

THE CHEMICAL FIXATION OF NITROGEN AT LOW TEMPERATURE AND ITS SIGNIFICANCE IN AGRICULTURE.

By B. RAMAMOORTHY, M.Sc.,

Post-Graduate Research Student, Imperial Agricultural Research Institute,
New Delhi.

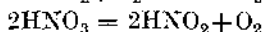
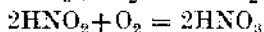
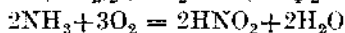
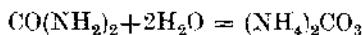
(Communicated by Rao Bahadur B. Viswa Nath.)

(Read November 6, 1937.)

Work on nitrogen transformations in the soil has established three facts.

1. Fixation of atmospheric nitrogen following the oxidation of carbohydrate materials.
2. Oxidation of ammonium compounds to nitrates.
3. Loss of nitrogen.

The oxidative processes which are believed to be mainly brought about by micro-organisms may be represented by the following main reactions. Starting with urea the degradation product of proteins :—



Energy relations are important considerations in the process and the micro-organisms concerned utilize carbohydrates and proteins of the organic matter present in the soil as sources of energy.

Recently Dhar and his associates (Influence of light on some Biochemical Processes, *Society of Biological Chemists, India*, 1935) have put forward the view that light energy is concerned in the process of nitrification and that the process is partly biological and partly photo-chemical. This view is corroborated by Corbet, (*Biochem. Jour.*, 28, 1934) and others, while Fraps and Sturges (*Soil. Sci.*, 1934), Joshi (*Sci. Reports, Imp. Agri. Res. Inst.*, 1934-35) and others deny photo-chemical nitrification. Joshi on the other hand observed decomposition of nitrates in light.

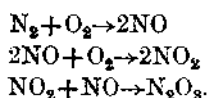
Viswa Nath (*Presidential Address, Agric. Section, Indian Science Congress*, 1937) has drawn attention to the apparently conflicting processes of oxidation of ammonia and losses and gains of nitrogen occurring in the soil, and suggested a re-examination of the whole problem, particularly in regard to the significance of nitrogen loss which appears to be followed by nitrogen fixation. In the course of studies on soil nitrogen which are in progress at the Imperial Agricultural

Research Institute, it occurred to the author that the chemical energy of oxidation might in some way be concerned in the process.

In reviewing the literature on chemical chain kinetics especially phosphorous oxidation first studied by Van Marum (*Ann. Chim. Phys.*, 1799) up to Melville (*Trans. Faraday Soc.*, 28, 2, 815 ; 1932), it is now established that Phosphorous oxidation is definitely propagated by energy chains and by collision with other molecules these chains are broken. The effect of various gases in changing the critical pressure limits has been thoroughly investigated by others. But the question as to what happens to the energy of these chains in a deactivating collision with another molecule has not been touched upon so far.

Experimental.—Shönbein (Poggendorf's *Annalen*, 1845, 65, 69) first recorded the formation of hydrogen peroxide and ozone when moist P is oxidized in air. Russel (*J.C.S.*, 33, p. 1263 ; 1903) studied oxidation of moist phosphorous and mentioned that, in addition to ozone and H_2O_2 , ammonium nitrite and nitrate are also formed, but he has not given experimental evidence in support of this statement.

Experiments with phosphorous.—5 gms. of phosphorous were placed in a bulb and air which was first purified by passing through two bottles each of sodium hydroxide, sulphuric acid and 5% solution of ferrous sulphate in dilute H_2SO_4 , was passed slowly over the phosphorous in the bulb and the products of reaction were caught in 2N solution of sodium hydroxide. There was a side feeder of purified air joining the products of reaction in their passage from the bulb to the absorption vessels. The sodium nitrite formed was estimated colorimetrically with Lunge's modification of Greiss-Illoway reagent. The oxidation may be represented as—



The rate of passage of air and the amount of moisture present appear to influence the yields. The yields are more with slower oxidation and greater moisture in the system. Typical results of several experiments are given below :—

Nature of experiments.	Mgm. N as N_2O_3 .
1. Very rapid flow of air	Nil.
2. At 150 bubbles of dry air per 2 minutes 40 seconds	0.01
3. At 150 bubbles of moist air per 2 minutes 40 seconds over phosphorous	0.02-0.03
4. At 150 bubbles of moist air per 2 minutes 40 seconds over moist phosphorous	0.04-0.05

Experiments with tin and magnesium.—The air feeder being out off, and when foils of metals, tin and magnesium were exposed to the issuing products

of the above reaction, the exposed surface of the metal was completely covered with the nitride.

When 0.8 gm. of tin was used in the experiment, 0.6 mgm. of nitrogen was fixed. If more surface could be exposed more nitrogen would probably have been fixed. The nitride of metals was also detected in the oxidation of potassium, but the rapid formation of a protective layer of the peroxide prevented further study.

Experiments with cane sugar.—When the products of phosphorous oxidation are passed over moistened Merck's extra pure cane sugar with the side feeder of air, 0.25 ± 0.01 mgm. of nitrogen was fixed.

Thus at ordinary air temperature the N_2 present in an oxidation system is capable of becoming active and combining with metals, oxygen and other substances as the case may be.

The next experiment was carried out with Merck's cane sugar oxidation instead of phosphorous, but in this case at high temperatures ($50^\circ C.$ and 96° – $98^\circ C.$) by passing a current of purified air for 48 to 72 hours. Large quantities of ammonia were detected with Nessler reagent as against blanks. The results of the experiments using 4 gms. of moist sugar spread in a U tube at $98^\circ C.$ were :—

No. of hours.	Mgm. of nitrogen as NH_3 .	Mgm. of nitrogen as N_2O_5 .
65	0.06	0.07
65	0.05	0.08
68	0.075	0.084

These results with cane sugar are in conformity with the findings of Dhar and his associates (*Proc. Nat. Acad. Sci., Allahabad*, 19th Dec., 1935), the only difference being that the influence of light, micro-organisms and catalysts (particularly ferrous iron itself in the oxidation of which ammonium salts were reported by Russel, 1903) is eliminated and that the nitrogen fixed is related simply to oxidation of sugar only. N_2O_5 was also detected with the Greiss reagent. Further work is in progress on the quantitative relationship between carbon oxidized and nitrogen fixed merely as the result of chemical oxidation.

An experiment on the reciprocal process of carbon fixation resulting from ammonia oxidation in unsterilized soil has given a result of 50 ± 4.5 mgm. of carbon fixation but this needs further verification.

These experiments point to the possibility of oxidation and reduction processes in biological systems by direct energy transfers. Apart from their theoretical interest in explaining some gaps in our existing knowledge, they are of considerable practical importance to agriculture. Considering for example the work on carbon assimilation *in vitro*, Baly (*Proc. Roy. Soc. A*, 116, p. 212; 1927) has worked out the energy relations of photo-synthesis. For a

single gram molecule of hexose sugar formed from CO_2 , 673,800 calories are at least required, while the living leaf achieves this by the absorption of 263,000 calories even, under the influence of red light. The question of absorbing more quanta is improbable (Kohn, *Nature*, 137, p. 706 ; 1936). This difference may at least be accounted for partly if the energy of simultaneous oxidative processes is taken into consideration. It may explain the Blackman reaction in a new fashion (Emerson, *Ann. Rev. Biochem.*, 1937, p. 538) and also the absolute necessity of O_2 for photo-synthesis emphasized by Willstätter and Stoll ('*Untersuchungen über die Assimilation*', Berlin, 1918, p. 348), the significance of the internal factors other than the chlorophyll content and the not always convincing results of photo-synthesis in vitro of several workers (Spoehr, *Ann. Rev. Biochem.*, 1933, p. 463) by merely exposing carbon dioxide and water without providing for an additional source of oxidation energy, just as, for example, ammonia oxidation is used by some micro-organisms for carbon fixation.

In regard to nitrogen fixation in soils, the fixation through chemical oxidation may explain nitrogen fixation in the hot months of May, June and July (Bal, *Proc. Nat. Inst. Sci. India*, 3, p. 57 ; 1937) ; it may also explain the beneficial advantages of moderate ploughing of the soil, although Leather (*Mem. Dep. Agri. India, Chem. Series*, 14, No. 3, p. 134 ; 1915) has shown that gaseous exchange occurs in soils to a depth of 1 ft. It suggests also a new orientation to the problem of nitrogen conservation from the point of view of the conditions necessary for an effective transfer of chemical energy by means of collision between molecules of nitrogen and the energy source.

I take this opportunity to record my deep sense of gratitude to Rao Bahadur B. Viswa Nath, F.I.C., F.N.I., for his great help and valuable guidance in the entire course of this work.