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Denitrification and Lime Precipitation by Marine Bacteria

On the Precipitation of Calcium Carbonate in the Sea by Marine Bacteria, and on the Action of Denitrifying Bacteria in Tropical and Temperate Seas. by G. H. Drew

*Journal of Ecology*, Vol. 1, No. 3 (Sep., 1913), pp. 186-189

Published by: [British Ecological Society](#)

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supplied denitrification takes place, especially in light soils. The author's experiments on the bacterial content of three originally similar soils treated differently for three years (unmanured, ammonium sulphate added, sodium nitrate added) lead him to the conclusion that the bacterial differences between such differently treated soils are relatively slight, and that a much more natural expression of the bacterial character of a soil is given by soil experiments than by water-cultures.

#### NITRATE DECOMPOSITION APART FROM BACTERIAL ACTION

**Vogel, I.** "Neue Beobachtungen über das Verhalten von Nitrat im Ackerboden." *Centralbl. f. Bakteriologie*, **34**, Abt. 2, 1912, p. 540.

The author makes the new and interesting observation that nitrates in the soil may undergo decomposition quite apart from the action of denitrifying bacteria. If sodium nitrate is distributed in thin layers of soil containing 15 to 20 per cent. water, active decomposition of the nitrate takes place, the nitrogen loss amounting to as much as 80 or 90 per cent. The decomposition of the nitrate occurred in various different soils, free nitric acid being formed; in the case of soils rich in humus, nitrous acid was also produced, along with oxides of nitrogen. The sodium was combined to form sodium carbonate, leading to material alteration in the physical properties of the soil. This non-bacterial decomposition of nitrate is probably related to colloidal phenomena.

#### DENITRIFICATION AND LIME PRECIPITATION BY MARINE BACTERIA

**Drew, G. H.** "On the precipitation of calcium carbonate in the sea by marine bacteria, and on the action of denitrifying bacteria in tropical and temperate seas." *Journ. Marine Biol. Assoc.*, **9**, 1913, pp. 479—524, 2 figures.

The primary object of the author's investigations was to study the action of marine denitrifying bacteria in tropical and temperate seas; but his discovery that these bacteria also possess the power of precipitating calcium carbonate from soluble salts present in sea-water has perhaps, by its geological significance, somewhat overshadowed the interest of the original object of the work. This paper is of great general ecological interest in many respects, as will be seen from the following summary.

The author's main contentions are as follows (1) That in the seas of the American tropics bacteria exist which are actively precipitating calcium carbonate from dissolved calcium salts. It is suggested that this bacterial action has been a very considerable factor in the formation of chalk and many other varieties of sedimentary rock chiefly, or in part, composed of calcium carbonate. It is also contended that the vast deposits of chalky mud now being formed to the west of the Bahamas, and in the neighbourhood of some of the Florida Keys, are being precipitated by bacterial agency, and that a similar process plays an important part in the cementation of fragments of coral and other detritus into compact coralline rock. (2) That the destruction of nitrates by bacterial action in the seas of the American tropics is far in excess of that occurring in temperate waters. Hence an explanation is afforded of the relative scarcity of plant life (and consequently of animal life) in tropical as compared with temperate seas, in accordance with the terms of Brandt's hypothesis (see below).

It has been found that the plankton of tropical and subtropical seas is far less in quantity than that found in colder waters. The zooplankton depends ultimately for its food on the phytoplankton, hence any factor limiting the growth of the phytoplankton, which was capable of exercising its influence in tropical and not in temperate or arctic waters, might offer an explanation of this phenomenon. It has been shown by various investigators that this factor is not temperature, light, or salinity, and it has been suggested that the explanation may lie in the relative deficiency in tropical seas of the nitrates or nitrogenous compounds which are so

## *Denitrification and Lime Precipitation by Marine Bacteria* 187

essential for all plant life. A matter of common observation in support of this view is the remarkable scarcity of algal growth in the shallow waters of tropical shores as compared with that in temperate regions, and the fact that in the tropics, wherever sewage or other nitrogenous waste is poured into the sea, a free growth of algae is found. Though, in the absence at present of a really reliable and accurate chemical method of estimating the combined nitrogen in sea-water, this theory cannot be directly tested, the existence of denitrifying bacteria in temperate waters has long been known, and it would seem a fair deduction that should this bacterial destruction of nitrates take place with greater intensity and completeness in tropical than in temperate waters, an explanation of the relative scarcity of phytoplankton in the former would be offered.

This suggestion was first made by Brandt ("Ueber die Bedeutung der Stickstoffverbindungen für die Produktion im Meere," *Beih. z. Bot. Centralbl.*, **16**, 1904, pp. 383—402), who argued that if the denitrifying bacteria of the sea, like those of the land, develop a strongly disturbing activity at higher temperatures, only a relatively small production of phytoplankton would take place in the warm seas in spite of much more favourable conditions, according to the law of the minimum, while in the cold seas more nitrogen compounds would be at the disposal of the phytoplankton producers owing to the retardation or suppression of the disturbing process. All the denitrifying species that have been found in temperate seas have a higher temperature optimum than that of their natural environment, and this fact is obviously a point strongly in favour of Brandt's hypothesis. Various recent attempts have been made to correlate quantitative plankton observations with direct analysis of the amount of combined nitrogen in sea-water in different localities, but the limits of error in all methods hitherto used for determining the nitrate content of sea-water are so large as to make them quite unreliable; for instance, Raben (*Wiss. Meeresunters.*, Kiel, **8**, 1905) states that his error in control experiments averaged over 30 per cent. Since chemical methods are at present inadequate to give evidence on this hypothetical deficiency of nitrates in warmer seas, the author's primary object was to investigate the distribution and relative activity of denitrifying bacteria in tropical waters in comparison with those found in temperate seas.

The previous researches most closely related to the author's investigations are those of Gran (*Bergens Mus. Aarb.*, **10**, 1901), who isolated a number of species of denitrifying bacteria from the inshore waters of the coast of Holland. Gran, who made use of solutions of nitrates, nitrites, or ammonium salts as the sole source of nitrogen in his culture media, which contained only a dilute solution of calcium malate as organic nutrient material for the bacteria, classified the bacteria into four groups according to the reactions in pure cultures towards nitrates or nitrites. (1) Those which reduce nitrates and nitrites to free nitrogen without any ammonia formation. (2) Those which readily reduce nitrates to nitrites; the nitrite disappears slowly without perceptible formation of free nitrogen, and some ammonia is formed. (3) Those which cannot reduce nitrates to nitrites, but which are capable of slowly removing the nitrate without any perceptible formation of free nitrogen; though the nitrites are not reduced, yet they can serve as the sole source of nitrogen for the growth of the bacteria. (4) Those which cannot reduce and are not capable of assimilating either nitrates or nitrites, but will flourish when ammonium salts are present.

In samples of water taken in the English Channel some ten miles off Plymouth, the author recognised species belonging to Group 2 of Gran's classification, but could not detect the presence of species belonging to any of the other groups, which are probably composed chiefly of littoral forms. In fluid culture media inoculated with sea-water samples and kept at 28° C., Gran found that the formation of nitrite was detectable in from one to two days, and that eventually all the nitrate and nitrite were destroyed in the majority of cases, especially if the cultures were reinoculated at intervals. The author obtained similar results in cultures kept at 30° C. after eight days; in cultures kept at 15° C. the first formation of nitrite was detected in from five to six days but denitrification never proceeded beyond this stage. Baur (*Wiss. Meeresunters.*, Kiel, **6**, 1901) showed that the optimum temperature for growth and denitrification of the species described by him lay between 20° and 25° C., when the bacteria were grown in fluid culture media containing peptone.

The author next considers the origin of the nitrate supply of the sea. Nitrates are absorbed by diatoms and the phytoplankton in general and built up into complex nitrogenous compounds

within the plant. If these compounds, on the death of the plant, are broken up and the nitrogen again rendered available for use in the form of nitrates, a series of reactions must be gone through which may well be performed by bacterial agency, and this also applies to the waste nitrogenous products of animal metabolism. In addition, it has been shown that nitrates are actually decomposed by the denitrifying bacteria, which would thus tend to keep the nitrate concentration down to the level necessary for their own existence, and would come into competition for this essential with other forms of plant life. If the bacteria are successful in decomposing nitrates to the extent of entirely removing the nitrogen from all chemical combination, as seems probable from the experiments in cultures, it follows that there must be some source of nitrates in order that the concentration in the sea may remain constant. The existence of nitrifying bacteria, capable of absorbing and combining with the free nitrogen of the air and eventually giving rise to nitrates, has been shown by several observers, but these bacteria have so far only been found on the bottom close to the shore, or apparently living in symbiosis with algae or plankton organisms. On the bottom of inshore waters, also, there have been found bacteria capable of forming nitrites from ammonium salts and others which can convert nitrites into nitrates. It is possible that similar bacteria having a nitrifying action remain to be discovered in the open sea.

The precipitation of calcium carbonate in the sea by bacterial agency is apparently a line of investigation that has not been previously suggested or followed. Both Baur and Gran made use of calcium salts in their culture solutions in order to obviate the great increase in alkalinity that resulted if potassium or sodium salts were used, but they have not called attention to, or apparently realised, the probable significance of this precipitation of calcium carbonate by bacterial agency as an important factor in the formation of various sedimentary calcareous rocks in tropical seas. Murray and others ascribe the precipitation of calcium carbonate in sea-water to the interaction of ammonium carbonate, derived as an ultimate product of the decomposition of nitrogenous organic matter, with the calcium sulphate present in sea-water. Though this reaction has been shown conclusively to occur under experimental conditions, where nitrogenous organic matter has been allowed to putrefy for some time in sea-water, its effect must be purely local and confined to the immediate neighbourhood of the decaying organic body which gives rise to the formation of ammonium carbonate.

After describing in detail the apparatus used in the collection and cultivation of samples of sea-water, the author reports on the results obtained in various localities. In samples taken off Port Royal, Jamaica, it was found that the rate of denitrification was always more rapid in cultures taken from a depth of 3 or 6 fathoms than from the surface, and was also more rapid in the thick muddy water of a mangrove swamp where organic matter was abundant. Even under similar temperature conditions the marine bacteria in the tropical seas off Jamaica, the Bahamas, etc., are much more active in causing denitrification than those found in the English Channel and the Bay of Biscay, and since the rate of denitrification is a function of the temperature, it follows all the more that the destruction of nitrates by bacterial agency in the tropical seas must be far in excess of that occurring in the cooler waters of the temperate regions.

The remainder of the paper is largely occupied by a description of observations and experiments which go to show that the extensive deposits of fine unorganised calcareous matter, forming chalky mud-flats near the Bahamas and the Florida Keys, are now being precipitated by the action of the newly discovered organism *Bacterium calcis* on the calcium salts present in solution in sea-water. Hence the suggestion is obvious that the *Bacterium calcis*, or other bacteria having a similar action, may have been an important factor in the formation of various chalk strata, in addition to the part played by the shells of foraminifera and other organisms in the formation of these rocks, while oolitic rocks may owe their origin to the occurrence of some diagenetic change in the precipitate of finely divided calcium carbonate particles produced in this way by bacterial action. If this view as to the formation of chalk and oolite rocks is correct, it would seem probable that these strata must have been deposited in comparatively shallow seas, whose temperature approximated to that of tropical seas at the present time.

Details are given of the bacterial investigation of the chalky mud-flats near Andros Island, British West Indies, samples of which were shaken up with sterilised sea-water and the diluted fluid plated in peptone agar. The bacteria found in these cultures were nearly all *B. calcis*, calculation showing that the surface mud itself must contain at least 160 millions of bacteria

## *Denitrification and Lime Precipitation by Marine Bacteria* 189

per 1 c.c. Subcultures of *B. caldis* were made in various media, in all of which denitrification was rapid and eventually complete and was accompanied by the precipitation of calcium carbonate; in some cases relatively large particles were formed, and a sediment similar in appearance to the chalky mud of the mud-flats was produced. Examination of the precipitates showed the invariable presence, in addition to calcium carbonate, of small quantities of hydrated calcium sulphate. This sulphate is obviously derived from that in solution in the sea-water itself, but no such precipitation occurred in culture media kept uninoculated under similar conditions as control experiments. Hence it would appear that this deposition of calcium sulphate, along with the carbonate, must in some indirect way be the result of bacterial action, and the author suggests that the odour of sulphuretted hydrogen noticeable in the deeper layers of the mud-flats may be due to the reduction of the calcium sulphate to a sulphide and subsequent decomposition of the sulphide by bacterial action.

In conclusion the author points out that the investigation can at most be considered to offer a mere indication of the part played by bacterial growth in the metabolism of the sea. To obtain a real insight into the question it would be necessary to make more extensive bacterial and chemical observations in tropical, temperate and arctic waters, to study the bacteriology of other areas where calcium carbonate is being precipitated from the sea, and to make further investigations in the laboratory into the chemistry of the reactions that can be brought about by various species of marine bacteria.

It is greatly to be hoped that other investigators will take up the extremely interesting line of work opened up by the researches of the author, who unfortunately died suddenly before the paper itself was published.

### METABOLISM OF DECIDUOUS TREES

- (I) **Bauer, H.** "Zur Periodizität der Stoffbildung und Nährstoffaufnahme in jungen Laubhölzern." *Naturwiss. Zeitschr. Forst- und Landwirtschaft*, **10**, 1912, p. 188.
- (II) **Ramann, E.** "Die Wanderungen der Mineralstoffe beim herbstlichen Absterben der Blätter." *Landw. Versuchsstat.*, **76**, 1912, pp. 157—164.
- (III) ——— "Mineralstoff-Wanderungen beim Erfrieren von Baumblättern." *Ibid.*, pp. 165—168.

(I) The author has made a series of further observations on the metabolism of one-year-old oak trees, in continuation of the work on variations in dry weight, etc., of trees already carried on by him independently (*Nat. Zeitschr. Forst- u. Landw.*, 1910, pp. 457—498; *Ibid.*, 1911, pp. 409—419) and in conjunction with Ramann (*Jahrb. f. wiss. Bot.*, **50**, 1911). These researches showed that during the activity following the spring awakening of trees, while the leaves are unfolding, the great expenditure of energy is reflected in the fact that deciduous trees lose from 20 to 45 per cent. in total dry weight, and that during this period practically no nitrogen is absorbed; also that the time of maximum absorption of nitrogen, potassium, calcium, magnesium, and phosphorus varies from species to species, while for the same species the different elements are absorbed at different times and at independently varying rates. Results of this kind, showing the importance of the regulation of mineral supply by selective root-absorption, have obvious ecological bearings, and may help to explain the association of certain trees in woodland formations.

In this paper the author gives the results of his investigations on the periodicity of assimilation and mineral absorption during four periods in the life of the young oak sapling:—(1) from March 15 to May 24—70 days; (2) from May 24 to June 25—32 days; (3) from June 25 to July 31—36 days; (4) from July 31 to September 19—50 days. During period 1, there was practically no assimilation and only a very slight uptake of mineral salts. Period 2 is marked by the maximum absorption of magnesia, while assimilation approaches its culmination. In period 3, if no second burst of-growth (formation of "Johannistriebe" or "midsummer shoots")