XXXVIII. DETERMINATION OF CARBON AND NITROGEN BY THE ACTION OF CHROMIC ACID UNDER REDUCED PRESSURE.

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DURING the course of work on the anaerobic decomposition of plant materials in the soil, the need was felt for a rapid and accurate method for the determination of carbon in extracts and solid residues, which in certain cases, *e.g.* in the determination of lignin, could also estimate the amount of nitrogen present in the same sample. The chromic acid method suggested itself and was examined in some detail to ascertain its suitability for the purpose in view. This method has been used by numerous workers for such widely different materials as soils, industrial wastes, coals, iron, steel and various organic compounds.

The low results obtained for carbon by the earlier workers have been shown [Friedmann and Kendall, 1929] to be due to insufficient heating, low temperature, too much dilution *etc.*, and it is now generally admitted that with improved manipulation the method is capable of yielding results as accurate as those obtained by the dry combustion procedure.

Among the workers who have examined the suitability of the method for the simultaneous determination of nitrogen, Fritsch [1896], Kruger [1894], Anderson and Schutte [1924], Antipov-Karataiev and Fillipova [1932], Brown [1927] and Tiurin [1933] have reported satisfactory agreement between the Kjeldahl values and those obtained by alkaline distillation of the residue after chromic digestion, whilst others [Dafert, 1888; Robertson, 1916; Shewan, 1935; Subrahmanyan *et al.*, 1934] have found that the method yielded low results. The low values have been attributed to the presence of chlorine [Robertson, 1916; Anderson and Schutte, 1924], to the oxidising action of chromic acid, to the structure of the compound [Shewan, 1935] and to a portion of the nitrogen being retained by the chromium in the oxidising mixture [Narayanayya and Subrahmanyan, 1935]. The author has recently found that the recovery of ammonia by the method bears a definite relation to the structure of the compound and that the loss occurs by a portion of the nitrogen being oxidised to nitrate and the rest evolved in gaseous form, as nitrous oxide in the cases examined.

APPARATUS AND PROCEDURE.

The apparatus used in the present investigation, shown in Fig. 1, is a modification of that used by Adams [1934], though the manipulative details show much difference. The Kjeldahl flask A of about 500 ml. capacity is fitted in the neck with a boiling-tube B having its bottom punctured and packed with glass-wool to serve as a trap for the absorption of SO_3 fumes [Pollard and Forsee, 1935]. The inlet tube C connects with the annular space between B and A and carries a cock F by means of which the admission of air into the apparatus can be

regulated. The exit tube D is connected respectively to a test-tube L containing H_2SO_4 of constant boiling-point [White and Holben, 1925] for retaining SO_3 fumes, kept in a bath of cold water, a $CaCl_2$ drying tube M, two weighed tubes P and N packed with "sofnolite" and anhydrous $CaCl_2$, as shown in the figure, for the absorption of CO_2 and finally to a $CaCl_2$ drying tube Q and suction pump S.
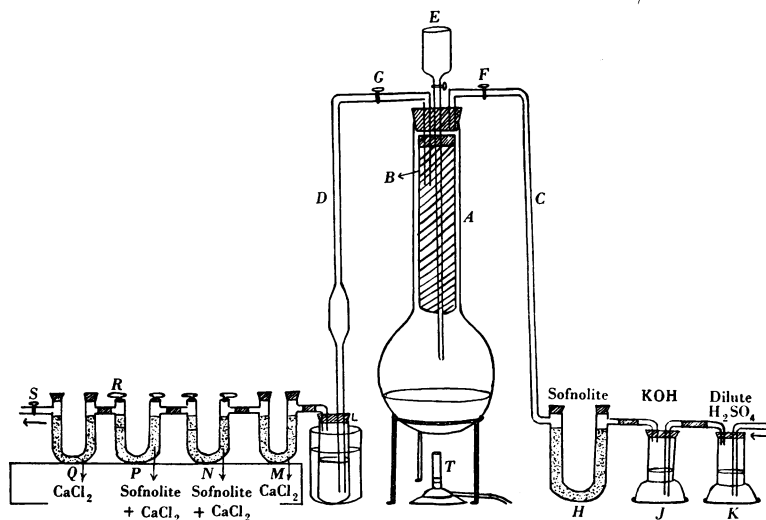

Fig. 1.

The manipulative details adopted were as follows. A weighed amount of the substance under investigation (to yield 0·2–0·4 g. of CO_2) is transferred into the Kjeldahl flask preferably through a glass tube which is subsequently washed with 5–10 ml. of distilled water. The flask is fitted into place, cock F is closed and the required amount of CrO_3 solution, at the rate of 1 ml. of aqueous 50% (wt./volume) CrO_3 solution per 100 mg. of CO_2 expected, is placed in the thistle funnel E. Suction is then started, most of the CrO_3 solution let in and the sides of the funnel washed with about 2 ml. of water, to prevent the formation of a precipitate on the subsequent addition of H_2SO_4 and possible jamming of the cock. Sufficient H_2SO_4 is then added, in small portions at a time, to make the ratio of H_2SO_4 : total water (including CrO_3 solution) equal to 2 : 1 by volume or slightly above. Higher ratios than 3 : 1 produce a cake at the bottom of the flask in the case of soils, which is hard to remove later by mechanical rubbing or warming with water. It can be removed, however, by warming with alkali followed by treatment with dil. HNO_3.

After all the H_2SO_4 has been added, cock F is partially opened to admit a slow stream of air, freed from NH_3 and CO_2, at the rate of 1 or 2 bubbles per second. The rate at which air is removed from the flask being much greater than that at which it is admitted, there is throughout the experiment low pressure maintained in the flask of the order of 3–4 in. of mercury. This low pressure, besides promoting vigorous boiling at a lower temperature and the avoidance of excessive fumes, markedly improves the efficiency of the absorption of the CO_2 evolved. The admission of air serves partly to carry off the CO_2 evolved and partly to cool the neck of the flask and condense fumes of SO_3 formed. In spite of this arrangement and the glass-wool trap B, a portion of the fumes was found always to be carried

forward into the exit tube D, but was effectively retained by the 98·3 % H_2SO_4 in tube L. It may be stated that used portions of the acid in L could be collected together, heated for several hours and used over again.

About 20–30 minutes' digestion of the contents of the flask at boiling-point was found to be sufficient for most soils and plant materials, but where derivatives of pyridine, quinoline, isoquinoline or the tetrammonium compounds such as betaine and choline are present, a longer time may be required depending on the nature of the compounds present. In these cases, completion of oxidation could be tested either by stopping the digestion at half-hour intervals and weighing the amount of CO_2 formed, or better without interrupting the oxidation, by having two sets of "sofnolite" tubes connected in parallel and collecting the CO_2 evolved in alternate sets per half-hour. Suitable blanks should be run in each case. At the end of the digestion, cocks R and S are closed and cock F is opened in stages so as to admit a regulated stream of air into the apparatus and equalise the pressure inside and outside, after which the "sofnolite" tubes P and N are detached and weighed.

Direct distillation with alkali, without pretreatment, of the digested residue gives only the amount of ammoniacal nitrogen present. It will be seen later that in the cases of soils and plant materials, for nitrogen values of 5 mg. and above, this value is uniformly lower than the Kjeldahl figure by 10 % and this correction could be applied. Where, however, the amount of nitrogen present is about 5 mg. or less, the difference between ammoniacal nitrogen formed and the total nitrogen found by the Kjeldahl method is due to a quantitative oxidation to nitrate which is held in solution by the H_2SO_4 present. The total nitrogen in such cases can be estimated as follows.

The excess of chromic acid is reduced by gentle warming and addition of solid sodium sulphite in small quantities at a time, testing for completion of reaction with starch-iodide solution used as an external indicator. A few pieces of solid paraffin and pumice stone are added and most of the H_2SO_4 neutralised by careful addition of NaOH in small quantities, keeping the flask cooled under the tap. 30–40 ml. of 50 % NaOH and 1–2 g. of Devarda's alloy are then added and the distillation conducted as usual. The addition of paraffin and removal of most of the acid by preliminary neutralisation avoids excessive frothing and a too vigorous reaction in the early stages. A trace of the paraffin usually passes over but this does not interfere with the clear definition of the end-point. Even this can be avoided by using paraffin which has been previously boiled for some time with alkali.

<div align="center">ESTIMATION OF CARBON.</div>

Influence of other substances. The addition of metallic catalysts was found to have little effect on the course of the reaction. H_3PO_4 was found to moderate the vigour of the reaction and hence a mixture of H_3PO_4 and H_2SO_4 recommended by some workers [Schollenberger, 1916; Assoc. Off. Agric. Chem., 1930] was not used. Sulphur, sulphide and sulphites did not interfere.

Chlorides seriously interfered with the accuracy of the carbon determination on account of chlorine passing over; this could be conveniently avoided by adding 1 to 2 g. of HgO to the substance in the Kjeldahl flask before the addition of CrO_3 and H_2SO_4 [cf. Florentin, 1924; Subrahmanyan et al., 1934] and by adding H_2SO_4 in small quantities with gentle rotation of the flask.

The following procedure was tried with success in cases where the addition of HgO is for other reasons undesirable. After the addition of CrO_3 solution, the suction is continued for 2 or 3 min. to ensure a sufficiently low pressure in the

flask, then cock G is closed and cock F opened slightly to allow entry of air at the rate of a bubble per second. H_2SO_4 is added, in small portions at a time, with gentle rotation of the flask. Only sufficient of the substance should be taken to yield 100–200 mg. of CO_2, to avoid escape of CO_2 by bubbling upward through the thistle funnel E. At first a white cloud of HCl is formed, which however is kept down by the flow of air from the inlet tube C, and after a few seconds the bottom of the flask is filled with greenish yellow vapours of chlorine. After all the H_2SO_4 has been added, cock F is closed and the flask allowed to remain with gentle shaking from time to time for about 4–5 min. In this interval the chlorine is reabsorbed by the CrO_3-H_2SO_4 mixture with the probable formation of a chromic derivative which is not re-evolved on heating, and the flask clears. Cock G is now gradually opened and the contents of the flask digested as usual.

Organic and inorganic carbon. The method can be conveniently applied to the determination of organic and inorganic carbon (carbonates) in the soil by the determination of total carbon on one sample and of carbonates on another [Schollenberger, 1930]. A preliminary removal of carbonates by the action of dilute H_2SO_4 or phosphoric acid followed by the estimation of organic carbon by chromic digestion (keeping the ultimate H_2SO_4: water ratio at the optimum point) has also been found to give concordant results.

The values for carbon obtained by this method for a selection of the large number of pure organic compounds, soils and plant materials examined by this method are given in Table I and show satisfactory agreement with the dry combustion values.

Table I. *Recoveries of carbon and nitrogen by chromic digestion.*

Soils	Materials	% carbon		% total N recovered as		
		Dry combustion	Chromic digestion	NH$_3$-N	NO$_3$-N	Gaseous N (lost)
1.	Arnish moor soil*	53·0	53·1	91	4·5	4·5
2.	Insch soil, Auchinbradie*	5·15	5·18	90	5	5
3.	Bangor soil*	3·15	3·11	85	7·5	7·5
4.	Hungarian Brown Chernozem†	2·36	2·29	90	Nil	10
5.	Ohehe tropical forest soil†	2·74	2·88	90	5	5
6.	Barmera Australian chloride soil, 4·6% Cl.†	0·53	0·56	65	35	Nil
7.	Ohio leaf mould layer†	17·83	18·01	95	2·5	2·5
8.	Hungarian Solonetz†	1·86	1·81	90	10	Nil
Plant materials						
1.	Rice straw	32·6	32·2	90	10	Nil
2.	Bracken leaves	44·7	45·0	90	10	Nil
3.	Grass (lawn mowings)	38·4	38·6	87·5	7·5	5
4.	Rape seed cake	40·0	40·4	90	5	5
Proteins						
1.	Egg albumin	42·1	42·4	90	5	5
2.	Caseinogen	43·6	43·2	92·5	2·5	5
3.	Blood albumin	43·2	43·0	90	5	5
4.	Edestin	—	—	82·5	7·5	10
5.	Gelatin	—	—	90	4	6
6.	Glutenin	—	—	92·5	3	4·5
7.	Haemoglobin	—	—	91	3	6
8.	Fibrin (blood)	—	—	90	4	6
9.	Keratin	—	—	92·5	3	4·5
10.	Peptone	—	—	88	4	8

* Soils from the A.E.A. Co-operative Analyses Series.
† Soils from the I.S.S.S. Organic Carbon Series, 1935.
The figures for carbon in soils by Dry Combustion are taken from: Report of Organic Carbon Committee, *Trans. of the Third Int. Congress of Soil Science*, 1, 1935; Report of Analysis of Soils Sub-Committee, *Agric. Progress*, 11, 1934.

ESTIMATION OF NITROGEN.

The residues after digestion with CrO_3-H_2SO_4 mixture yield low results for nitrogen, as compared with the Kjeldahl values, because a portion of the organic nitrogen is oxidised to nitrate. Added ammonium salts are quantitatively recovered under these conditions, showing that the phenomenon is not one of oxidation of ammonia by chromic acid. A more detailed examination of the course of oxidation of organic nitrogen in soils, plant materials, extracted proteins and simple organic substances has shown that of the total nitrogen in a substance a definite fraction is reduced to ammonia, the proportion depending on the structure of the substance. Of the fraction not reduced to ammonia a portion is oxidised to nitrate and the rest lost in gaseous form. These proportions of recovered ammonia expressed as percentages or fractions of total nitrogen have been defined as "oxidation constants" [Acharya, 1935]. Their values are fairly constant if amounts of the substances containing above 10 mg. of N in the case of soils and 20 mg. in the case of substances richer in nitrogen (e.g. proteins) are taken for oxidation. At lower levels a portion or the whole of the gaseous nitrogen which would otherwise be lost tends to be fixed as nitrate. The course of oxidation of pure organic substances will be dealt with in detail in a later communication.

The nitrogen values obtained by chromic digestion of some typical soils, plant materials and proteins are shown in Table I. It will be noted that in the case of soils and plant materials the recovery of nitrogen as ammonia is of the order of 90 % and hence an average correction of 10 % on the figure for direct distillation with alkali would give results agreeing within 1–2 % with the Kjeldahl figure. In the case of extracted proteins, however, there is more variation and this correction may not be found to be so satisfactory. It may be mentioned that in the case of organic compounds such as the purines, guanidine, iminazole and pyrimidine derivatives and others having two or more nitrogen atoms linked to the same C atom, the recovery of NH_3-N is much lower and varies greatly from one compound to another. The application of a correction in such cases would obviously be of no value.

Influence of the amount of substance taken. This is shown for some typical soils and plant materials in Table II. For amounts of soil containing 5 mg./g. and less of nitrogen, the gaseous nitrogen fraction is oxidised to nitrate and hence the modified procedure to include nitrates (p. 246) gives results agreeing with the Kjeldahl figure. As the usual soil aliquot taken for carbon estimation corresponds to 50–75 mg. of carbon and the C : N ratio in most soils is about 10 : 1, the nitrogen values of the residues in most digested soil samples would be of the order of 5–7 mg., and the modified procedure to include nitrates would give results agreeing to within 98–100 % of the Kjeldahl figure.

Influence of chlorine. The interference of chlorine in the estimation of carbon has been noted already. It was found that chlorine interferes also in the estimation of nitrogen in that it increases the amount of nitrate formed at the cost of ammonia (Table III). The low values obtained in presence of chlorine have been attributed [Anderson and Schutte, 1924] to the liberation of free nitrogen according to the equation

$$K_2Cr_2O_7 + 2NH_4Cl \rightarrow 2KCl + Cr_2O_3 + 4H_2O + N_2,$$

or to the interaction of hypochlorous acid formed with ammonia [Subrahmanyan et al., 1934]. The quantitative recovery of ammonium salts in presence of added chlorine under the experimental conditions shows, however, that the action of chlorine is on the amino-groups of protein rather than on the ammonia formed.

Table II. *Influence of the amount of substance taken on nitrogen recovery.*

			After chromic digestion			
Material	Amount of substance taken g.	Total N by Kjeldahl mg.	NH$_3$-N mg.	NO$_3$-N mg.	Total N recovered mg.	Gaseous N (lost) mg.
1. Insch soil, Auchin-bradie	1	3·8	3·5	0·3	3·8	Nil
	1·5	5·7	5·2	0·5	5·7	Nil
	2	7·5	6·8	0·6	7·4	0·1
	3	11·3	10·2	0·7	10·9	0·4
	4	15·0	13·5	0·8	14·3	0·7
	5	18·8	17·0	0·9	17·9	0·9
2. Arnish Moor soil	0·2	3·8	3·5	0·3	3·8	Nil
	0·3	5·7	5·2	0·5	5·7	Nil
	0·4	7·6	6·9	0·5	7·4	0·2
	0·5	9·5	8·6	0·5	9·1	0·4
	0·6	11·4	10·3	0·5	10·8	0·6
3. Grass (lawn mowings)	0·10	3·6	3·2	0·4	3·6	Nil
	0·15	5·4	4·8	0·6	5·4	Nil
	0·2	7·2	6·4	0·6	7·0	0·2
	0·3	10·8	9·5	0·7	10·2	0·6
	0·5	18·0	15·8	1·0	17·2	1·2
4. Rape seed cake	0·1	5·6	5·2	0·4	5·6	Nil
	0·2	11·3	10·2	0·6	10·8	0·5
	0·3	17·0	15·2	1·0	16·2	0·8
	0·4	22·6	20·2	1·2	21·4	1·2
5. Bracken leaves	0·2	3·8	3·5	0·3	3·8	Nil
	0·4	7·6	6·9	0·7	7·6	Nil
	0·5	9·5	8·6	0·9	9·5	Nil
	0·6	11·4	10·3	0·9	11·2	0·2
6. Egg albumin	0·03	3·7	3·4	0·3	3·7	Nil
	0·05	6·2	5·6	0·6	6·2	Nil
	0·10	12·3	11·1	1·2	11·7	0·6
	0·20	24·4	22·1	1·2	23·3	1·1
	0·30	36·6	33·1	1·8	34·9	1·7

Table III. *Influence of chlorine on the recovery of nitrogen.*

(Figures expressed as percentages of total N.)

Material	No KCl added		0·1 g. KCl added		0·2 g. KCl added	
	% NH$_3$-N	% NO$_3$-N	% NH$_3$-N	% NO$_3$-N	% NH$_3$-N	% NO$_3$-N
1. Egg albumin	90	5	87·2	7·6	84·3	10·4
2. Caseinogen	92·5	2·5	90·6	4·1	88·6	6·2
3. Rice straw	90	10	87·4	12·4	85·5	14·2
4. Rape seed cake	90	5	87·7	7·1	84·7	10·1
5. Ohio leaf mould	95	2·5	88·7	8·6	82·8	14·4
6. Insch soil, Auchin-bradie	90	5	83·5	11·2	74·4	20·2

Estimation of nitrate in presence of chromic and sulphuric acids. As other substances besides nitrate, *e.g.* hydroxylamine, yield ammonia on treatment with reducing agents, it seemed desirable to show the formation of nitrate and if possible to estimate it in the oxidising mixture by a more specific reaction. The amount of nitrate formed being usually of the order of 0·5–1 mg. in presence of 40–60 g. of H_2SO_4 and 3–5 g. of CrO_3, the phenoldisulphonic acid method of estimation was examined.

The method finally worked out was as follows. The digested residue (filtered through glass wool if necessary) is made up to a volume of 500 ml. or 1000 ml. (depending on the concentration of sulphuric and chromic acids) in a tall beaker.

It is warmed to 50° and the excess of CrO_3 reduced by Na_2SO_3 (solid) added little by little, testing externally with starch-iodide indicator. The liquid is rendered alkaline by the addition of solid $CaCO_3$ followed by $Ca(OH)_2$, stirred well, allowed to settle and an aliquot of 50 ml. or 100 ml. of the clear supernatant liquid pipetted off for evaporation and estimation of nitrate in the usual way [Assoc. Off. Agric. Chem., 1930].

The method was found to give quantitative recoveries of added nitrate and the results with soils and plant materials agreed with those obtained by the Devarda method.

SUMMARY.

1. A procedure is described for the estimation of carbon and nitrogen in soils, plant materials and organic compounds by the action of a mixture of chromic and sulphuric acids under reduced pressure.

2. The results for nitrogen in soils and plant materials are too low on account of one portion being oxidised to nitrate and another lost in gaseous form. Where, however, the aliquot taken contains about 5 mg. or less of nitrogen the gaseous portion is also fixed as nitrate and correct figures are obtained by the estimation of the ammoniacal and nitrate-nitrogen present. For amounts higher than 5 mg. an average correction of 10 % on the ammoniacal nitrogen formed gives results agreeing to within 98–100 % of the Kjeldahl figure.

3. A procedure is described for the estimation of small amounts of nitrate in presence of large quantities of sulphuric and chromic acids.

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