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INVESTIGATIONS UPON NITRIFICATION AND THE
NITRIFYING ORGANISM.¹

THE nitrogen of organic substances is, for the most part, liberated during decay in the form of ammonia or ammoniacal compounds; and these substances yield, by oxidation, nitrous acid and finally nitric acid, which, in turn, in the form of nitrates, feeds the living plant, and thus begins again the cycle of transformation.

The oxidation of the nitrogen of ammonia, and its ultimate conversion into nitric acid, is called nitrification. This change is especially active in soils near the surface, where nitrates are formed abundantly from percolating waters which contain much nitrogenous matter.

This phase of nitrification, the formation of nitrates in porous soil, has been attentively studied: but less attention has been given to the process of nitrification as it goes on in surface waters, such as streams and ponds; and it is to this side of the question, namely, nitrification as it occurs in natural waters, that our study has been chiefly directed.

Some eighty samples of water, selected from the two hundred and forty coming each month to the laboratory of the State Board of Health, were examined at intervals of from two to seven days for ammonia, nitrites, and nitrates. These samples were received from all parts of the State, and included all classes of surface water, rivers, ponds, and reservoirs. They were examined repeatedly during the months of June, July, and August, 1888.

The results may be briefly stated as follows. The organic matter in suspension decays in about seven days, as is shown by the increase in "free ammonia." In about fourteen days this "free ammonia" has disappeared, and nitrite has taken its place, reaching a maximum in about twenty-one days. Later the nitrite too disappears, and in twenty-eight days or

more all the nitrogen has been converted into the form of nitrate. When the suspended matter is removed by filtration through paper, or by precipitation with alumina, no change occurs unless free ammonia were present at the outset.

These changes were so universal, and so independent of the character of the water and of its condition of aeration, that it seemed important to avail ourselves of the unusual opportunity offered by the close proximity of the chemical and biological laboratories of the State Board of Health, to carry on a series of chemical and bacteriological investigations on solutions of known composition. Accordingly, we began a series of experiments covering a period of nearly two years, in which the daily and weekly changes caused by the growth of bacteria were watched from both the chemical and the bacteriological standpoint, in order to determine the sequence and rate of such changes. Other points came up in the course of the work, as will appear from the following pages.

It has long been known that the first step — the decomposition of nitrogenous matter, and consequent production of ammonia — is due to the vital activity of bacteria. The early experiments of Schwann and Schultze (1839), and the later and thoroughly conclusive work of Pasteur, showed that putrefaction of organic matter is brought about solely by the small vegetable organisms known as bacteria. Even after this fact became generally known, it was some time before the importance of the complete range of this discovery was suspected. It was still maintained that the process of nitrification proper — the oxidation of ammonia to nitric acid — was of a purely chemical nature, although the burden of proof was soon thrown on those who upheld this view. The close dependence of nitrification upon a rather narrow range of temperature, the cessation of the process on the addition of antiseptics, the operation of "seeding" one solution with another, the impossibility of effecting rapid nitrification by chemicals, the analogous phenomena of putrefaction, — all pointed clearly to the fact that nitrification depends on the presence of living organisms.

The first conclusive proof that such was the case, however, came from the work of Schloësing and Muntz in 1877 (*Comptes Rendus*, 1877, Tome 84, p. 301). The work of these observers rendered it practically certain that living organisms of some kind are the true agents of nitrification. "It now remains for us," they said, "to discover and isolate the nitrifying organisms." Schloësing and Muntz, in their subsequent investigations, believed that they had succeeded in making this discovery; but, in view of the facts of modern bacteriology, we are unfortunately unable to assign much value to this part of their work. It is not easy to satisfy one's self that Schloësing and Muntz ever worked with really "pure cultures" of isolated species. While the work of these investigators established beyond all question the fact that nitrification, like the analogous phenomena of fermentation and putrefaction, is caused by living organisms, it left entirely open the precise nature of these organisms.

The first experiments with species of bacteria isolated by modern methods, and therefore undoubtedly pure cultivations, are those recorded by Heræus (*Zeitschr. für Hygiene*, I., 1886, p. 193). Heræus experimented with fourteen well-known species of bacteria, and with about as many others freshly isolated by himself from water and soil. He cultivated these in an ammoniacal solution, and obtained in the case of several familiar species good qualitative tests for nitrous acid. Among these species were *Bacillus prodigiosus*,

¹ Edwin O. Jordan and Ellen H. Richards, in report on water supply and sewerage to the State Board of Health of Massachusetts. (The series of experiments detailed in this paper were planned and carried out jointly by the authors, the bacteriological portion of the work being done by Mr. Jordan, and the chemical portion by Mrs. Richards.)