

TABLE XVI.
Effect of 0.4 cc. Normal KOH for Different Periods of Contact.

Time after adding H ₂ O ₂ . (Minutes.)	Control.	0.4 cc. normal KOH added. Time of contact in minutes.			
		0.	15.	30.	60.
1.....	7.9	2.9	1.4	1.0	0.3
2.....	14.1	6.1	2.9	1.8	1.0
3.....	18.5	9.3	4.1	2.7	1.4
4.....	21.8	11.8	5.2	3.9	1.8
5.....	24.5	13.7	6.0	4.1	2.0
6.....	26.7	15.3	6.7	4.6	2.2
7.....	29.3	16.4	7.2	5.1	2.4
8.....	29.8	17.6	7.5	5.3	2.5
9.....	30.9	18.3	7.7	5.6	2.6
10.....	31.8	18.8	8.0	5.7	2.7

ing alkalis. Ammonium hydroxide, potassium carbonate, sodium carbonate and sodium bicarbonate show only a slight depressing effect. It is a noticeable fact that neutralization of the alkali by a standard solution of hydrochloric acid increases the retarding effect in every instance.

The duration of contact with sodium and potassium hydroxide has a decided effect upon the enzyme activity. The results show a lowering of enzyme activity when the time of contact is increased.

The results obtained with catalase preparations from different sources, however, are hardly comparable, as it is only reasonable to suppose that the purity of the catalase solutions materially affects the results.

Summary.

I. The inhibiting effect of acids and alkalis upon the catalase of taka-diastase follows the order of their ionization constants.

II. After fifteen-minute contact with acid or alkali, the activity of the enzyme cannot be restored by neutralization.

III. The effect of acids was not influenced by the time of contact within the limits of these experiments. The effect of caustic alkalis, on the other hand, was proportional to the period of contact.

In conclusion, the writer takes pleasure in acknowledging his indebtedness to A. W. Dox for his kind suggestions in carrying out this work.

AMES, IOWA.

[CONTRIBUTION FROM THE HAWAII EXPERIMENT STATION NO. 11.]

THE ORGANIC NITROGEN OF HAWAIIAN SOILS. I. THE PRODUCTS OF ACID HYDROLYSIS.

By W. P. KELLEY.

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Introduction.

The vegetable proteins occurring in different plants constitute the principal source of the soil nitrogen. These undergo biological decompo-

sitions of a hydrolytic nature, which probably take place in a gradational manner similar to that which occurs during animal digestion. The chemistry of soil nitrogen, therefore, may be looked upon as being the chemistry of protein undergoing hydrolysis.

For some time it has been the practice among soil bacteriologists to determine the ammonia split off by bacterial action and the rates of its oxidation to nitrate under varying conditions. But ammonia is the final hydrolytic product and is probably split off, in the main, directly from the acid amides and amino acids which have themselves arisen from hydrolysis of the proteins. Hence, the determination of the organic nitrogen compounds that are obtainable from a soil, ought to throw some light on the possibilities of subsequent ammonification, and indicate the nature of the decompositions that have already taken place. This view of the subject receives support from the recent researches of Jodidi¹ who found that different acid amides and amino acids undergo ammonification in soils at different rates, depending upon their structure. He found, for example, that glycocoll and leucine were ammonified more rapidly than phenylalanine, which he attributes to the resistant character of the benzene ring occurring in the last named compound.

The vegetable proteins, upon hydrolysis, yield amide, basic and non-basic nitrogen in quite variable amounts, as shown by Osborne.² The amide nitrogen ranges from 6% in legumelin from the horse bean to 24.5% in gliadin from wheat. The basic nitrogen varies from 3% in zein from maize to 32.8% in globulin from the cocoanut; while the non-basic nitrogen occurs in more constant and always much larger amounts. The average of the 34 different vegetable proteins reported contain 11.6% amide, 23.6% basic and 63.5% non-basic nitrogen.

It is evident, then, that soil organic matter originating from different plants would probably yield, upon hydrolysis, varying amounts of amide, basic and non-basic nitrogen compounds. During recent years some investigation has been made on this subject. From the researches of Shorey,³ Jodidi,⁴ Robinson⁵ and Lathrop, and Brown,⁶ it has been shown that the acid soluble nitrogen of soils varies considerably. On the average, the hydrolytic products have been found to be composed of approximately 25% amide, 10% basic and 65% non-basic nitrogen.

Schreiner,⁷ Shorey and their associates in the Bureau of Soils have isolated a number of nitrogen compounds from soils, the specific nature

¹ Iowa Sta., *Research Bull.* 9 (1912).

² The vegetable proteins.

³ U. S. D. A., Hawaii Sta., *Ann. Rept.* 1906, 37-59.

⁴ Mich. Sta., *Tech. Bull.* 4 (1909); Iowa Sta., *Research Bulls.* 1 and 3 (1911).

⁵ Mich. Sta., *Tech. Bull.* 7 (1911).

⁶ *J. Ind. Eng. Chem.*, 3, 657-60 (1911).

⁷ U. S. D. A., Bur. of Soils, *Bulls.* 47, 53, 74, 80, 87, 88, 89.

of which indicates that the biological decompositions going on produce cleavages analogous to those brought about upon acid hydrolysis of the proteins.

In view of the limited number of soils previously studied in this connection, and the strong probability that under bacterial action hydrolysis of the proteins takes place previous to the deamidization process proper, it is desirable that a greater range of soils be investigated with reference to their nitrogen constituents.

The Nitrogen of Hawaiian Soils.

The soils used in this investigation belong to the laterite class common to the islands. Sample No. 379 is a silty loam, containing considerable amounts of organic matter, and was taken from an old pasture where semi-arid conditions prevail.

Sample No. 428 is a highly organic sandy soil, recently cleared of a tropical jungle, and may be considered virgin fern forest land.

Sample No. 446 is a silty loam now being used for pineapples, but formerly devoted to pasture for many years.

Sample No. 292 represents a loam soil containing considerable gravel, and has been devoted to aquatic agriculture for many years.

Samples Nos. 343, 405, 406 and 345 are silty soils, and No. 347 is a highly humous soil, each of which have been continuously cultivated in rice for thirty or more years.

Nitrate and Ammonia.—Nitrates and ammonia, generally comprising not more than a small percentage of the total nitrogen in soils, were determined as follows: Nitrate was determined in water solutions, by the use of the phenol-disulfonic acid method, while the ammonia was determined by the direct distillation of separate portions of the soil after adding magnesium oxide. All the data presented in this paper have been calculated to the water-free basis.

TOTAL, NITRATE AND AMMONIA NITROGEN.

Total nitrogen.		Nitrate nitrogen.		Ammonia nitrogen.	
Lab. No.	Per cent.	Pts. per million.	Per cent. of total nitrogen.	Pts. per million.	Per cent. of total nitrogen.
379	0.592	10	0.169	10	0.169
428	0.770	45	0.519	220	2.857
447	0.354	62	0.751	10	0.282
292	0.122	4	0.328	10	0.819
343	0.220	0	0.000	22	1.000
345	0.218	0	0.000	32	1.468
347	1.241	70	0.537	130	1.048
405	0.195	1	0.049	50	2.564
406	0.456	15	0.325	60	1.316

It will be observed that in contrast to soils elsewhere the ammonia content in most instances was found to be considerably greater than the

nitrate,¹ and the nitrogen present as nitrate and ammonia constitutes only a small percentage of the total nitrogen. The nitrogen of these soils, as was expected, exists, therefore, very largely in organic combinations.

The Organic Nitrogen.—In this work the Osborne-Harris² modification of the Hausmann³ method, as outlined by Jodidi⁴ in his studies on Iowa soils, has been used. The hydrolysis was conducted by heating to boiling under a reflux condenser for 10 hours, 50 gram portions of the air-dried soils with 750 cc. strong hydrochloric acid, filtering and making the filtrate to one liter. Aliquot parts of the solution thus obtained were used for the determination of the amide, basic, and non-basic nitrogen. Since the amide nitrogen was determined as ammonia by the direct distillation of the solution after making alkaline with magnesium oxide, the ammonia originally present in the soil was deducted from the amounts found.

The basic nitrogen was determined by precipitation with phosphotungstic acid in water solutions, obtained by filtering the residue left after the distillation with magnesium oxide, and, as shown by previous investigators,⁵ this precipitate contains nitrogen largely in the form of diamino acids, although it should be remembered that alkaloids, etc., if present, would also be precipitated. The non-basic nitrogen was determined by subtracting the sum of the other two groups from the total nitrogen dissolved, and while this group is now commonly referred to as mono-amino acids, Jodidi and Robinson have each brought forth evidence that it is not made up wholly of mono-amino acids. Just what other bodies occur in this group is not yet known. The results follow:

ORGANIC NITROGEN OF HAWAIIAN SOILS.

Soluble in Hydrochloric Acid.

Lab. No.	Per cent. of total N.	Per cent. of the soil.				Per cent. of nitrogen in solution.		
		Amide N.	Basic N.	Non-basic N.	Total N.	Amide N.	Basic N.	Non-basic N.
379	71.96	0.095	0.030	0.300	0.426	22.30	7.04	70.42
428	72.59	0.141	0.028	0.368	0.559	24.79	5.01	66.27
447	67.51	0.074	0.033	0.131	0.239	30.96	13.81	54.82
292	91.80	0.024	0.012	0.075	0.112	21.43	10.71	66.96
343	90.91	0.054	0.020	0.124	0.200	27.00	10.00	62.00
345	88.52	0.042	0.017	0.131	0.193	21.76	8.81	67.88
347	80.42	0.225	0.097	0.663	0.998	22.55	9.72	65.43
405	84.62	0.042	0.019	0.099	0.165	25.45	11.51	60.00
406	91.22	0.079	0.055	0.276	0.416	18.99	13.22	66.35
						Av. 23.91	9.98	64.57

¹ The occurrence of large quantities of ammonium compounds is a phenomenon common to many Hawaiian soils and will be further discussed in a separate publication.

² THIS JOURNAL, 25, 323 (1903).

³ Z. physiol. Chem., 27, 95 (1899).

⁴ Iowa Sta., Research Bull. 1 (1911).

⁵ Jodidi, loc. cit.; Robinson, *Ibid.*

It will be seen from the above table that there is considerable variation in the amounts of nitrogen dissolved from the different soils. In soil No. 447 only 67.51% of the total nitrogen was dissolved, while No. 292 yielded 91.80%. Concerning the insoluble nitrogen very little indeed is known.

Amides.—The amide nitrogen in all the soils studied, with the exception of Nos. 447, 343 and 406 occurred in approximately the same relative amounts, being on the average 23.91% of the soluble nitrogen. It is noteworthy that the amides of soils comprise a considerably higher percentage of the nitrogen than occur in most of the vegetable proteins previously studied. A direct comparison of the amide nitrogen of the above soils with that found in soils elsewhere is possible in a few cases only, for the reason that the strengths of the acid, and the lengths of the time of digestion, used in the investigations on this subject vary too greatly. With the results obtained by Jodidi, however, these data are comparable, and show that Hawaiian soils contain approximately the same relative amounts of amide as Michigan peat and Iowa soils.

Basic Nitrogen.—The nitrogen compounds precipitated by phosphotungstic acid were found to vary considerably in the different soils, amounting on the average to 9.98% of the soluble nitrogen. It is noteworthy that the percentage of basic nitrogen in soils falls far below the percentage found in the majority of vegetable proteins. In these studies no attempt was made to prove the nature of these nitrogen compounds, but from the work of others it seems permissible to consider the basic nitrogen as being composed principally of diamino acids.

Non-basic Nitrogen.—The percentages of non-basic nitrogen, with the exception of that from soil No. 447, were found to be remarkably similar in every instance, amounting to about two-thirds of the nitrogen dissolved by boiling hydrochloric acid. In this respect the soluble nitrogen of soils is quite similar to that of vegetable proteins. The non-basic nitrogen of soils as stated above is looked upon as being composed largely, but not entirely, of mono-amino acids, and probably such as are obtained in the hydrolysis of protein. Robinson,¹ for example, isolated leucine and iso-leucine from hydrochloric acid solutions of Michigan peat.

Discussion.

As shown above, the relative amounts of amide nitrogen split off from the soils studied was about double that previously found in the seed proteins, while less than one-third as much basic nitrogen was found. On the other hand, the relative amounts of non-basic nitrogen proved to be about equal to that of the seed proteins. These variations may be due to the bacteria's attacking protein more largely at certain points than others, resulting in the splitting off of relatively greater amounts of basic

¹ *Loc. cit.*

nitrogen compounds than of the other groups,¹ and the basic nitrogen compounds then becoming diamidized, thus materially reducing the amounts of basic nitrogen present.

It is also possible for certain diamino acids to become transformed into diamines, as for example, arginine can be decomposed into urea and ornithine, then into putrescine, while lysine can be converted into cadaverine by the action of certain putrefactive bacteria. The urea that would thus be formed is known to be susceptible to the attack of a specific group of bacteria which convert it into ammonium compounds.

These views should not be interpreted to mean that bacteria attack protein at the diamino linkings only; there is much evidence that hydrolysis also takes place all along the line, resulting in the cleavage of amide, diamino and mono-amino acid compounds, all of which doubtless undergo ammonification, although probably at different rates.²

It should also be remembered that certain organic nitrogen compounds are readily assimilated by plants, others to a less extent, while still others are incapable of being assimilated.³ Therefore, it is possible that the nitrogen compounds belonging to one group may be absorbed by plants to a greater extent than those of other groups. In view of these facts it is apparent that an explanation of the striking differences found in the percentages of nitrogen split off in the different groups in soils, on the one hand, and vegetable proteins, on the other, can only be surmised at the present time. Further investigation on this subject will be undertaken.

HONOLULU, HAWAII.

[CONTRIBUTION FROM THE HAWAII EXPERIMENT STATION NO. 13.]

THE ORGANIC NITROGEN OF HAWAIIAN SOILS. II. THE EFFECTS OF HEAT ON SOIL NITROGEN.

By W. P. KELLEY.

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Introduction.

It has been observed frequently that soil heating produces notable effects on the growth of crops. With the exception of the studies carried out in the Bureau of Soils, the investigations on this subject have dealt largely with bacteriological and physiological phases of the question. In this connection, Lyon and Bizzel⁴ observed that an increase in the solubility

¹ Since this paper was written results have been obtained from ammonification experiments, employing different nitrogenous materials, which strengthen the view that the basic nitrogen group is eliminated from soils at a greater rate than the amide and non-basic nitrogen compounds.

² See Jodidi, Iowa Sta., *Research Bull.* 9 (1912).

³ See Hutchinson and Miller, *Jour. Agr. Sci.*, 4, 262-89; also, Schreiner and Skinner, U. S. D. A., Bur. of Soils, *Bull.* 77.

⁴ Cornell Sta., *Bulls.* 275 and 326.