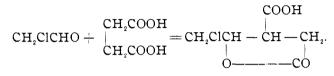
Monochlordiparaconic acid on reduction forms an acid, $C_{9}H_{12}O_{2}$, which I found too unstable for investigation. Considering that these reductions do not lead in the direction of methylparaconic acid and that aldehyde as well as chloral condenses with sodium succinate in the presence of dehydrating agents, it would be of interest to treat similarly the monochlor aldehyde. Possibly the following change might result:



This monochlormethylparaconic acid, which I believe has never been prepared, might reduce to methylparaconic acid and yet the tendency of these compounds seems to be to double the molecular weight on giving up chlorine. On account of the extreme complexity of these changes and the instability of resulting compounds, as well as the great length of time necessary to prepare material, very little progress can be made unless one is willing to devote a year to the queston as it now stands.

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[CONTRIBUTION FROM THE NORTH CAROLINA EXPERIMENT STATION.] NITRIFICATION IN DIFFERENT SOILS.¹

> BY W. A. WITHERS AND G. S. FRAPS. Received February 22, 1902.

In a previous article² the authors communicated some work on the rate of nitrification of some fertilizers in a pasture soil, in which it was found that the percentages of nitrogen in them nitrified in a definite time corresponded with their availability as measured by vegetation tests, with one exception. This exception was ammonium sulphate, which was nitrified to a very small extent, although it has a high availability.

Inasmuch as the prevailing opinion in regard to the process of nitrification is that organic nitrogen is first converted into am-

¹ An account of this work was read before the Association of Official Agricultural Chemists in November, 1901.

² This Journal, **23**, 318 (1901).

monium salts, then into nitrites, then to nitrates, it seemed strange that ammonium sulphate should possess a low rate of nitrification. Results of experiments by other workers were cited, in one of which ammonium sulphate was nitrified to a greater extent than dried blood, in the other to a less extent.

The explanation we then put forward to account for the unexpected behavior of ammonium sulphate is as follows:

1. Ammonium sulphate may hinder the action of the nitrifying organisms. This explanation does not account for the beneficial action of calcium carbonate.

2. The nitric and sulphuric acids formed by the action of the nitrifying organisms are detrimental to them, being neutralized only in part by the bases of the soil. This does not explain why a soil should nitrify dried blood more rapidly than ammonium sulphate, even when calcium carbonate is added.

3. Different soils contain different nitrifying organisms, some of which convert organic matter directly to nitrites, while others change ammonium salts to nitrites more readily.

All three of the above causes may be in operation. The work to be described is a continuation of that mentioned above, and was designed to test the hypotheses then put forward.

PLAN OF WORK.

The work was confined to a study of the comparative rate of nitrification of ammonium sulphate and cottonseed meal in different soils, both with and without the addition of calcium carbonate. The soils were obtained from different sections of the country and varied widely in their composition and properties.

The procedure was that described in the paper already referred to. The sample of soil was sifted through a coarse sieve, and the quantity of cottonseed meal or ammonium sulphate, containing 0.3 gram nitrogen, was intimately mixed with 500 grams of it. In some cases, additional tests were made with half this quantity of nitrogen. The mixture was placed in precipitating jars, and kept in a dark closet. When calcium carbonate was added, the amount taken was exactly sufficient to neutralize all the nitricand sulphuric acids which would be produced if the ammonium sulphate were completely nitrified. The same quantity was used for the cottonseed meal. The amount of water in the soils was about 15 per cent.; at suitable periods, one or more jars in each set was weighed, and the estimated loss of moisture was replaced in all the jars. At the end of three weeks (a little longer in some cases), the nitrates were leached out, and determined by the Tiemann-Schulze method, correction being made for the nitrates formed in the soil to which no fertilizer had been added.

DESCRIPTION OF SOILS.

1667. Pasture soil from the farm of this college. A light loam, containing humus and not acid to litmus.

1668. Heavy clay soil from college farm. Contains very little humus, possesses low fertility, and is slightly acid to litmus.

1669. Black garden soil from Florida Experiment Station. Contains much humus and is acid to litmus.

1670. Soil from the Massachusetts Hatch Experiment Station. This soil is from a plot in field C, fertilized with ammonium sulphate, muriate of potash, dissolved bone-black, and stable manure since 1891. This is the plot giving the least satisfactory growth, especially upon lettuce, beets, spinach and onions. It contained little humus and was acid to litmus.

1674. Soil from Rhode Island Experiment Station, used in plot experiments. Slightly acid to litmus, contains humus.

1675. Sandy soil from the Red Springs test farm of the North Carolina Department of Agriculture. Contains little humus.

1676. Soil from plot 23, Rhode Island Experiment Station, fertilized with ammonium sulphate. This soil, and also 1677, 1678, and 1679, is from a series of plots which have been in use for some time to test the effect of lime on an acid upland soil.

1677. Soil from plot 25, fertilized with ammonium sulphate and lime.

1678. Soil from plot 27, fertilized with sodium nitrate.

1679. Soil from plot 29, fertilized with sodium nitrate and lime.

1680. Sandy soil from Tarboro test farm of the North Carolina Department of Agriculture.

Acknowledgment is hereby made to Director H. J. Wheeler, of Rhode Island, Chemist H. K. Miller, of Florida, Agriculturalist W. P. Brooks, of Massachusetts, and State Chemist B. W. Kilgore, of North Carolina, for their kindness in furnishing samples of soil.

EXTENT OF NITRIFICATION.

The table contains the result of the nitrification tests, expressed in percentage of the total nitrogen nitrified. It also gives the temperature and the water content of the soils.

Tests were made, with lead acetate paper, to see if any hydrogen sulphide was evolved during the nitrification. The soil extract was also examined. The results were negative.

		Average moisture. Per cent.	Temperature.		Per cent. nitrified.	
			Extremes.	Mean. °C.	Alone.	With CaCO ₃ .
1667.	Ammonium sulphate	10.5	25-30	27	7.2	57.2
	Cottonseed meal	•••	•••	••	32.4	43.6
	1/2 ammonium sulphate	•••		••	•••	71.8
	½ cottonseed meal Ammonium sulphate and	l	•••	••	•••	43.0
	cottonseed meal	•••	•••	••	•••	22.3
€ 668.	Ammonium sulphate	13.0	25-30	27	0.6	11.6
	Cottonseed meal	•••	•••	••	0.0	I.I
	½ ammonium sulphate .	• •••	•••	••	•••	43 2
£669.	Ammonium sulphate	13.0	25-30	27	0.2	15.8
	Cottonseed meal	• • • •	• · •	••	17.3	41.5
1670.	Ammonium sulphate	15.0	23-28	26	2.2	45.7
	Cottonseed meal Ammonium sulphate and		•••		14.8	32.6
	cottonseed meal	• •••	•••	••	0.5	•••
1 674.	Ammonium sulphate	το. 7	20-26	22	2. I	2.3
	Cottonseed meal		•••	••	3.4	4.9
	1/2 ammonium sulphate.	• •••		••	•••	16.5
	1/2 cottonseed meal Ammonium sulphate and		•••	••	•••	10 .9
	cottonseed meal	• •••	•••	••	1.3	•••
1675.	Ammonium sulphate	· 8.o	19-24	20	0. 0	0.0
	Cottonseed meal		•••	••	0.0	0.0
1 676.	Ammonium sulphate	• 12.2	18-23	19	0. 0	1.5
	Cottonseed meal		• • •	••	1. 9	-1.3
	1/2 ammonium sulphate.		•••	••	••	1.5
	1/2 cottonseed meal	• •••	•••	••	••	0.6

NITRIFICATION IN SOILS.

	Average	Temperature.		Per cent. nitrified.	
	moisture. Per cent.	Extremes. °C.	Mean. °C.	Alone.	With CaCO ₃ .
1677. Ammonium sulphate	. 11.7	18-23	19	1.0	5.6
Cottonseed meal		•••	••	1.8	6.2
1678. Ammonium sulphate		18-23	19	0.0	0.6
Cottonseed meal	• •••	• • •	••	0,0	0.0
1679. Ammonium sulphate	•	18-23	19	1.2	3.0
Cottonseed meal	• •••	• • •	••	2.9	10.6
1680. Ammonium sulphate		19-24	20	0.0	-1.3
Cottonseed meal	• •••	• • •	••	1.3	o.8
1⁄2 ammonium sulphate .	• •••	•••	• •	••	o.8
$\frac{1}{2}$ cottonseed meal	• •••	•••		••	4.5

DISCUSSION OF RESULTS.

Effect of Temperature.—The nitrification was much more active in the first four soils, in which the temperature was $23^{\circ}-30^{\circ}$ C., than in the others, in which the temperature was lower.

Effect of Calcium Carbonate.—The addition of calcium carbonate invariably caused increased nitrification, if any nitrification at all took place. For example, the increase was from 100 to 366, 100 to 240 with cottonseed meal, and 100 to 800, 100 to 2100 with ammonium sulphate. In some soils, nitrification did not occur when calcium carbonate was not added. Previous liming of the soil did not keep the calcium carbonate from being effective.

Effect of Previous Liming of the Soil.—Practically no nitrification occurred in the acid unlimed soils (1676 and 1678), even in presence of calcium carbonate, while the same soil previously limed (1677 and 1679) nitrified the fertilizers.

Effect of Previous Fertilizers.—A limed soil (1677) fertilized with ammonium sulphate for some years nitrifies ammonium sulphate much more readily than the same soil which had received sodium nitrate (1679).

Variation in the Nitrifying Power of Soils.—All our results on different soils are not comparable, since the nitrification did not all take place at the same temperature. But we can easily see, that while some soils nitrify ammonium sulphate to a greater extent in three weeks than cottonseed meal, there are others in which the reverse is the case.

Effect of Increasing Ration of Soil to Fertilizer.-This usually resulted in a slight increase in the quantity of nitrates formed. thus involving a very great increase in the percentage of nitrification

EXPLANATION OF RESULTS.

We will now discuss the bearing of these results upon the explanations we have previously put forward.

I. Ammonium sulphate may hinder the action of the nitrifying organisms. Additional evidence in favor of this hypothesis is afforded by the fact that the process of nitrification is more rapid when the quantity of ammonium sulphate is reduced.

2. Detrimental action of the nitric and sulphuric acids. This is in accord with the fact that calcium carbonate was always bene-This explanation has been generally accepted for a long ficial time

3. Different soils contain different organisms. This is in accord with our observation that some soils nitrify ammonium sulphate more rapidly than cottonseed meal, and with others the reverse is true. This is exactly what was expected when the experiments were instituted.

THE NITRIFYING ORGANISMS.

Our third hypothesis seems to be in opposition to the prevailing opinion that organic matter must necessarily be converted into ammonia as the first stage in nitrification. It does not seem to be in opposition to the facts as recorded in the literature on this subject. however, so far as we have been able to find. Warington, Frankland, and Stutzer each found that the pure nitrous organism is capable of nitrifying organic nitrogen. Omelianski¹ disputes this, and says that pure cultures of nitrifying bacteria are incapable of nitrifying organic nitrogen, but it must be converted into ammonia first. He claims that the opposite conclusions reached by Frankland, Warington, and Stutzer and his associates were based upon inaccurate observations. But it is possible that the organisms are different.

But even if it is true that Omelianski is right, it does not neces-¹ Experiment Station Record. 12, 115 (1901), abs.

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sarily follow that bacteria which nitrify organic nitrogen to nitrites do not exist. All the conditions under which the nitrous organisms have been isolated tend to eliminate such germs, the cultures always taking place in the presence of ammonium salts as a source of nitrogen, and organic nitrogen being excluded. Such a medium is unfavorable to the nitrate bacterium, also. The isolation of the bacteria which oxidize organic nitrogen must be conducted in a medium containing organic nitrogen, but not gelatine, since it has been found heretofore that species of bacteria separated by culture on gelatine do not nitrify at all.

SUMMARY.

I. Addition of calcium carbonate invariably accelerates the nitrification of cottonseed meal and ammonium sulphate, especially the latter.

2. In some soils a greater percentage of the nitrogen in ammonium sulphate is nitrified than that in cottonseed meal, and in other soils, the contrary is the case, even in the presence of calcium carbonate.

3. The factors which produce this result are probably as follows: a. The presence of the ammonium sulphate diminishes the activity of the nitrifying organisms; b. The acids produced also hinder them; c. Different soils contain different classes of organisms, some of which nitrify organic in preference to ammoniacal nitrogen.

4. We have found no evidence on record that organisms which nitrify organic nitrogen directly do not exist. The fact that they have not been isolated by present methods may be due to their elimination by the use of ammonium salts, on which they cannot feed, in the nutritive medium.

5. Liming acid soils is favorable to nitrification.

6. Continuous application of ammonium sulphate to a soil previously limed increases its power of nitrifying ammonium sulphate.