Nitrogen Gas Production by the Reaction of Nitrites with Amino Acids in Slightly Acidic Media

FRANKLIN E. ALLISON and JANET DOETSCH

Three possible methods of loss of nitrogen in gaseous forms from soils and other media have been emphasized. These are (a) by volatilization of compounds such as ammonia, nicotine (from plants), and possibly oxides of nitrogen; (b) by formation of elemental nitrogen through the action of denitrifying bacteria; and (c) by production of nitrogen gas as a result of the interaction of nitrous acid with amino acids or ammonia. The present paper deals with the last of these methods.

The reaction of nitrous acid with amino acids or ammonia is a strictly chemical one but the reactants are formed in Nature chiefly by biological agencies. This reaction, which is utilized in the Van Slyke apparatus for the quantitative determination of amino acids, is as follows:

\[ \text{RNH}_2 + \text{HNO}_2 \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{N}_2 \]

It will be noted that the nitrogen gas is formed in equal amounts from the reduced and the oxidized forms of nitrogen present.

Wilson (7) and Allison and Sterling (2) have recently discussed this reaction from the standpoint of its possible occurrence in soils and plants. In previous work (1, 2) at this laboratory, using cultures of Chlorella and Lemma and excised leaves of several plants, there was little evidence that the reaction occurs in cultures or in the sap of green plants. Other studies in soils (6) maintained under anaerobic conditions, and in cultures of rhizobia (3), also gave negative results. Wilson (7), on the other hand, concluded that the reaction apparently occurs throughout Nature and is limited in scope primarily by the small quantity of nitrite present. He points out that an organism may merely reduce nitrates to nitrites under one set of conditions, but in the presence of suitable substances with which the nitrite can react nitrogen gas may be formed. From studies with excised tobacco shoots Dawson (4) recently concluded that the data support but do not prove that nitrogen may be lost from plants as a consequence of this reaction.

The chemical experiments reported below were performed for the purpose of determining the extent to which nitrogen gas can be formed under conditions somewhat similar to those found in Nature. It was necessary, however, to use higher concentrations of reacting substances, especially of nitrite, than normal soil concentrations for the obvious reason that nitrite is seldom present in acid soils in more than traces. The concentration used experimentally was one that was adequate to give quantities of gas that could be measured manometrically with a fair degree of accuracy.

All determinations of gas production were made in respiration vessels. Two procedures were used, namely direct and a direct method. These two methods and the results obtained will be discussed separately.

Results obtained by the indirect method.—The procedure followed was to add a phosphate buffer to a nitrite solution, with the presence and absence of alanine and observe the manometric changes taking place in separate vessels, both of which contained air. In practice the buffer and alanine were added from separate side arms after the vessels had come to equilibrium in the water bath. The final volume of gas in the vessels was 4 ml. The buffers, consisting of phosphoric acid and its potassium salts, were usually added in such amounts as to make the final concentration of each 0.2%. The sodium nitrite concentration was 0.05%. To oxidize the nitrite to nitrate, such amounts were added to require 524 cm of \( \text{O}_2 \) per respiration vessel. Two grams of alanine was used as needed to react with the nitrite to form \( \text{N}_2 \). In a separate experiment it was determined that the amino acid reacts readily and quantitatively with nitrous acid under highly acid conditions occurring in the Van Slyke apparatus. When an experiment as outlined above is performed, the manometric changes recorded where no alanine is present represent the uptake of oxygen as the NO formed from sodium nitrite in acid solution, is oxidized to nitrate. Ultimately, all of the NO so formed is converted to \( \text{N}_2 \) and removed from the gaseous phase.

When this experiment is performed in the presence of an amino acid a second reaction is possible, namely the oxidation of the nitrogen of both the nitrous acid and the amino acid that reacts with it to free nitrogen gas as mentioned above. If this proceeds then a positive manometric reading is observed for each mole of reacting nitrite. Readings intermediate between the two extremes (negative due to \( \text{N}_2 \) evolution in the one case and positive due to \( \text{N}_2 \) evolution in the other) may be interpreted to show the extent to which the two reactions proceed with the amino acid. Results given below are in agreement with this interpretation.

The decreases in gas volume equivalent to the manometric changes observed in the presence and absence of alanine at pH 2.2 are shown in Fig. 1. It is observed that approximately half of the added nitrite was decomposed and oxidized to nitrate in 1 hour. After 6 hours 81% of the nitrite had been oxidized. When alanine was added, together with sodium nitrite, the decrease in gas volume was less than with nitrite alone, showing that some of the two materials reacted to form nitrogen gas.

Experiments similar to those reported in Fig. 1 were performed at various pH values over the range 2.2 to 6.0. The curves for the data obtained at pH 6.0 for a 24-hour period are shown in Fig. 2. At this pH the percentage of nitrite decomposed and oxidized was much less than at pH 2.2.