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CHEMICAL FACTORS IN DENITRIFICATION.

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Gilbert J. Fowler and Y. N. Kotwal.

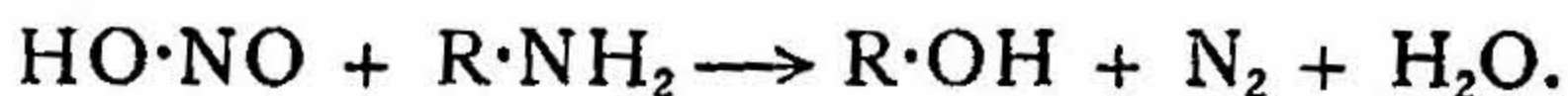
CHEMICAL FACTORS IN DENITRIFICATION.

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INTRODUCTION.

In studying the possible losses of nitrogen incurred by nitrogenous manure during (a) storage, (b) incorporation with the soil, or (c) operations concerned with sewage purification, those arising from purely chemical reactions and resulting in the production of free nitrogen require careful examination.

Ammonium nitrite itself is an unstable substance and nitrous acid reacts easily with urea and with amino-acids, under ordinary laboratory conditions, with elimination of nitrogen according to the well-known general equation:—



It has been commonly assumed¹ that the amino-compounds formed by the natural decomposition of proteins do actually react with nitrous acid produced during the nitrification of the ammonium compounds also present.

A search through the voluminous literature concerned with denitrification changes shows that published researches have been almost exclusively concerned with the changes occurring in presence of bacteria and that the possible purely chemical factors have received little or no attention. Thus it is an assumption to conclude that reactions taking place in comparatively strong solutions and in presence of mineral acids will necessarily occur under conditions obtaining in agricultural or sewage disposal practice, when the solutions are much more dilute, and the reaction of the medium is either alkaline or, if acid, the acidity is due to the presence of organic or other 'weak' acids.

The object of the experiments described in the present paper was to discover whether such elimination of nitrogen occurs under conditions comparable with those encountered in practice. For this purpose in the first place dilute solutions of ammonium nitrite were

¹ Fowler, *Bacteriological and Enzyme Chemistry*, p. 230. The Conservation of Nitrogen, *This Journal*, Vol. 3, Part viii. p. 246.

Dilling and Pearse, *Report on Industrial Wastes from Stock-yards and Packingtown in Chicago*, p. 154.

Haigh Johnson, *Annual Report Sewage Works Managers Association*, 1922, p. 58.

employed, prepared by the interaction of ammonium chloride and silver nitrite, the solution being afterwards standardised by permanganate. Solutions of ammonium nitrite of concentrations varying from 0.15 to 0.0015 per cent. or from 32.8 to 0.328 parts of nitrous nitrogen per 100,000 were thus prepared, even the weaker solution containing more nitrous nitrogen than is generally found in sewage effluents.

The stability of the solutions was tested by (1) Heating to various temperatures alone. (2) Heating in presence of carbon dioxide, either combined as bicarbonate or in the gaseous state. (3) Interaction with urea and various acids, mineral and organic.

A further set of experiments was conducted to reveal the interaction of hydrogen sulphide, or of sulphides liable to be formed when putrefactive change is taking place, with nitrites produced by oxidation of nitrogenous compounds. A condition of things rendering possible such reactions may readily arise, e.g., in a sewage filter containing pockets of water-logged material in which putrefactive changes predominate and the liquid percolates through this layer from other portions where there is free aeration.

In small pools of sewage or effluent such as are formed on imperfectly drained sewage farms, oxidation may occur on the surface and reduction in contact with the film of sewage mud deposited at the bottom of the pool. In the course of such reduction processes hydroxylamine and hyponitrous acid may also possibly be formed by the action of ferrous compounds on the nitrite present and Dunstan and Dymond¹ have shown that with concentrated solutions, evolution of nitrogen may take place at the same time. Experiments have consequently been undertaken with a view to imitating practical conditions involving such possibilities and determining whether any loss of nitrogen then took place. Incidentally in the course of some of the foregoing experiments an interesting reaction has been noticed between sodium nitrite and hydrogen sulphide which is worthy of further study from the purely chemical point of view.

Finally, trials were made of the stability of dilute solutions of ammonium nitrate when warmed with various oxidisable carbohydrates, etc.

The general result of the work has been to show that under practice conditions, the loss of nitrogen from the purely chemical reactions which might occur during the decomposition and oxidation of nitrogenous organic matter is negligible. These results are in

harmony with those of Russell and Norman Smith,¹ who showed that the corresponding process of nitrification only took place in presence of living agencies.

EXPERIMENTAL.

PREPARATION OF AMMONIUM NITRITE.²

The preparation of ammonium nitrite by the interaction of ammonium chloride and silver nitrite involved the preparation in the first place of silver nitrite. An aqueous solution of ammonium nitrite was prepared by adding ammonium chloride, in the form of a concentrated solution to a cold saturated solution of silver nitrite and removal of the precipitated silver chloride after the addition of a little Fuller's earth. The solution of ammonium nitrite, thus obtained, from a cold saturated solution of silver nitrite does not exceed 0.167 per cent.

ESTIMATION OF NITRITE.

For this purpose, a given volume of the nitrite solution was added to an excess of standard permanganate solution, the mixture acidified by the addition of dilute sulphuric acid (20 c.c.) and the excess of permanganate estimated by titration with ferrous ammonium sulphate solution. This method was devised before our attention was directed to the practically identical method of Blanchard.³ According to him if ammonium salts are present a small amount of these is oxidised at the same time, but the amount is constant so that results are comparable.

EXPERIMENTS ON THE STABILITY OF DILUTE SOLUTIONS OF AMMONIUM NITRITE.

(a) *Effect of temperature.*

Our experiments have been made with concentrations likely to be met with in agricultural or sewage purification practice, and at temperatures between 25° and 50°. The concentrations actually used were :—

1.	0.15	per cent.,	or	32.8	parts nitrous nitrogen per 100,000
2.	0.015	"	or	3.28	" "
3.	0.0015	"	or	0.328	" "

¹ *J. Agric. Sci.*, 1905-6, 1, 444.

² cf. Abegg, 1908, ii, 1, 721. Neogi and Adhikary, *J. Chem. Soc.*, 1911, 99, 116. P. C. Ray, *ibid*, 1909, 95, 345.

³ *Z. physikal. Chem.*, 1902, 41, 692.

Arndt¹ and Blanchard² have studied in some detail the decomposition of fairly concentrated solutions (0.1 to 0.6 molar) of ammonium nitrite at temperatures between 60° and 90° and the latter has shown that in addition to nitrogen, small amounts of nitric oxide are formed, and also that traces of acid have a marked accelerating effect.

The results of our experiments show that solutions containing 0.15, 0.015, and 0.0015 per cent. of ammonium nitrite can be kept for months without any loss of nitrite, and that after 15 minutes at temperatures between 35° and 50° no change could be detected, or even after 5 hours at 50°.

At 96° the more dilute solutions, viz., 0.014 and 0.0014 per cent., showed no change in concentration after 5 hours, but the more concentrated solution, viz., 0.16 per cent., decomposed slowly, as shown by the figures given in Table I.

TABLE I.

Temperature 96°. Original strength of solution 0.16 per cent. ammonium nitrite.

	Period of heating				Final percentage strength	Percentage decomposition.
1 hour	0.15	6.3
2 hours	0.142	11.3
5 hours	0.114	28.8

As the two lower concentrations, corresponding with 3.06 and 0.306 parts of nitrous nitrogen per 100,000, both exceed those commonly occurring in practice, it may be concluded, that dilute solutions of ammonium nitrite are quite stable at temperatures likely to be encountered in practice.

Experiments on the stability of ammonium nitrite in presence of carbon dioxide.

It has been shown by R. B. Moore³ that when carbon dioxide is passed through strong solutions of potassium nitrite, nitrous fumes are liberated.

Our experiments showed that when solutions of ammonium nitrite of the concentration mentioned on p. 31 were mixed with equal

¹ *Z. physikal. Chem.*, 1901, 39, 64.

³ *J. Amer. Chem. Soc.*, 1904, 26, 959.

² *Ibid.*, 1902, 41, 681.

volumes of calcium bicarbonate solutions, no loss of nitrite could be detected after maintaining the mixture for 5 hours at 50°. Similarly no reduction of the amount of nitrite present could be detected by bubbling carbon dioxide through the solutions.

Experiments on the interaction of dilute solutions of nitrites with urea in presence of various acids.

As already explained, the object of these experiments was to ascertain whether any elimination of nitrogen by the interaction of dilute solutions of ammonium nitrite and various amino-compounds resulting from the decomposition of proteins was likely to occur in practice.

The experiments on the interaction of nitrites, urea and various acids were made in an ordinary nitrometer, the following solutions, containing the indicated number of grams per litre, being employed:—(1) Hydrochloric, 36.5 (2) Sulphuric, 49.0 (3) Acetic, 60.0 (4) Amino-acetic, 75.0 (5) Tartaric, 70.0 (6) Metaphosphoric, 80.0; together with saturated solutions of (7) Hippuric, (8) Uric and (9) Carbonic acid.

The general procedure was as follows:—5 c. c. of 0.16 per cent. ammonium nitrite were placed in the nitrometer, then 5 c. c. of 1 per cent. urea and finally 10 c. c. of the acid under examination. The mixture was shaken three times every fifteen minutes and the volume of the gas read at the end of one hour. In the case of carbonic acid 20 c. c. of the saturated solution were used instead of 10 c. c.

The volumes of gas measured in c. c. at N. T. P. evolved after an hour were as follows:—Sulphuric (3.2) hydrochloric (2.7-3.2) metaphosphoric (2.3) and tartaric (1.6), whereas the other acids gave negligible amounts of gas after 1 hour, and even after 18 hours, the amount of gas with acetic and hippuric acids was less than 0.5 c. c. Assuming that all the gas evolved is pure nitrogen, the theoretical yield is 2.8 c. c.

Experiments have also been made on the action of amino-acids, prepared by hydrolysing glue with concentrated hydrochloric acid, on dilute solutions of ammonium nitrite. The amino-acids used liberated large amounts of gas from a 1 per cent. sodium nitrite solution in the presence of free hydrochloric acid, but the same amino-acids, either neutralised or in the presence of free mineral acid, gave no gas when tested with 0.16 per cent. ammonium nitrite solution in a nitrometer.

An experiment on similar lines was tried later in a modified form of apparatus which allowed the use of a greater volume of liquid.

In the apparatus figured, the bottle A can be of any size according to the volume of liquid used and can be filled completely or partially, according to the character of the experiment.

Finally an experiment was made involving 2200 c. c. of effluent from the activated sludge tank; sufficient ammonium nitrite being added to make the concentration of nitrite equivalent to 0.7 parts of nitrous nitrogen per 100,000; 100 c.c. of saturated thymol solution were added to inhibit bacterial activity and 100 c. c. of the neutralised mixture of amino-acids added. The total possible volume of gaseous nitrogen which might be evolved was about 25 c. c. No gas collected during three hours, and on adding 10 c. c. of strong sulphuric acid at the end of this period, only a very few bubbles of gas were evolved.

It would seem therefore that in such dilute solutions as are likely to occur in practice, no appreciable evolution of gas takes place through the interaction of nitrous acid and amino-compounds. Just as ammonium nitrite is stable in dilute solutions, so it would appear are the other possible combinations of the amino-group and nitrous acid.

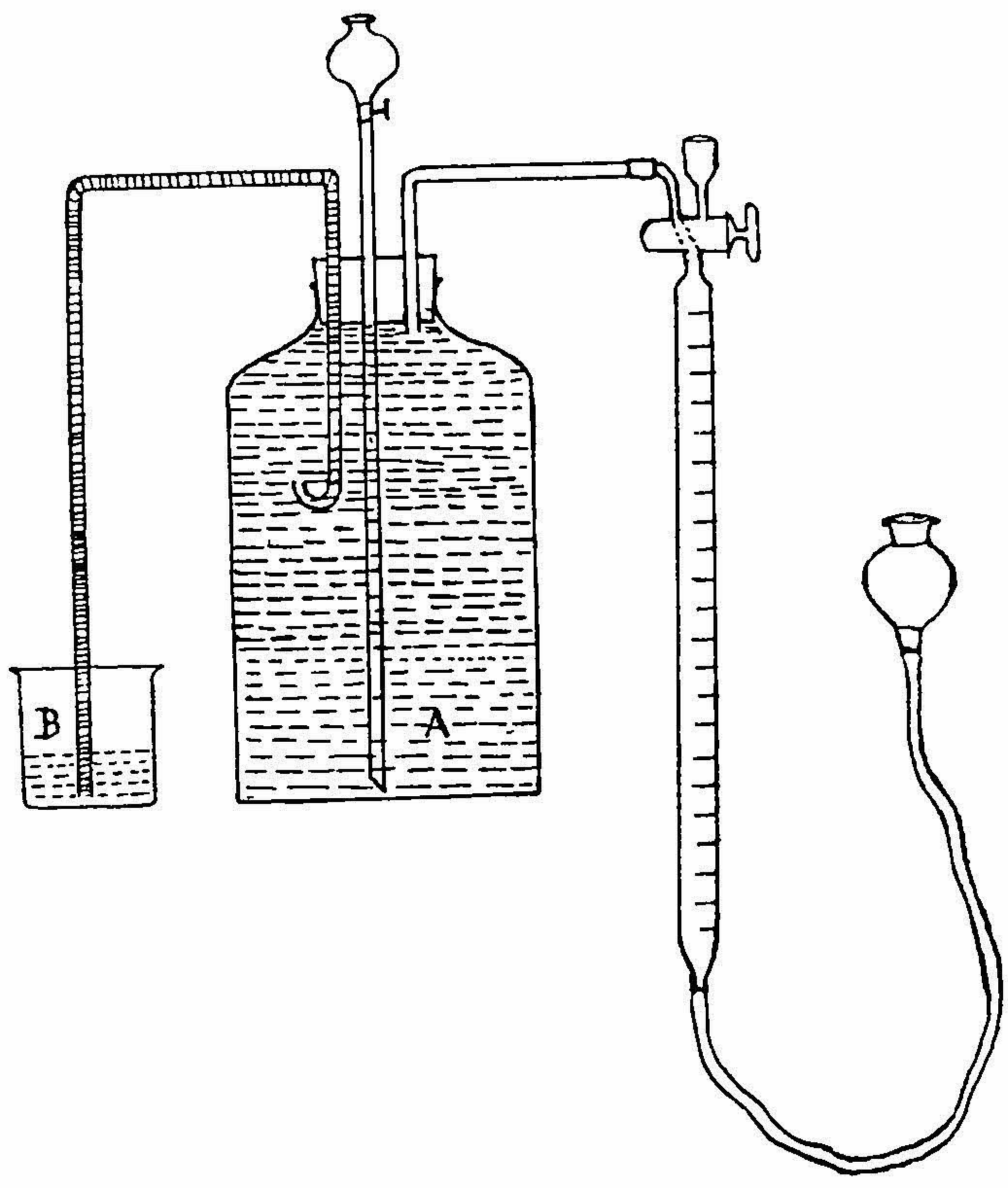
Possibilities of supersaturation.

A natural criticism of some of the foregoing results suggests itself, viz., that the true extent of gas evolution is masked by supersaturation effects. It has, indeed been shown by Lamplough¹ that considerable supersaturation occurs in reactions where gases are evolved, unless the mixture is either well stirred or well shaken. As, however, shaking or stirring was adopted in our experiments the errors due to supersaturation must have been small. This conclusion was confirmed by a number of experiments made directly to investigate this point by adding various inert substances to solutions containing a mixture of urea, nitrite and a mineral acid in suitable concentration, and observing their effect in increasing or diminishing the actual amount of gas given off or its rate of evolution. The greatest difference observed between the blank and the highest evolution of gas was roughly 30 per cent. so that the results do not support the suggestion that the non-evolution of gas in the case of large volumes of solution was due to supersaturation.

Stability of ammonium or sodium nitrite in presence of hydrogen sulphide.

Hydrogen sulphide is not infrequently produced by the ultimate decomposition of the protein matter in sewage, and also by the action

¹ *Proc. Chem. Soc.*, 1906, 22, 280; 1909, 25, 23, 166.



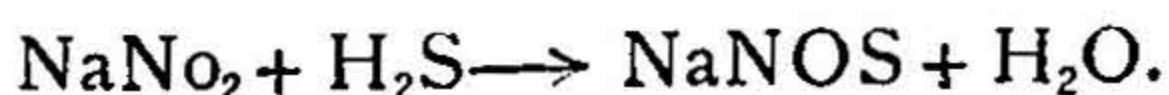
of carbon dioxide on sulphide of iron, which is often formed in sewage filter beds where there is defective aeration. Under certain conditions such sulphide may be oxidised with production of sulphuric acid, in which case there would seem a possibility of loss of nitrogen by the interaction of amino-compounds of nitrites as in the foregoing experiments.

On making the preliminary experiment of passing hydrogen sulphide through a solution of an alkali nitrite, a milky precipitate of sulphur appeared almost immediately and the solution soon became yellow. It was naturally assumed that this was due to a catalytic oxidation of the hydrogen sulphide by traces of oxygen in presence of nitrous acid.

Quantitative experiments, however, showed in the first place that no appreciable loss of nitrite occurred during the reaction. This was proved by removing all sulphide present by means of lead carbonate or cadmium sulphate and determining the nitrite in the solution either colorimetrically by Griess-Ilosvay reagent or by actual titration in the usual manner with permanganate.

With dilute solutions of sodium nitrite, hydrogen sulphide produces an opalescence in presence of air, but the yellow colour appears only after sometime. The opalescence is probably due to the formation of a small amount of sulphur owing to the oxidation of the hydrogen sulphide either by the nitrite, or catalytically by the oxygen of the air in the presence of nitrite. If the former then the oxidation is so small that the concentration of nitrite, as determined colorimetrically, is not affected.

With more concentrated solutions of nitrite the solution becomes deep yellow in colour, and after a time the odour of hydrogen sulphide cannot be detected. This colour may be due to the formation of polysulphides of sodium from the small amounts of sulphur formed, or due to the formation of a thionitrite:—



The yellow colour appears to be readily destroyed by the addition of a drop of a dilute solution of a mineral acid.

A yellow product is also formed together with water, when dry hydrogen sulphide is passed over dry sodium nitrite, but the reaction has to be carried out with care, as violent explosions may occur.

The reaction between sodium nitrite and hydrogen sulphide is worthy of further examination, but as it is clear that hydrogen sulphide is not oxidised to sulphuric acid by the presence of nitrites, no further experiments were made for the purpose of the present inquiry.

Experiments on the possible loss of nitrogen through the reduction of nitrites by ferrous hydroxide.

Wherever sewage is allowed to decompose in presence of soil or other material containing iron there is a tendency for a reducing action to take place and for ferrous compounds to be formed. Thus the black mud at the bottom of sewage-polluted streams, the black mud of the septic tank in fact, and the black patches that are met with in water—logged sewage filters all owe their blackness to the presence of reduced iron either as ferrous sulphide or ferrous hydroxide or a mixture of both.

It has been shown by Dunstan and Dymond¹ that ferrous hydroxide when allowed to act upon solutions of sodium nitrite at the ordinary temperature gives rise to hyponitrite, nitrous oxide, ammonia or free nitrogen according to the conditions which obtain. They also indicate that hydroxylamine is decomposed by ferrous hydroxide. It seemed of interest therefore to study the interaction of dilute solutions of nitrite and ferrous hydroxide either as a precipitate in mass or covering portions of materials such as pumice in such a way as to imitate the conditions sometimes occurring in bacterial filters. As well as ferrous hydroxide, ferrous sulphide was used in some of the later experiments.

The following solutions were used, all being much more dilute than those employed by Dunstan and Dymond, viz., ammonium nitrate (0.17 per cent.) hydroxylamine nitrite (1 per cent.) at laboratory temperature (25° approx.) but no evolution of gas could be detected. For the experiments with ferrous hydroxide in bulk, the apparatus as figured was used.

In order to imitate the conditions occurring in a water-logged filter, pieces of pumice stone were saturated with ferrous hydrate by immersing first in ferrous sulphate and then in caustic soda solution, (the reverse method of effecting the saturation was not satisfactory) and packed in an ordinary drying tower, fitted with a tap funnel and attached to a nitrometer. The air was completely driven out of the apparatus by carbon dioxide and 175 c. c. of 0.17 per cent. ammonium nitrite solution were then allowed to trickle over the saturated pumice. Although this was enough to have produced 100 c. c. of gaseous nitrogen, no appreciable quantity was found to be present after absorbing with caustic soda the carbon dioxide forced by the liquid into the nitrometer.

¹ *J. Chem. Soc.*, 1887, 51, 646.

Experiments on the stability of dilute solution of ammonium nitrate.

A number of experiments were made to inquire whether dilute solutions of ammonium nitrate were reduced on being heated to a temperature above that likely to occur in practice, with oxidisable carbohydrate or other substances which may occur in polluted liquids.

For this purpose the following strengths of ammonium nitrite solution were prepared, viz. 1.0, 0.1, 0.01, and 0.001 per cent. and also solutions of corresponding strength containing glucose and starch.

Three sets of mixtures of these various solutions were made in the following proportions :—

Set No. 1.—ammonium nitrate and glucose 1 : 1

Set No. 2.—ammonium nitrate and starch : 1 : 1

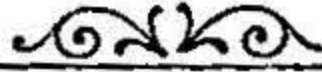
Set No. 3.—ammonium nitrate, starch and glucose
2 : 2 : 1

and heated in closed bottles in the thermostat at 50°, the solutions being carefully tested for nitrites by the Griess-Ilosvay test every hour up to 6 hours. Not even a faint reaction was obtained.

Mixtures of finely divided paper pulp and ammonium nitrate when heated to 50° gave no sign of interaction or nitrite production, even when carbon dioxide was bubbled through the mixture. Moreover, no sign of production of ammonium nitrite or of the oxidation of hydrogen sulphide with deposition of sulphur could be detected in a mixture of ammonium nitrate and hydrogen sulphide after more than 6 hours at room temperature, or with sodium nitrate after 48 hours a striking contrast with the behaviour of hydrogen sulphide and ammonium or sodium nitrite. It would thus appear that the rapid reduction of nitrates and loss of nitrogen which take place when a solution containing nitrates is incubated with septic tank sludge or other similar material, is a bio-chemical, rather than a purely chemical reaction.

GENERAL CONCLUSIONS.

The general conclusion of all the foregoing experiments is that the evolution and consequent loss of gaseous nitrogen taking place in nature, or in the operations of agriculture and sewage purification due to purely chemical causes is negligible, as far as the reactions investigated are concerned. The losses of nitrogen due to bio-chemical changes occur in a great variety of ways and much study has been given to the subject. A great deal of systematic work, however remains to be done if these losses are to be controlled and prevented, and it is hoped that the experiments described in the foregoing pages may clear the way for future bio-chemical research.



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