alkali found and the caustic will, of course, give the carbonate. For example: 2 grams of soap and 15 cc. N/10 stearic acid; run in 3.2 cc. N/10 caustic soda to back titrate. Consequently, 15 — 3.2 = 11.8 cc. N/10 stearic acid equivalent to total free alkali.

To neutralize the caustic in the sample treated with barium chloride was required 4.1 cc. N/10 stearic acid. Fifty cc. of the alcohol used required 0.2 cc. N/10 caustic soda, then 4.1 + 0.2.

4.3 cc. N/10 stearic acid to neutralize free caustic alkali.

11.8 — 4.3 = 7.5 cc N/10 stearic acid to neutralize carbonated alkali.

1 cc. N/10 stearic acid = 0.004 gram caustic soda or 0.0053 gram sodium carbonate.

The above figures calculated to percentage would be:

0.86 per cent. caustic soda and 1.99 per cent. sodium carbonate.

It is to be noted that a rubber stopper cannot be used in the flasks for dissolving the soap on account of the sulphur in the rubber, which decolorizes an alcoholic solution of phenolphthalein. The method is applicable to all soaps which do not contain fillers which react with the standard solutions employed.

BUFFALO, July 24, 1900.

INVESTIGATIONS ON THE DETERMINATION AND COMPOSITION OF HUMUS AND ITS NITRIFICATION.

BY CHARLES RIMBACH.

Received July 30, 1900.

THE investigation on the nitrification of the *matière noire* of Grandeau, here discussed, was undertaken on the suggestion of Prof. Hilgard, to test the question whether or not that substance is to be regarded as a source of nitrates under the influence of nitrifying bacteria as has been assumed, but never definitely proved. The method of preparation consists in treating the soil with dilute hydrochloric acid to remove the lime and magnesia with which the humus substance forms insoluble compounds; and after washing with distilled water, until the filtrate is neutral, extracting it with 6 per cent. ammonia. On evaporation the solution leaves the *matière noire* behind.

As a medium containing the carbonates of lime and magnesia is most favorable to nitrifying bacteria, the compound of the humus with these metals was employed for the experiment in order to simplify conditions. For this purpose the ammonia extract from a soil was precipitated with hot solutions of gypsum and magnesium sulphate ; the compound, which settles very slowly, was washed and decanted several times with gypsum solution to keep it flocculated. and then put on a linen filter. After the liquid had passed, the mass was further washed by shaking up in a bottle with gypsum solution, this operation being repeated until the filtrate was nearly colorless. The precipitate was dried at 100°, whereby it lost considerably in volume, forming a hard, brilliant, brittle mass with conchoidal fracture. It was free from ready-formed ammonia expellable by magnesia. The lime-magnesia compound thus prepared naturally contained gypsum. In the air-dried condition the precipitates prepared from two different soils had the following composition :

West Berkeley Soil.—An excessively stiff clay or "black adobe" soil, rich in lime, containing over 2 per cent. of *matière noire.*

CALCIUM-MAGNESIUM HUMATE.

	Per cent.
Water	17.95
Ash	14.34
Organic matter	67.71
Nitrogen	3.06 of the organic matter.

Redwood Soil.—From Marin County (sandy humus soil).

CALCIUM-MAGNESIUM HUMATE.

	Per cent.
Water	17.80
Ash	15.37
Organic matter	68.57
Nitrogen	4.01 of the organic matter.

The ash of the latter was soluble in dilute hydrochloric acid, leaving an insignificant white residue. It contained :

	Per cent.
Lime	47.07
Magnesia	13.65
Phosphoric acid	6.15

Potash in noticeable quantity.

The humate from the Redwood soil, being the richer in nitrogen, was used for the nitrification experiment. Twenty-eight grams humate = 22.8816 grams dry matter with 0.767 gram nitrogen, were mixed in a beaker with 2 kilos of sand taken from drifting sand dunes, a short distance from the ocean near San Francisco; it had been previously ignited, and freed from nitrates by leaching with hydrochloric acid and distilled water.

The material for infection was prepared from soil of the ten-acre tract, Southern California Experiment Station. This soil contains from 1,560 to 12,280 pounds of alkali salts per acre to the depth of 1 foot; up to 23 per cent. of these are nitrates = 0.062 per cent. of the soil. After having been freed from nitrates by washing with distilled water, it was shaken with water, and the turbid liquid filtered through linen; the above mixture was moistened with the filtrate.

The material was kept for two months at a temperature of about 25°. Every other day, with some interruptions, the sand was moistened so that the content of free water amounted to about 2 per cent., stirred up with a glass rod, and thus kept in a mellow, flocculated condition, to facilitate the access of the air. After that lapse of time the moistened sand was mixed thoroughly, and a part of it was dried, weighed, and leached with water. The extract, subjected to the colorimetric determination of nitric acid, showed that 5.94 per cent. of the nitrogen of the humus substance had been nitrified.

Further experiments are in progress for comparing the nitrification in a natural soil, and in the same freed from *matière noire*, in which the microscope showed only slightly browned vegetable débris.

Conclusions.—The assumption made heretofore by Prof. Hilgard and others that Grandeau's *matière noire* is a direct source of nitrogen through nitrification, is definitely verified by the above experiment, in which all other possible sources were eliminated. It also justifies, presumptively, Prof. Hilgard's thesis that humus containing a high percentage of nitrogen, such as is found in the soils of the arid regions, will yield larger amounts of nitrates in the same time than will that of lower nitrogen percentages; and that conversely, low nitrogen percentages may fail to supply a sufficiency for plant growth.

Determination of Humus.[1]—An investigation was also made to test the Grandeau method for the determination of humus, and particularly that of nitrogen in humus, as hitherto used. The method is the following :

Two portions of 5 or 10 grams of air-dried soil are weighed off into prepared filters. They are treated with 1 per cent. hydrochloric acid to dissolve out the lime and magnesia, as already explained. The treatment with acid is continued until there is no reaction for lime ; the acid is then washed out with water to neutral reaction. The funnels containing the washed soils are now stoppered and one of them is treated with ammonia water (6 per cent.) for the determination of humus by evaporation, and the other with sodium hydroxide (4 per cent.) for the estimation of nitrogen. The lye, whether ammonia or soda, remains in the respective funnels a few hours ; the solution is then allowed to pass through and the filter washed once with the lye ; the funnel is then restoppered and a fresh quantity of the solvent put on the filter. The treatment is repeated until the filtrate remains colorless. The solutions are then made up to 1 liter, and aliquot parts are taken for the determination.

The ammonia solution is evaporated in a weighed platinum dish, the residue dried at 100°, weighed, ignited, and reweighed, the loss in weight showing the amount of humus.

The soda solution is treated with sulphuric acid and mercuric oxide to determine the nitrogen in the usual way.

The percentage of nitrogen in the sodium extract is referred to the percentage of humus in the soil as determined by ammonia extraction, for obtaining the percentage of nitrogen in the humus.

It was to be decided whether, as assumed in this method, ammonia and soda extract the same quantities of humus and nitrogen ; *i. e.*, the same substances. For this purpose two soils, peaty soil from Westminster, Orange Co., Cal., with 19.12 per cent. humus, and heavy black adobe soil from the University grounds with 1.20 per cent. humus, were treated as above described. A duplicate of the Westminster soil served for determining the quantity of humus lost for the analysis by the extrac-

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[1] The word "humus," as used in this paper, is intended to designate Grandeau's *matière noire*.

tion with hydrochloric acid and water, which was prolonged until 1 liter of filtrate was obtained. Three hundred cc. of this filtrate on evaporation gave 0.0375 gram residue, containing 0.0367 gram organic matter with 7.45 per cent. nitrogen. This quantity of organic matter is 4.53 per cent. of that contained in the ammonia extract.

For determining the organic matter of the ammonia solution, aliquot parts of it were evaporated. The residue always contains combined ammonia, which was determined in another aliquot part (previously boiled to expel free ammonia) by distillation with magnesia. The quantity found, subtracted from the loss of weight by ignition, gives the organic matter. In the residue from the distillation the organic nitrogen was determined by the Kjeldahl method.

	Gram.
Residue of 200 cc. ammonia solution	0.1795
Ash	0.0108
Ash-free ammonia humate	0.1687
Less combined ammonia	0.00834
Organic matter	0.16036

The combined ammonia taken up from the ammonia solution amounts to 4.93 per cent. of the ash-free ammonia humate; the organic nitrogen, to 6.98 per cent. of the organic matter.

The determination of the organic nitrogen in the ammonia and soda solutions respectively, when applying the above correction, gave the following results:

	Mg.
Organic nitrogen in 100 cc. ammonia solution	5.33
Organic nitrogen in 100 cc. soda solution	8.23

These results are important in that they prove that soda solution extracts more nitrogen than does ammonia.

On account of the impossibility of determining the organic matter in the neutralized soda solution by evaporation and calcination, because of the large quantity of sodium salt, which hinders the combustion, it was decided to determine by the precipitation of the humus, the quantity and quality of humus-substances in both extracts. In order to determine which reagent precipitates the humus most completely, ammonia-humate extract from University adobe soil was treated with solutions of copper and magnesium sulphates, and with hydrochloric acid. Before adding the salt solutions, the humus extract was freed from uncombined ammonia by boiling; the precipitates were washed with boiling water, in which the magnesium humate was rather soluble, while the filtrate from the copper humate was perfectly clear and nearly colorless. The hydrochloric precipitates were washed with 2 per cent. hydrochloric acid until 500 cc. had passed. All precipitates were free from ammonia. The nitrogen content of the filter was considered. For all precipitations the same quantity of ammonia extract was used.

	Precipitants used.		
	Copper sulphate.	Magnesium sulphate.	Hydrochloric acid.
Organic matter of the precipitates in per cent. of the total organic matter of the extracts	89.16	65.16	85.21
Nitrogen of the precipitates in per cent. of the organic matter of the precipitates	....	4.48	4.93
Nitrogen of the precipitates in per cent. of the total organic nitrogen of the extract	....	....	88.24
Ash in per cent. of the precipitates	23.40	14.60	5.21

These results show that magnesium sulphate (because of the solubility of humates in water) is not suitable as a precipitant, while copper sulphate precipitates the humus-substances most completely. Hydrochloric acid has nearly the same complete effect as copper sulphate, and as it can be added to the extract without previous elimination of ready-formed ammonia, it was used for the determinations described above.

The precipitation of the humus with hydrochloric acid in the ammonia and soda extracts from the Westminster and University soils, gave the following results:

		Precipitate of the humus acids by hydrochloric acid.	Organic matter of the same.	Nitrogen of the precipitates in per cents of	
				the organic matter of the precipitates.	the total organic nitrogen of the extract.
Westminster Soil—	cc.	Gram.	Gram.		
Ammonia solution	200	0.1127	0.2110	5.84	54.78
Soda solution	200	0.1495	0.1490	3.75	22.78
University Soil—					
Ammonia solution	300	0.0299	0.0233	....	....
Soda solution	300	0.0103	0.0077	....	....

It is thus seen that the humus-substances of these two soils behave altogether differently toward ammonium and sodium hydroxides as solvents.

The nitrogen of these precipitates was not determined on account of the small amounts obtained. The organic matter of the hydrochloric acid precipitate from the Westminster ammonia solution is 70.24 per cent. of the total quantity of organic matter contained in the same.

Even if the chlorides of ammonium and sodium should influence differently the solubility of the precipitates, the ratio of the latter, which is 3 : 4 in the first, and 3 : 1 in the second case, shows that substances of different nature are thus obtained.

Organic Matter in the Soda Extract.—For determining approximatively the ratio of nitrogen and organic matter in the soda extract, a weaker solution, which, of course, may have a different solvent power, was used in the extraction. 140 grams of University adobe soil, after being extracted with hydrochloric acid and water (250 cc. of the latter contained 0.0133 gram organic matter) was treated during twelve hours with 1 per cent. soda solution, then washed with 0.25 per cent. soda-lye, this operation being repeated until 1 liter filtrate was reached. 50 cc. of this were evaporated, the residue ignited, a solution of ammonium carbonate added in order to convert lime and magnesia into insoluble carbonates, evaporated again, and the residue gently heated. Then the ash was extracted with water and the quantity of sulphuric acid necessary for neutralizing the soda determined by titration. With the same quantity of acid 50 cc. of the soda solution was evaporated. The residue dried, weighed, ignited, and reweighed showed the organic matter. The determination of nitrogen was made with an aliquot part.

For comparison, 140 grams of the same soil were treated with the same quantities of acid, water, and 6 per cent. ammonia, and the extract was analyzed in the usual way. The ash-free residue contained 5.88 per cent. of ready-formed ammonia.

	cc.	Organic matter. Gram.	Organic nitrogen in per cents. of the organic matter.
Ammonia solution	50	0.0589	4.73
Soda solution	50	0.0543	5.37

These figures, however, do not allow a definite conclusion as to the total amount of soluble humic matter in the soil : for it was found that in the ammonia filtrates obtained at different times during the extraction (which lasted six days), the nitrogen percentages of the organic matter vary considerably. The differences in the percentages of combined, ready-formed ammonia are small.

AMMONIA SOLUTION FROM SOIL FROM THE TEN-ACRE TRACT.

	Organic nitrogen.[1]	Combined ammonia.[2]
Filtrate from the first four hours....	4.90	6.75
Filtrate from the seventh and eighth day	9.35	7.26

Apparently the humus-substances that are poor in nitrogen are readily soluble in ammonia and consequently form the main part of the first extract, while the substance with higher nitrogen percentages are less soluble and need a longer time for entire extraction by ammonia, but are more quickly dissolved by soda.

CONCLUSIONS.

1. By leaching with hydrochloric acid and water a certain quantity of humus, varying in the different soils, is lost to the subsequent extraction with ammonia.

2. The content of nitrogen of the soda extract, being different from that of the ammonia extract, cannot be directly referred to the content of organic matter of the latter.

3. In the determination of organic matter and nitrogen in the ammonia extract, the quantity of combined ammonia must be considered, as has been described in the analysis of Westminster humus.

It is thus evident that the percentages of humus heretofore determined are too high, inasmuch as the combined ammonia, which is about 5 per cent. of the ash-free ammonium humate, has been erroneously calculated as organic matter. Furthermore, if all soils behave like Westminster soil, from which soda extracts about one and a half times as much nitrogen as does ammonia, the nitrogen percentages in humus as heretofore determined, even though they are referred to organic matter and combined ammonia, would be too high.

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[1] In per cents. of the organic matter contained in the solution.

[2] In per cents. of the ash-free ammonia humates.

4. The question still remains whether soda or ammonia solution, and in what strength, satisfy best the requirements of a solvent for humus substance, regarded as the sum of organic substances, decomposed or not, that are immediately available for the plants.

After concluding this investigation, we received a publication of A. L. Emery entitled "Soil Humus: Some Sources of Error in Analytical Methods."[1] As to the absorption of ammonia by the humus solution, there mentioned, it is, as we have explained, at least partially a simple process of combination of the humus acids (liberated by hydrochloric acid from earth bases) with the ammonia; the quantity of the latter has been regularly determined by Prof. Hilgard for some time past, by distillation with magnesia (not lime, which decomposes organic nitrogen compounds). A question, however, still to be decided is whether the ammonia used forms amido-compounds with the humus substances during the digestion or evaporation.

NOTES.

The Brown-Taylor-Richards Method for the Microscopical Identification of Butter.—In the June number of the Journal appears an article by John A. Hummel on "Brown and Taylor's Official" method of identifying butter, with plates showing the appearance of butter, oleomargarine, and renovated butter, when viewed by polarized light. This is the first time the method used by the Bureau of Internal Revenue for identifying oleomargarine has been formally christened, and I would suggest, if the designation is to stand in chemical literature, the addition of the name of Mr. Richards, formerly microscopist of this office, to whom is due the credit for its adoption and successful employment as a field test for the past twelve years. His report on this subject will be found on page CLXII of the Annual Report of the Commissioner of Interval Revenue for the fiscal year ended June 30, 1888. Directions for the use of the form of (unmounted) microscope officially adopted and its polarizing attachments are given in Series 7, No. 9, revised, of the Regulations of the Internal Revenue Office, everything being simplified as much as possible, so that the test can be performed by

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[1] This Journal, 22, 285 (1900).