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TRANSFORMATION OF ACETATE CARBON INTO CARBOHYDRATE AND AMINO ACID METABOLITES DURING DECOMPOSITION IN SOIL

L. H. SØRENSEN

Danish Atomic Energy Commission Research Establishment Risö, Roskilde, Denmark

and

E. A. PAUL

Department of Soil Science, University of Saskatchewan, Saskatoon, Canada

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Summary—Carbon-14-labelled acetate was added to a heavy clay soil of pH 7·6 to study the transformation of acetate carbon into carbohydrate and amino acid metabolites during decomposition. The acetate was totally metabolized after 6 days of incubation at 25°C when 70% of the labelled carbon had been evolved as CO_2. Maximum incorporation of trace-C into the various organic fractions was observed after 4 days when 19% of residual, labelled carbon in the soil was located in carbohydrates, 29% in amino acids and 21% in the insoluble residue of the soil. The curves showing the amounts of labelled carbon located in carbohydrates and amino acid metabolites show a curvilinear form during the first 30 days of incubation, indicating a variety of chemical compounds decaying at different rates. After this time, the decay curves became straight lines indicating a greater homogeneity of the metabolites. After 200 days of incubation, 2·2% of the labelled carbon originally added to the soil was located in carbohydrate metabolites, 7% in amino acid metabolites and 5% in the insoluble residue. The carbon in these fractions accounted for 77% of the total, residual, labelled carbon in the soil; 12% in carbohydrates, 40% in amino acids and 25% in the insoluble residue. The remainder was non-indentified, acid-soluble material.

The carbohydrate metabolites originating from the labelled carbon decayed at a faster rate than the amino acid metabolites, indicating different protective mechanisms for these materials.

INTRODUCTION

A SUBSTANTIAL amount of the carbon in soil organic matter is located in carbohydrates and amino acids. The relation of these compounds to other soil organic matter constituents is unknown; they are generally considered to be constituents of large molecules such as polysaccharides, peptides and proteins, which by reaction with other soil constituents have obtained a certain degree of stability in the soil (Bremner, 1967; Gupta, 1967). Former investigations by the senior author (Sørensen, 1967) utilizing ^{14}C-labelled cellulose indicated that a part of the added cellulose carbon was transformed into amino acid metabolites during the decomposition, and that these metabolites decayed slowly, and that the stabilizing factor could be adsorption to clay minerals.

The current investigation was designed to measure the amounts of carbohydrate and amino acid metabolites formed in a prairie soil during decomposition of an added carbon source, and of the persistence of these metabolites in the soil. Such an investigation, performed under laboratory conditions, is a necessary prerequisite: to a better understanding of the factors involved in stabilization of the metabolic products which arise during decay of plant residues under field conditions, and to a measure of their turnover rate (Paul, 1970).

The carbon was added as ^{14}C-labelled acetate. Acetate is readily decomposed in the soil (Ivarson and Stevenson, 1964). Being chemically different from carbohydrates and amino acids, its microbial transformation into these compounds can be determined by normal isolation and radioactive counting techniques.

METHODS AND MATERIALS

Soil

The upper 15 cm of soil was sampled from a virgin short grass prairie site on the Matador Field Station of the Canadian International Biological Programme, situated in the southern part of Saskatchewan. The pH of the soil was 7·6 (water-saturated paste); the organic carbon content 1·7%; the inorganic carbon content 0·3%; the total nitrogen content 0·24%; the total clay content 65%.

Radioactive substrate

Equal amounts of sodium acetate-2-^{14}C and sodium acetate-1-^{14}C were mixed and added to acetic acid in an amount yielding 100 μCi/g of carbon.

The decomposition experiment

Air-dry soil passed through a 1 mm sieve, was moistened to 40 per cent of the soils waterholding capacity. This water contained enough of the above-mentioned acetic acid to add 500 mg of carbon per 100 g of soil, and ^{15}N-labelled $(NH_4)_2SO_4$ in an amount corresponding to 4 mg N/100 mg of added carbon. The addition of the acetic acid decreased the pH of the soil to 6·8, but after 12 hr storage of the soil sample at 4°C, the soil pH was again 7·6. A soil sample removed and dried immediately after the addition of the acetic acid is referred to as the zero incubation sample.

Three samples, each corresponding to 50 g of dry soil, were removed from the main portion and placed in 150 ml Erlenmeyer flasks. These flasks were fitted to an aeration train and the CO_2 collected in 0·2 N NaOH. After removal of an aliquot for the determination of radioactivity, the CO_2 was precipitated and titrated as barium carbonate. The moisture content of the soil samples was kept constant by addition of water after weighing the flasks. The incubation temperature was 25°C. Samples were withdrawn at intervals, from the main portion of soil, for the chemical determinations.

Extraction and determination of soil carbohydrates

Five grammes of ground, air-dry soil were moistened with 4 ml of 72% (v/v) H_2SO_4 and left for 4 hr at room temperature with occasional stirring; 96 ml of water were then added and the mixture boiled for 16 hr under reflux. After cooling, the mixture was filtered through glass fibre paper and the residue washed with 150 ml of water. Activated charcoal was added to an aliquot of this extract. After stirring for 5 min, the charcoal was removed by filtration, and washed with 1 N acetic acid. The carbohydrate content of the filtrate, from the charcoal, was determined by the anthrone method using glucose as a standard (Oades, 1967). The blanks and the standard had the same concentration of acetic acid as the filtrate.

The remaining H_2SO_4 extract was treated with the Dowex 2 (X-8), saturated with OH^-, until the pH was 1–2. The resin was removed by filtration and washed with water until no reducing sugars were present in the washings. This partly neutralized extract was then passed through a column of Dowex 50 (X-8), H^+ saturated, and Dowex 2 (X-8),

acetate saturated. The effluent plus washings were evaporated to dryness *in vacuo*. The residue was taken up in water, filtered, and samples were withdrawn for carbohydrate, carbon and radioactivity measurements.

The carbohydrate content in the non-deionized extract, as measured by the anthrone method, was fully recovered in the deionized extract, but only 80 per cent of the total carbon content of the deionized extract could be accounted for as glucose carbon as determined by the anthrone method. The glucose–carbon values obtained by the anthrone method, together with the values for specific radioactivity obtained from the carbon determinations have been used in the calculations.

No radioactivity was measured in the carbohydrate extract obtained from the soil sample taken at zero incubation time; this indicates that non-metabolized acetic acid did not contaminate the extract.

Extraction and determination of soil amino acids

Ten grammes of finely ground, air-dry soil were boiled with 100 ml of 0·5 N HCl for 16 hr, under reflux. The mixture was filtered, washed with water, returned to the boiling flask, and boiled with 100 ml of 6 N HCl for another 16 hr. The residue was removed by filtration, washed with water and dried. The combined hydrolysates were evaporated to 10 ml *in vacuo*, water was then added and the evaporation repeated twice. The residue was eventually taken up in water, filtered, and an aliquot reserved for nitrogen determinations, the remnant was run through a column of the Dowex 50 (X-8), H^+ saturated. The column was rinsed with water until the effluent was neutral, and the amino acids displaced with 2 N NH_4OH. The ammonia was removed from the effluent by evaporation to dryness *in vacuo*. The residue was taken up in water, filtered, and samples were withdrawn for determination of carbon, radioactivity and amino acid nitrogen (Bremner, 1965).

Alpha amino acid nitrogen and amino sugar nitrogen were determined in the extracts. Only about 60 per cent of the α-amino acid nitrogen added to the column in the non-deionized extract was recovered from the column. Total amino acid carbon was calculated by multiplying the amount of α-amino acid nitrogen plus amino sugar nitrogen in the non-deionized extract by five (Sørensen, 1967).

Carbon and radioactivity determinations

Carbon was determined by wet combustion *in vacuo* in an appparatus of the type described by Lindenbaum *et al.* (1948). The reagents were potassium dichromate and a mixture consisting of 600 ml concentrated H_2SO_4 and 400 ml 85% H_3PO_4 (Shaw, 1959). The carbon content of solutions were determined on samples evaporated to dryness *in vacuo* in the combustion tubes.

One millilitre of the 0·2 N NaOH solutions used for adsorption of CO_2 was transferred to counting vials. Addition of 10 ml of a toluene-Triton-X-100 scintillant (Turner, 1968) yielded a clear, homogeneous fluid, which was counted in a scintillation counter (Picker-Nuclear), with a counting efficiency of 77 per cent.

Carbonates were determined by boiling the soil with 1·25 N HCl and carbon liberated by decarboxylation was measured during a further 4·5 hr boiling period with 12% HCl (Reuszer, 1968).

RESULTS

Acetate carbon evolved as CO_2

The CO_2 collected in the NaOH traps did not fully measure the CO_2 production of the soil during decomposition of the added acetate. The lower solid curve in Fig. 1 indicates

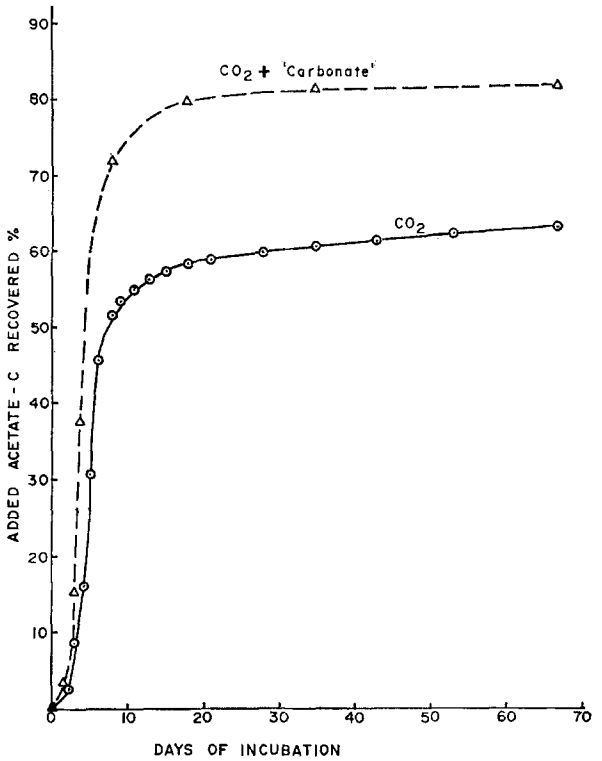

FIG. 1. Evolution of labelled CO_2-C during decomposition of ^{14}C-labelled acetate in soil. The lower solid line curve indicates the amounts collected in NaOH traps. The upper dotted curve indicates this amount plus labelled C released from inorganic carbonates in the soil by HCl treatment.

labelled-C collected as CO_2 in the NaOH traps. The upper dotted curve indicates this amount plus labelled-C in CO_2 released from carbonates in the soil by boiling with 1·25 N HCl. No labelled carbon was recovered by HCl treatment of the soil sample at zero incubation. After 2 days of incubation 3 mg of labelled carbon per 100 g of soil were recovered, after 4 days 30 mg, and after 5 days 103 mg. The amounts did not increase beyond this level; after 200 days of incubation 90 mg adsorbed labelled CO_2-C was recovered from 100 g of soil.

The soil contained 0·3 per cent inorganic carbonate carbon and had a high pH. Because of the high pH the substrate carbon was added as acetic acid which could have formed calcium acetate in the soil. Decomposition of the acetate by soil organisms would result in the replacement of the acetate ions by bicarbonate, resulting from the dissolved radioactive CO_2 in the soil water. The addition of 1·25 g of acetic acid (= 500 mg C), added per 100 g of soil, could theoretically account for the release of 125 mg of carbonate carbon.

Acetate carbon released by decarboxylation

The amount of labelled carbon released by decarboxylation increased from zero at the beginning of the incubation period to an amount corresponding to 1·7 per cent of residual labelled carbon in the soil after 5 days of incubation. The values remained at this level during the incubation period with a slight tendency to increase; after 200 days of incubation 3·7 per cent of residual labelled carbon in the soil was released by decarboxylation.

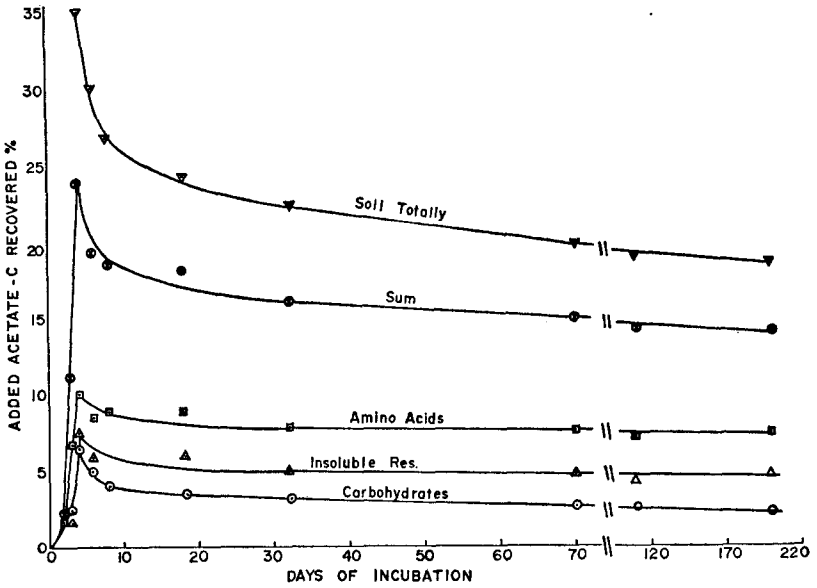

FIG. 2. Recovery of labelled carbon from soil during decomposition of ^{14}C-labelled acetate. The upper curve indicates total amounts of labelled carbon in organic form recovered from the soil. The three lower curves indicate the amounts recovered in carbohydrate and amino acid metabolites, and in the insoluble residue of the soil after 16 hr boiling with 6 N HCl. The curve marked 'sum' indicates the sum of the amounts in the three mentioned fractions.

The amount of carbon released by decarboxylation from native soil organic matter accounted for 2·5 per cent of the organic soil carbon.

Acetate carbon recovered from various fractions of soil organic matter

The upper curve in Fig. 2 indicates that 35, 27 and 18 per cent of the labelled carbon remained in the soil after 4, 30 and 200 days of incubation, respectively. These determinations of labelled carbon remaining in the soil were in good agreement with the data obtained from CO_2 evolution determinations if the labelled carbon in inorganic carbonates was taken into account.

The three lower curves in Fig. 2 indicate the amounts of labelled carbon recovered in carbohydrates, insoluble residue after HCl treatment, and amino acids, respectively, at various sampling intervals. Maximum incorporation of labelled carbon into these fractions was observed after 4 days of incubation when the amino acids accounted for 10% of the added labelled carbon, the insoluble residue for 7% and the carbohydrates for 6·6%; these values decreased to 8, 5 and 3% after 32 days of incubation, and to 7, 4·6 and 2·3% after 200 days of incubation.

The sum of the carbohydrates, insoluble residue and amino acids as shown in Fig. 2 by the curve marked 'sum' does not equal the total labelled carbon remaining in the soil. The difference is attributable to acid hydrolysable organic carbon not present as carbohydrates or amino acids and to loss of carbon during the acid hydrolysis.

Table 1 shows the amounts of labelled carbon in the three fractions expressed on the basis of total residual labelled carbon in the soil in organic form. After 4 days of incubation 19% of total residual labelled carbon was located in carbohydrates, 29% in amino acids and 21% in insoluble residue, and 31% was unaccounted for by these three fractions. After

TABLE 1. THE AMOUNTS OF THE ADDED ACETATE-^{14}C LOCATED IN CARBOHYDRATE METABOLITES, AMINO ACID METABOLITES AND INSOLUBLE RESIDUE

Days of incubation	Percent of total residual acetate-^{14}C in soil located in: carbohydrates	amino acids	insoluble residue	non-identified materials
2	1·9	8·4	0·8	*
3	8·4	21·4	6·0	*
4	18·8	28·6	20·9	31·7
6	16·4	28·0	19·5	36·1
8	14·9	33·1	21·0	31·0
18	14·2	35·5	24·9	25·3
32	13·8	35·1	22·6	28·6
70	13·4	38·6	24·1	23·9
108	13·5	37·4	22·2	26·9
200	12·3	40·4	25·4	22·1

* Acetate still present.

TABLE 2. THE CONTRIBUTION OF LABELLED METABOLITES TO VARIOUS FRACTIONS OF SOIL CARBON

Days of incubation	Acetate-^{14}C as percent of total C in: carbohydrates	amino acids	insoluble residue
2	0·8	2·2	0·1
3	5·1	8·3	1·5
4	11·4	12·4	5·8
6	9·3	10·5	4·7
8	7·8	11·0	4·7
18	6·7	10·7	5·1
32	6·1	9·7	4·4
70	5·7	9·4	4·5
108	5·4	8·8	3·8
200	5·2	9·2	4·3

32 days of incubation, 14% was located in carbohydrates, 35% in amino acids, 23% in insoluble residue and 29% unaccounted for. After 200 days, 12% was located in carbohydrates, 40% in amino acids, 25% in insoluble residue and 22% unaccounted for. It is seen from these figures that the carbohydrate metabolites in the soil turn over at a faster rate than the amino acid metabolites and the compounds comprising the insoluble residue. The slope of the curves in Fig. 2 indicates the same trend.

Table 2 shows the amounts of labelled carbon in the three fractions, expressed on the basis of total carbon (labelled + unlabelled). These figures also indicate that the newly-formed carbohydrate metabolites decay at a more rapid rate than the two other fractions. The sugars constituting the carbohydrate fraction were detected by means of paper chromatography, using 2 different solvent systems (Sørensen, 1963); glucose was by far the most abundant constituent; galactose, mannose, arabinose, xylose, ribose and uronic acid were also observed, besides some unidentified spots.

The amino acids constituting the amino acid fraction were not identified, but Ivarson and Stevenson (1964) who studied the decomposition of ^{14}C-labelled acetate in soil found labelled carbon in 15 amino acids liberated from soil organic matter by acid hydrolysis.

Similar results were obtained by Sørensen (1963) and others who have studied the decomposition of ^{14}C-labelled carbohydrates in soil.

The insoluble residue was not investigated in detail. About 30 per cent of the labelled carbon could be extracted with 0·1 N NaOH by shaking for 3 hr at room temperature (3 g of residue plus 20 ml of NaOH solution). Other work conducted in this department, utilizing ^{15}N, has indicated that this residue is very low in total nitrogen and contains an insignificant amount of ^{15}N immobilized during the incubation period (W. McGill, private communication, University of Saskatchewan).

Direct microscopic counts of microorganisms in the soil samples removed at intervals indicated that the fungal population increased rapidly during the initial stages of the incubation, whereas, the bacterial population reached their peak after about 20 days of incubation.

DISCUSSION

The data presented in this paper show that acetate added to a soil was rapidly metabolized, and calculations have indicated a half-life of 3·4 days. Maximum incorporation in carbohydrate and amino acid metabolites was observed after 4 days of incubation. This newly synthesized material was also subject to decomposition as indicated by the slope of the decay curves shown in Fig. 2. The curves have a curvilinear form during the first 30 days of the incubation period. This part of the curves is presumably a summation of a number of straight lines each indicating the decay rate of individual compounds. During the latter part of the incubation period the decay curves become straight lines indicating a greater homogeneity of the metabolites. The half-lives of the metabolites left in the soil after 90 days have been calculated to be 800 days for the carbohydrates, 1500 days for the compounds in the insoluble residue and 1600 days for the amino acid metabolites. These half-lives of 2–4·5 years will possibly be lengthened somewhat when soil from longer incubation periods is analysed.

Jansson (1963) observed over a period of 6 years, in pot experiments, a half-life of 15–22 years for ^{15}N fixed in organic form in the soil. These half-life figures were obtained on the basis of net mineralization rates. Jenkinson (1965) observed in field experiments a half-life of 4 years for carbon added to the soil in ^{14}C-labelled ryegrass. The senior author (Sørensen, 1967, and unpublished results) observed in laboratory experiments over a period of 5 years, a half-life of about 8 years for amino acid metabolites formed in a loam soil during decomposition of ^{14}C-labelled cellulose.

The chemical nature of the compounds in which the amino acids and the carbohydrates are located is unknown. Jenkinson (1966) has, by means of partial sterilization of the soil, followed by reinoculation, obtained evidence that a part of such metabolites could be located in living cells, the biomass; and calculated a half-life of 1·5 years for the biomass. Extra-cellular products, as for example enzymes, are however produced in the soil during decomposition of fresh organic material. Sørensen (1969) found that a part of the amino acid metabolites formed in soil during decomposition of added hemi-cellulose, could be located in enzyme-protein stabilized by adsorption to clay minerals.

The different half lives of the carbohydrate metabolites, and the amino acid metabolites observed in this investigation indicate a different form of stabilization for these two groups of compounds. If microbial cells were the major form for these two components the half-lives should be similar.

The metabolites located in the insoluble residue are of an unknown chemical nature. The

low incorporation of labelled nitrogen in the insoluble residue indicates that their nitrogen content is low. A part of the compounds might be artefacts produced during the acid treatment of the soil.

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