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The oxidation of sulfur by microörganisms.

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Two groups of phenomena are to be considered in the study of the sulfur cycle: (1) the reduction phenomena and (2) the oxidation phenomena. The first result in the production of hydrogen sulfide and the second in the oxidation of H_2S to S and of the latter to H_2SO_4. Both groups of phenomena result from activities of microörganisms. Winogradsky was the first investigator to have definitely demonstrated the rôle of microbes in the oxidation of the H_2S to S, and of the latter to H_2SO_4, but it is characteristic that the organisms studied by Winogradsky (*Beggiatoa* and *Thiothrix*), never turned the medium acid: this was explained by the presence of sufficient $CaCO_3$ in the culture to neutralize any acid formed. Keil isolated the two organisms in pure culture only about thirty years after the work of Winogradsky was carried out.

The sulfur oxidizing bacteria were usually divided into four groups, namely (1) Thread-forming colorless bacteria, accumulating sulfur within their cells. The *Beggiatoa* and *Thiothrix* are representative of this group. (2) Non-thread forming, colorless bacteria, accumulating sulfur within their cells. Here are referred forms (*Thioploca*, *Thiovulum* etc.) of various sizes and shapes, the distinguishing differences being the facts that they oxidize H_2S, accumulate sulfur within their cells, are colorless and non-thread forming. Some of these have been isolated in pure culture. (3) Purple bacteria. Some of these seem to play a part in the sulfur cycle, although none of the sulfur forms have yet been isolated in pure culture. (4) Colorless, non-thread forming sulfur oxidizing bacteria which do not accumulate sulfur within their cells, but which produce an abundance of sulfur (from H_2S and thiosulfates) outside of their cells. The two characteristic and most important forms belonging to this group are the *Thiobacillus denitrificans*, anaerobic, deriving its oxygen from the decomposition of nitrates; and *Thiobacillus thioparus*, which oxidizes thiosulfates, H_2S, and S, allows an extensive accumulation of sulfur from the first two and allows the medium to become distinctly acid. The work on this group of organisms has been carried on chiefly by Beijerinck and associates.

It is suggested here to add another group of sulfur-oxidizing bacteria, which was isolated and is being extensively studied at the New Jersey Agricultural Experiment Station. This group (5) will comprise bacteria similar to group 4 in their morphology, although much smaller in size (less than 1 μ. in length) and distinctly different physiologically. They do not act upon thiosulfates and H_2S, only upon elementary sulfur and allow the medium to become acid up to a P_H of 0.8–1.2.

These organisms have been isolated from composts consisting of sulfur, phosphate rock and soil, where the sulfur oxidation is very strong; they were isolated by means of a purely inorganic culture medium, consisting of minerals with elementary sulfur as the only source of energy. These organisms are autotrophic and do not need any organic substances for their development, the carbon being derived from the CO_2 of the air. The sulfur is oxidized very rapidly with the production of sulfuric acid. When the medium is poor in neutralizing substances as well as in inorganic buffering substances the accumulation of acids will soon reach such a concentration that the growth of the organisms may be stopped; in the presence of the proper neutralizing agents, such as tricalcium phosphate, sodium bicarbonate, or in the presence of sufficient amount of buffering agents, such as di-basic-phosphates and bicarbonates, the acid produced interacts with the basic element giving salts or acid salts tending to make the medium less acid. But as soon as the neutralizing agent is used up, the acid begins to accumulate, if there is an excess of elementary sulfur in the medium. It is characteristic to note here one thing: while the lack of acid production in the work of Winogradsky was explained by the fact that the presence of sufficient carbonates in the medium kept it at a neutral point, we find, in the case of our organism, that the neutralization of the basic substances is accomplished at a P_H 3.6 to 2.0, which is a distinctly acid zone, in fact even a more acid zone than the final acidity of the majority of acid producing bacteria (lactic, acetic, etc.) and yeasts.

We reported elsewhere that the sulfur-oxidizing bacteria, which we have isolated and a complete description of which will be published soon, produce a greater concentration of acids and remain alive in that acid medium, than any biological phenomena ever known.

Age of Culture.	P_H.	Titrat. Acidity c.c. N/10 Alkali per 5 c.c. of Culture.	Mgs. in 100 c.c. of Solution.		Sulfur Dissolved Mgs.	Soluble + Phosphates (as P) Mgs in 100 c.c.
			Sol. SO_4	Insol. SO_4		
At start..........	5.4	0.8	209	0	0	45
2 days..........	5.3	0.9	211	0	—	47
4 "	4.0	1.3	240	40	20	60
8 "	2.7	3.9	444	56	87	87
12 "	2.3	5.0	253	378	140	183
23 "	2.4	5.4	300	275[1]	115	194
"	2.1	6.2	314	310	158	196
30 "	1.6	9.6	530	360	210	194

What we attempted in our work is not only the isolation of strong sulfur-oxidizing organisms, but the production of acid at such a rate and under such conditions that it should transform insoluble tri-calcium phosphate into soluble phosphates and phosphoric acid. The insoluble phosphates are used as neutralizing agents.

[1] The decrease is due to the fact that the determinations were based on separate flasks, in which the ratio between the soluble and insoluble $SO_4^=$ was not alike, for some reason or another.