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Brief communication

MICROBIAL FORMATION OF NITRITE IN VENTILATING SHAFTS

Cases have been described of fatal nitrite poisoning in swine resulting from the intake of condensation water from ventilating shafts (*Andersen* 1962, *Olsson & Rosberg* 1964).

Formation of nitrite, through oxidation of NH_3 present in the outflowing air, may take place in the accumulation of the deposits on the inside surfaces of the ventilating shafts. *Karlog* (1966) found that the rate of nitrite formation was independent of the kind of material used for construction of the shafts, and therefore considered the oxidation of NH_3 to be brought about by microbial activity rather than by direct catalysis.

In February 1967, the ventilation systems in 23 pighouses were examined and 26 samples were taken from inside deposits for bacteriological examination and for determination of nitrite content (*Rider & Mellon* 1946) and pH.

In well-ventilated houses the deposits were dry and could be seen to consist mainly of fine cereal dust. The nitrite content in deposits of this kind varied from 0.03 to 0.09 mg/g, the pH from 7.2 to 7.7.

In poorly ventilated houses the deposits were moist and mouldering, with a nitrite content varying from 0.1 to 4.9 mg/g and a pH varying from 8.0 to 8.5.

From each of 16 samples, 1 g was inoculated into flasks with 150 ml of a salt solution for cultivation of autotrophic bacteria, with NH_4Cl as a source of nitrogen and energy, and $CaCO_3$ granules as a source of carbon (*Stainer et al.* 1958). After adjustment of the pH to 8.5 the flasks were placed in a mechanical shaker and incubated at 28°C.

In 2 of the 16 cultures no nitrite was formed after 40 days. In 9 cultures a slight formation of nitrite was demonstrable after 1—2 weeks. After 6 weeks, however, the nitrite content was still very low in these cultures, which were therefore discarded.

In the remaining 5 cultures, nitrite contents of from 0.34 to 0.67 mg/ml were found after 4 weeks.

Fresh cultures from the 5 samples in question, prepared in the same medium and incubated under the same conditions as

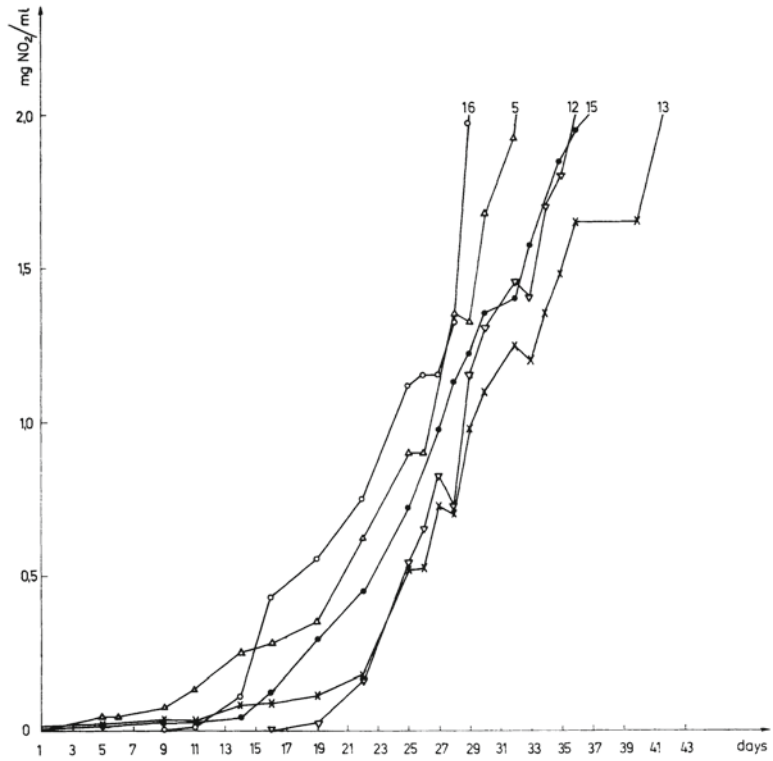

Figure 1. Formation of nitrite in 5 cultures of Nitrosomonas.

before were examined for nitrite daily or at frequent intervals, and maintained at a pH of 8.5.

After a lag period of 4—14 days, nitrite was formed in all of the 5 cultures. As appears from Fig. 1, the accumulation of nitrite, expressed as a function of time, followed a curve analogous to the growth curve of a bacterial culture.

From 1 of the positive samples, a further 4 cultures were made, 2 of which were heated to 95° and 127°C respectively before being incubated. All 4 cultures were constantly aerated during incubation and kept at a pH of 8.5. Considerable amounts of nitrite were formed within 2 weeks in the 2 unheated cultures, but not in the 2 that had been heated.

After some failures, nitrite formation was obtained also in a medium free of insoluble constituents (*Engel & Alexander* 1958). After incubation for 36 days and repated adjustment of the pH to 8.0 by means of K_2CO_3, there was a faint turbidity and a nitrite

Figure 2. Electron-micrograph showing Nitrosomonas europaea in division, amphitrichous rod and slender atrichous rod. 13800 ×.

content of 0.87 mg/ml. Electron microscopy of deposit from this culture showed a large number of slender amphitrichous rods and a lesser number of non-ciliated ovoid rods. The latter were ab. 1.6 μ by 1.3 μ. Deposit from a 39-day-old, distinctly turbid subculture with a nitrite content of 13.6 mg/ml consisted mainly of such ovoid rods. These rods were Gram-negative. In the same deposit there were also a few slender, Gram-negative rods and a very few slender, granular staining, Gram-positive rods.

The large, ovoid, Gram-negative rods conform to the description of Nitrosomonas europaea, which in the general nitrogen cycle in soil metabolizes NH_3 to nitrite.

Nitrite which is toxic to most plants and microorganisms, under most conditions is immediately further metabolized to nitrate by Nitrobacter spp., but under certain circumstances, viz., a high pH and a high concentration of NH_3, this process may be blocked (*Alexander* 1961).

The present observations thus give evidence to suggest that the nitrite present in deposits on the inside of many ventilating shafts is produced by Nitrosomonas spp., and that the NH_3, which forms the substrate for its production in excess, also is preventing it from being further metabolized to nitrate. The final proof hereof must await further research, however.

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