oxygen-concentration cells were developed by coupling of steel exposed to strongly reducing environments created by sulfate reduction with steel exposed to oxygen-containing solutions. Corrosion cells produced by coupling of metal sections exposed to iron sulfide with those free from the sulfide were suggested as a contributing corrosion factor. The process of anaerobic corrosion appears to be of sufficient practical importance to justify further study to explain the mechanism of the process.

8. Our studies of microbial transformation of sulfur extended into two additional areas. One of these was concerned with microbial breakdown of organic sulfur compounds, several of which were examined. The dissimilation of the amino acid methionine was particularly

interesting. First, we found a bacterium that could grow on methonine as the only organic material with formation of the sulfur products, methyl thiol and dimethyl disulfide. We were surprised that there seemed to be few microorganisms in soil that could grow on this amino acid. But we continued our search and found cultures that could decompose the amino acid in media supplemented with some organic nutrient such as glucose. One of these was a fungus which when cultivated in the medium containing both methionine and glucose, stripped the amino acid of the amino and hydrosulfide groups leaving a carbon residue of $\alpha$-hydroxy butyric acid. The explanation of its inability to grow on methionine was its failure to metabolize $\alpha$-keto butyric acid. This was an example of co-metabolism, the ability of a microorganism to transform a compound only in the presence of another metabolizable compound.

9. The second area of sulfur studies dealt with an investigation of the origin of natural sulfur deposits such as those that occur in Texas, Louisiana, and neighboring states. According to the theory of their origin, the elemental sulfur in the deposits was formed from the sulfate that occurs in the brines that saturate the formations. The sulfate is reduced to sulfide by action of the sulfate-reducing bacteria and the elemental sulfur is produced by chemical oxidation of the sulfide. It had been found by others that the ratios of the stable sulfur isotopes S32 and S34 in the sulfate of the brines and the elemental sulfur differed, and that there was enrichment of the isotope S32 in the elemental sulfur over that in the sulfate. Our studies were made to determine what changes occurred in the S32:S34 ratio during the reduction of sulfate to sulfide by development of sulfate-reducing bacteria. Enrichment increased the S32 isotope, thus increasing the S32:S34 ratio. Furthermore, under suitable growth conditions, the amount of this increase was within the range of the apparent fractionation that occurred during deposition of the elemental sulfur in the natural deposits (2.7%). The results support the hypothesis that sulfate-reducing bacteria participated in the formation of the deposits and indicate that their contribution was the initial reaction of reduction of sulfate to sulfide.

10. An entirely different project dealt with the nutrition of chickens. A commercial company located in northern Maine processed fish. Fish and fish waste was cooked, the solids and oil were removed, and the remaining liquid was concentrated to yield a product called "condensed fish solubles." This contained 50% soluble matter. The material had proved to be an important supplement to chicken feed due to its content of amino acids, trace elements, and vitamins of the B-complex, one of the most important of which was $B_{12}$. We undertook to increase this $B_{12}$ content. For this purpose the actinomycete *Streptomyces fradiae* was grown in diluted fish solubles and a 10- to 14-fold increase in $B_{12}$ was obtained. There was evidence that other growth factors for chicks were produced as well.

11. The scene shifts once more to an entirely different subject area. I received a culture from Dr. De of India with a request for aid in identifying a bacterium which he thought might be a species of the nitrogen-fixing bacteria of the genus *Azotobacter*. I found that it did fix nitrogen and, because it resembled bacteria of this genus, we named it *Azotobacter indicum*. It was an aerobic, Gram-negative, nonsporulating bacterium with oval cells, and it grew on simple carbohydrates and failed to grow on peptone and related nitrogen compounds. It had several distinguishing characteristics, one of which was the morphology of the cells. The oval cells were considerably smaller than those of other species of *Azotobacter* and they were unusual in that at each end of the cells there were refractive globules that occupied about 50% of the cell volume. These globules proved to be lipid. Another characteristic was its production of an abundance of slime which made colonies on agar plates soft and watery when the bacterium was grown on alkaline media but firm and rubbery on acid media. Upon further

examination, it was found remarkable that the culture grew well and fixed about the same amount of nitrogen over the range of pH 3.1 to 8.9. This was distinctly different from other species of the genus *Azotobacter* which required a nearly neutral reaction. At the time the studies were made, an important requirement of all species of the genus was that they grow at a reaction above pH 6.0.

This unusual bacterium has seldom been recovered from soils outside of tropical and subtropical areas. I did isolate it from a New Jersey soil and there have been other exceptions. In recent years, several related bacteria have been detected and they have been grouped in a new genus *Beijerinckia,* of which the type species is the culture we named *Azotobacter indicum.*

12. Finally, I wish to mention an additional experience with microorganisms having remarkable tolerance to conditions inhibitive to growth of most, if not all, other living things. I was requested to find the cause of slime production in a unit of a plant of the Bemberg Corporation. The company produced a silky fabric woven from the fibers of a modified cellulose. Cotton dissolved in ammoniacal copper hydrate was forced through fine holes of a spinneret into a solution of sulfuric acid at pH 0.4. The slime which appeared in the acid spinning liquor fouled the precipitated threads. Two fungi were isolated from the spinning liquor and from dead ends of the pipes. One of these identified as *Acontium velatum* was principally responsible for the slime formation. The other could not be identified readily because it produced no asexual spores but it has been named recently *Scytalidium acidophilum,* a new species of the genus.

The two fungi were found to have remarkable tolerance both to high concentrations of hydrogen-ions and copper sulfate. As already mentioned, the spinning liquor had a reaction of pH 0.4. Part of the copper sulfate was removed during recycling of the liquor so that the concentration was kept at 0.4%. Since the fungi developed in a solution that was abnormal for microbial growth, studies were made of their actual tolerance to acid and copper sulfate. Surprisingly, we found that both fungi could grow in media saturated with copper sulfate at pH 0 to 0.05. Growth was scant under these extreme conditions but good in media saturated with copper sulfate at pH 0 to 0.5. To my knowledge, this is the highest recorded combined concentrations of copper sulfate and hydrogen-ions that permit growth.

Further studies carried out with one of these fungi (*S. acidophilum*) provided an additional surprise. In spite of its tolerance to copper sulfate under acid conditions, it was exceedingly sensitive to copper near neutrality even though most of the copper became precipitated as copper hydrate at these reactions. There was a distinct loss of tolerance to copper starting at about pH 4.0 and in neutral media it was even more sensitive than several other common filamentous fungi. It seems likely that copper tolerance is controlled by cell permeability. Apparently, copper is excluded under strongly acid conditions but enters the cells and becomes toxic near neutrality.

## Concluding Remarks

This rambling discourse surely must convey the impression that my research interests in microbiology have been diverse. I have not included the various projects in soil microbiology that dominated most of my career. If there is one subject area with which I have been most concerned, it is the transformations of sulfur, its oxidation, reduction, the dissimilation of organic compounds, and the practical application of these processes. At least my presentation offers evidence to confirm my original thesis that a soil microbiologist can become involved in many problems far removed from those intimately connected with soil processes.

My microbiological career has been a fascinating, alluring, and stimulating experience. I have enjoyed encounters with the unexpected. Therefore it was with regret that it all ended with my retirement. There is still much to be done and important problems to be solved. To adapt an oft-quoted passage of Shakespeare, there are more things in heaven and earth than are dreamt of in our philosophy. I often have remarked that had I been blessed with independent means to choose any career, I would have been fortunate to have chosen the one I did.

I am grateful to you for this opportunity to speak about some of the things that have been among my principal concerns.