While the picture was completely clarified, however, Winogradsky left this field to work on the nitrifying and nitrogen fixing bacteria (see below). He profited in this from his earlier work on the sulfur bacteria, since he was able to view the nitrifying bacteria as chemosynthetic organisms quite analogous to the sulfur bacteria.

On the nitrifying organisms

1890 • S. Winogradsky


Before summarizing the work on nitrification which has occupied me for the past year, I would like to recall several of my previous works which were the point of departure for the present report.

Besides the organisms which are the subject of the present note, two groups of organisms have been studied which have the ability to oxidize inorganic substances. I have designated them by the names sulfur bacteria and iron bacteria.

The first group live in natural waters which contain hydrogen sulfide and do not grow in media lacking this substance. This gas is absorbed extensively and oxidized by their cells and is converted into sulfur granules. These latter are in turn degraded and sulfuric acid is excreted. The second group are able to oxidize iron salts, and their life is also closely connected with the presence of these compounds in their nutrient medium.

My efforts to elucidate the physiological significance of these phenomena have led me to the concept that these inorganic compounds are the fermentable materials (in the largest sense of the word) in the life of these beings, instead of the organic materials which are the fermentable substances for the large majority of microbes. This concept leads to the logical conclusion, confirmed by experience, that these beings comprise a group with certain physiological properties which can be summarized as follows. All of the energy necessary for their vital activity would be furnished by the oxidation of mineral substances, and their dependence on organic compounds for growth would be quite slight. In addition, inorganic compounds of carbon which are not utilizable by other organisms that lack chlorophyll would be used by them as a source of carbon.

The remarkable work of MM. Schloesing and Müntz has thrown light on the role of lower organisms in the process of nitrification. However, although their work makes it highly likely that a special agent exists for nitrification, they have not succeeded in demonstrating the process away from the soil, which is a natural medium with a wide variety of microorganisms. The principal requirement
for all microbiological experiments today is the isolation in pure culture of the agent responsible for the process. Because of the difficulties involved, a number of workers have failed to isolate the nitrification ferment, so that the conclusion of MM. Schloesing and Müntz concerning the existence of this ferment has not been confirmed by bacteriologists and botanists.

This question must be clarified first. I have found that the failures of my predecessors are due to the fact that they used media which had been solidified with gelatin, such as are used so often today for the isolation and culture of microbes. The nitrifying organisms will not grow on such media, so that if a mixture of microbes taken from a soil that is in the process of nitrifying are placed in such a medium, all of the organisms that are active die, and one only isolates those which are ineffective. It is possible, with some difficulty, to eliminate one by one all of the foreign species and to obtain pure and in large numbers the nitrifying species, by using a medium that is favorable to it but unfavorable to the other organisms. These cultures are able under the usual microbiological experimental conditions to carry out the nitrification process just as intensely as M. Schloesing has recently shown it to occur in the soil.

This organism has been more difficult to experiment with than any of the other very delicate organisms which I had previously worked with. However, its physiological properties not only confirm my conclusions, but have revealed a new fact which I would like to report to the Academy.

I applied to this study the ideas which I had already acquired concerning the nutrition of organisms which oxidize mineral substances. I cultivated the nitrifying microbe from the beginning in a liquid which did not contain organic matter, but only a natural water that was very pure. Since the addition of organic compounds did not seem to promote its growth, I have used for its culture a mineral solution that is completely devoid of organic carbon. Although this medium does not have any other carbon compounds in it but carbonic acid and carbonates, the action of the nitrifying organism has not diminished in its intensity over several months.

We must conclude that this organism is able to assimilate carbon from carbonic acid, and this conclusion is confirmed by the amounts of organic carbon in the cultures. This demonstrates that there has been an accumulation of organic carbon by the action of this organism.

The nitrifying organism, which is colorless, is able to synthesize completely its cell substance from carbonic acid and ammonia. It carries out these syntheses independently of the light, and without other sources of energy than the oxidation of ammonia. This new fact is contradictory to that fundamental doctrine of physiology which states that a complete synthesis of organic matter cannot take place in nature except through chlorophyll-containing plants by the action of light.

It is hardly likely that the nitrifying organism exhibits a chlorophyllous action, since a release of oxygen has never been observed. Another hypothesis, that it is an amide, perhaps urea, that is the first stage in the synthesis occurring in this organism, seems to me to be the only plausible one.

Further studies on the physiology and morphology of the nitrifying organism are in progress.
Winogradsky was able to show in a clear way for the nitrifying organisms that they obtained their energy from the oxidation of ammonia and use this energy for the assimilation of carbon dioxide. His earlier studies on the sulfur and iron bacteria had pointed this way, but these organisms had proven harder to work with. This discovery is really one of the most important in physiology, since it shows, as Winogradsky realized, that carbon dioxide is convertible into organic carbon without the intervention of light energy through chlorophyll. With the addition here of a third group of bacteria that could obtain energy from the oxidation of inorganic compounds, the chemosynthetic bacteria appeared to be fairly common.

The process of nitrification turned out to be more complicated than it appears here, and Winogradsky was instrumental in clarifying this picture. He described two genera of bacteria, one which oxidized ammonia to nitrite, and the other which oxidized nitrite to nitrate. This process is important agriculturally, since ammonia is easily lost from the soil, while nitrate is more stable and serves as a good nitrogen source for plants. As he mentioned, the isolation of these organisms in pure culture was quite difficult, mainly because the soil is so rich in bacteria that other forms, which grow much faster than the nitrifying bacteria, will take over on agar plates containing organic media. Further, the nitrifying bacteria seem to be inhibited by organic matter, so that it is necessary to find a substitute for agar or gelatin. Winogradsky later did this, using silica gel, and succeeded in this way in isolating pure cultures of each of the nitrifying bacteria. He was then able to demonstrate this process in pure culture and show that a different organism was responsible for each stage.

The biochemical aspects of chemosynthetic organisms are just beginning to be worked out. We know that the process of carbon dioxide fixation in the sulfur bacteria is quite similar to the process in green plants, using the same enzyme systems. The difference is in the source of energy. The sulfur and nitrifying bacteria derive their energy from the oxidation of these inorganic compounds, and these oxidations are coupled to phosphorylation, giving ATP. The energy from ATP is used in the process of carbon dioxide fixation. Only a small amount of modern work has been done on these interesting organisms, and many new things remain to be discovered.