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THE DECOMPOSITION OF ORGANIC COMPOUNDS IN SOIL

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KEY WORDS

Amico acids Carbon dioxide evolution Casein Decomposition Glucose Humification Mineralization Respiration rate Phenolic compounds

SUMMARY

The course of the CO_2 evolution rates of soil samples has been followed continuously in the absence and in the presence of various organic compounds. After an incubation period of 300 hours at 13 and 20°C the CO_2 evolution from pasture soil (containing 1.76% soil organic carbon) amounted to 0.13 and 0.44 μg CO_2–C.g soil^{-1}.h^{-1}, respectively. For arable soil (containing 1.20% soil organic carbon) the rates amounted to 0.04 and 0.09 μg CO_2–C.g soil^{-1}.h^{-1}, respectively.

At 20°C larger amounts of the organic substrates added to the soil supplied with 20 μg NH_4NO_3–N.g soil^{-1} were lost as CO_2 than at 13°C, indicating a higher efficiency of the growth of microorganisms at lower temperatures. In the absence of NH_4NO_3 the respiration rates were initially higher than in its presence, suggesting that a part of the soil microflora is inhibited by low concentrations of NH_4NO_3. The amounts of carbon lost were low for phenolcarboxylic acids with OH groups in the ortho position. The replacement of one of these groups by a methoxyl group resulted in a larger amount of the C lost as CO_2. The replacement of the COOH group by a C=C-COOH group had a decreasing effect on the decomposition of the phenolic acids tested. The decomposition of vanillic acid, *p*-hydroxybenzoic acid, and of the benzoic acids with OH groups in the meta position was as complete as that of glucose, amino acids or casein. The decomposition of bacterial cells to CO_2 was considerably less than that of glucose.

No evidence could be obtained that the low percentage of substrate converted to CO_2 at the time of maximal respiration rate was due to the decreasing diffusion rate of substrate to the microbial colonies in the soil during the consumption of substrate.

INTRODUCTION

Soil respiration can be used as a measure of microbial activity[11]. The course of the respiration rate is generally obtained by measuring either O_2 uptake or CO_2 accumulation during successively short periods of time, which means that the respiration rate is determined periodically. The use of a differential infrared CO_2 analyzer, however, makes it possible to measure the CO_2 evolution rate of soil

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samples continuously[6]. The respiration curves obtained using this apparatus present a clear picture of the biological activity in the soil during the time of incubation.

In this investigation the CO_2 evolution rate has been measured continuously. The purpose of these studies is to obtain more information on the microbial activity of the soil under various experimental conditions. The following experiments have been performed: (1) The effect of temperature on the CO_2 evolution rates of samples of arable soil and pasture soil; (2) the effect of the addition of glucose and a number of phenolic compounds to soil samples on the CO_2 evolution rate in the absence and presence of NH_4NO_3 at 13 and 20°C. Phenolic compounds are included as these may be present in the soil as a result of the decomposition of lignin and the synthesis by plants and microorganisms[5]. Moreover, phenolic compounds are especially important with respect to soil humus formation[4]; (3) the CO_2 evolution rates in soil samples treated with glucose, casamino acids, casein and dead microbial cells have been compared.

Previously[6], it was found that less than 27% of glucose-C had been converted to CO_2 at the moment that the maximum respiration rate was reached. It was thought that the rate of respiration would be determined by the diffusion rate of glucose towards the microbial cells in the soil. Theoretically, this diffusion rate may be positively effected by the increase of the moisture content and negatively effected by an increase of the distances between the microbial colonies in the soil. Therefore, the following additional experiments have been performed: (4) The effect of water content of soil samples treated with glucose and the effect of re-inoculation of sterile soil in the absence and presence of glucose on the CO_2 evolution patterns have been measured; (5) finally, a comparison has been made between the course of the CO_2 evolution rate and the course of the glucose concentration. The same has been performed with a water culture of an *Arthrobacter* sp., since in this case diffusion problems as occurring in soil are absent.

MATERIALS AND METHODS

Pasture soil, obtained from the 1–5 cm layer of a 22-years old pasture on sandy soil, and arable soil from the upper 5 cm of arable land on sandy soil, were dried to 30% of waterholding capacity and passed through a 2-mm sieve. The pasture soil had a pH of 6.7 and contained 1.76% organic carbon. The pH of the arable soil was 6.2 and the organic carbon content was 1.20%.

The organic substrates were given as a powder, with the exception of casamino acids which were added as a neutral solution containing 2.0 g per 100 ml. Prior to the addition of phenolic compounds an equivalent amount of a solution of NaOH 0.1 *M* was added and mixed throughout the soil samples. Where KH_2PO_4 and NH_4NO_3 were added these compounds were supplied as a neutral solution. Bacterial cells were obtained by growing a bacterium (short rods), isolated from soil, in a medium consisting of 0.7% yeast extract and 1% glucose. After growth the cells were washed 3 times,

sterilized at 120°C and subsequently dried by lyophilization. The amounts of KH_2PO_4, NH_4NO_3, and organic compounds supplied to the soil samples are recorded in the figures and expressed as μg per g of dry soil. After thoroughly mixing of the added nutrients the soil samples were adjusted to 60% of their waterholding capacity, unless otherwise stated. Water was added periodically to keep the water content at this level.

For the re-inoculation experiments, portions of 36, 39 and 39 g of arable soil were sterilized at 120°C and subsequently mixed with 4, 1 and 0.1 g of fresh soil, respectively. When these mixtures were supplied with glucose, this was added after the sterilization of the soil and subsequently mixed. For comparison 40 g of fresh soil were also taken into consideration.

The sizes of the soil samples varied and amounted to 10, 40, 50 and 100 g of soil. Ten-g portions were transferred to 25-ml Erlenmeyer flasks and the other portions to tubes (11 by 4 cm i.d.).

For determining the CO_2 evolution rate of a water culture, an *Arthrobacter* sp., isolated from soil, was grown in a medium containing 1.0 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g yeast extract and 0.57 g NH_4NO_3 per liter of tap water. The following trace elements were added: 10 mg $FeCl_3.6H_2O$ and 1 ml of a mixture containing 0.1 g $CuSO_4.5H_2O$, 1.0 g $ZnSO_4.7H_2O$, 1.0 g $MnSO_4.7H_2O$, 10 mg Na_2MoO_4, 10 mg H_3BO_3 and 10 mg $CoCl_2$ per liter. The pH of the medium was adjusted to 6.8 with 10% NaOH. The glucose was sterilized separately and added to 10-ml portions of sterile medium contained in conical flasks of 50 ml capacity in which a Teflon stirring bar was present. Inoculation was performed with cells from 2-day old cultures on yeast extract-glucose agar. Each conical flask (plugged with cotton wool) were transferred into a large Erlenmeyer flask which was connected to the gas handling system and put on a magnetic stirrer to aerate the liquid culture.

A description of the method used to measure the CO_2 evolution rate is given elsewhere[6].

To determine the glucose concentration in soil, a mixture of 10 g of soil and 100 ml distilled water was stirred vigorously for 15 min. The suspension was then centrifuged. After filtering the supernatant through a glass fibre paper the clear solution was used for the determination of the glucose concentration by the method of Somogyi-Nelson[10]. For the liquid cultures the glucose concentration was determined in the culture filtrate.

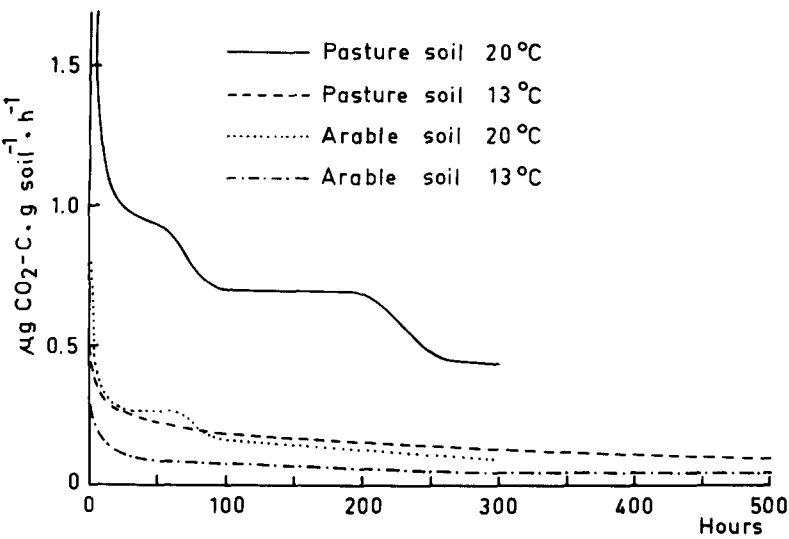

Fig. 1. CO_2 evolution rates in pasture soil and arable soil at 13 and 20°C. The sample size amounted to 100 g of soil.

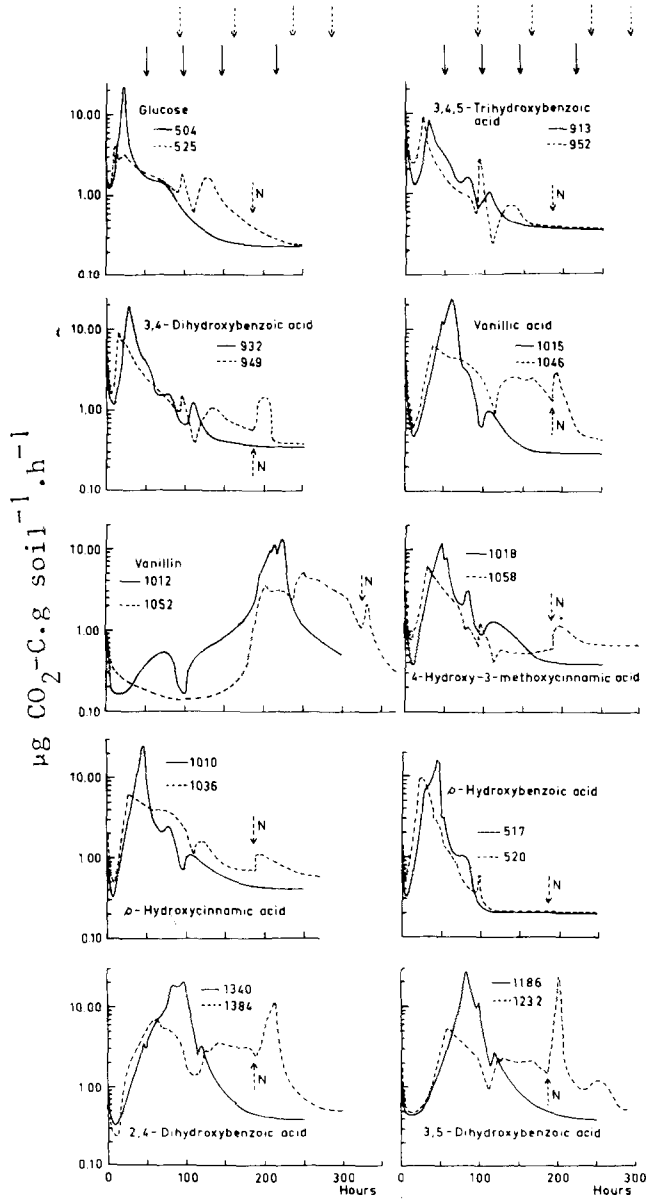

Fig. 2. The CO_2 evolution rates of arable soil after addition of glucose or phenolic compounds (room temperature). The figures in the graphs represent the amounts of substrate added (µg C.g soil^{-1}). The soil samples received 450 µg KH_2PO_4.g soil^{-1} supplied as a neutral solution. The sample size amounted to 10 g of soil. ——: Nitrogen was added at the start of the experiment (20 µg NH_4NO_3–N.g soil^{-1}); – – – –: N-deficient systems. Nitrogen was added at the times indicated by (broken) arrows + N (↓N). The *full* and *broken* arrows at the top of the figure indicate the times of water application to the NH_4NO_3 and the N-deficient systems, respectively.

The carbon content of the organic substrates used was determined with a Coleman Carbon Hydrogen Analyzer, Model 33 (Perkin Elmer Corp., Maywood, Il.) Prior to this determination the soil was ground by ball-milling. At the end of the incubation period the pH of the soil samples and media was determined.

RESULTS

The influence of temperature on the decomposition of soil organic matter

The CO_2 evolution decreased rapidly during the first 20 hours of the incubation period. After 20 hours the decrease was very small (Fig. 1). At 300 hours, the CO_2 evolution rates of pasture soil and arable soil at 20°C amounted to 0.44 and 0.09 $\mu g\ CO_2.g\ soil^{-1}.h^{-1}$, respectively. At 13°C the rates at 300 hours amounted to 0.13 for the pasture soil and 0.04 $\mu g\ CO_2$–C.g $soil^{-1}.h^{-1}$ for the arable soil.

The effect of NH_4NO_3 on the decomposition of glucose and phenolic compounds in soil at 20°C

This experiment was performed at about 20°C (room temperature). The irregular course of the respiration rate as illustrated in Fig. 2 is due to experimental errors (during some nights the central heating did not operate resulting in a lower temperature and consequently a lower respiration rate; where samples were not supplied with NH_4NO_3 the first addition of water had been delayed too long).

The decomposition of glucose, 3,4,5-trihydroxybenzoic acid (gallic acid), 3,4-dihydroxybenzoic acid, vanillic acid, 4-hydroxy-3-methoxycinnamic acid (ferulic acid), *p*-hydroxycinnamic acid, *p*-hydroxybenzoic acid and 2,4-dihydroxybenzoic acid at room temperature started to become exponential within 10 hours, while for 3,5-dihydroxybenzoic acid the exponential CO_2 production started some hours later (Fig. 2). Vanillin was decomposed very slowly during the first 100 hours. It is curious that for all the compounds tested the addition of 20 μg NH_4NO_3–N per g of soil at the start of the experiment resulted in a lower CO_2 evolution rate during the first stages of decomposition than without this addition.

During the exponential phase the rate of CO_2 evolution doubled in 3 to 4 hours when glucose, gallic acid, 3,4-dihydroxybenzoic acid or *p*-hydroxybenzoic acid were supplied (Fig. 2). For *p*-hydroxycinnamic acid, ferulic acid, vanillic acid, 3,5-dihydroxybenzoic acid and 2,4-dihydroxybenzoic acid the doubling times amounted to 5, 6, 8, 8 and 12 hours, respectively. Comparison of Fig. 2 with Fig. 3 shows that without the addition of NH_4NO_3 nitrogen became limiting

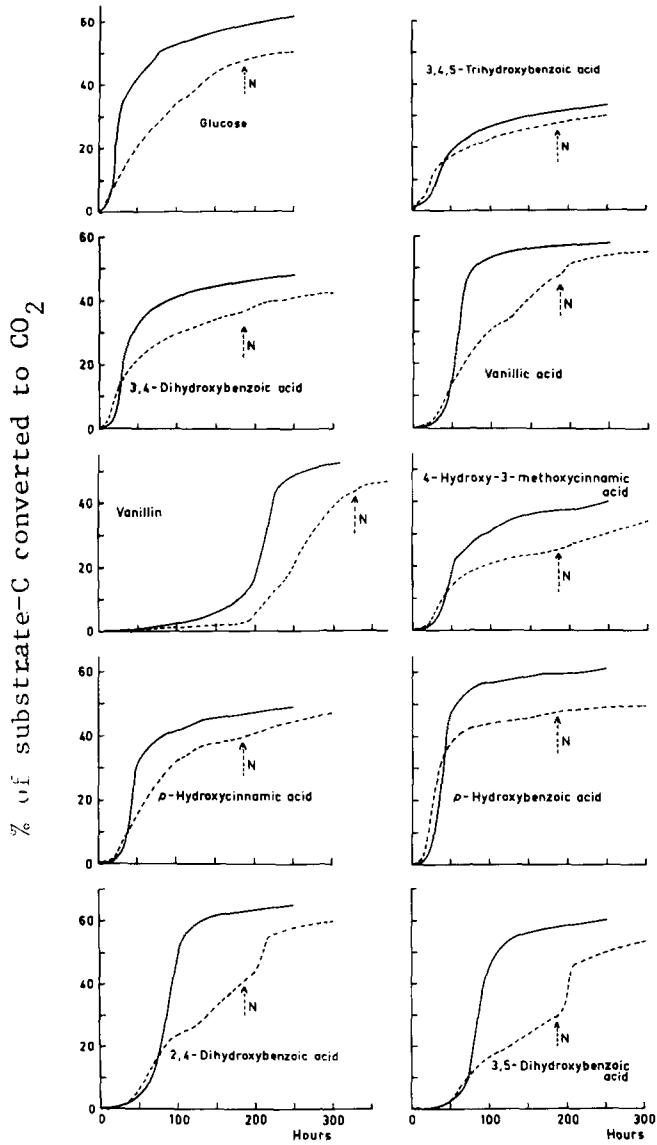

Fig. 3. The cumulative recovery of substrate-C as CO_2 at room temperature. Values of the produced CO_2 measured in variants without substrate have been subtracted. Further details are in the subscript of Fig. 2.

when 25 μg glucose-C and generally 50 μg C of the phenolic compounds per g of soil had been converted to CO_2. When the CO_2 evolution rate in the soil with NH_4NO_3 was maximal, less than 22% of the substrate-C had been converted to CO_2 for those substrates with a doubling time of the CO_2 evolution rate of 6 hours or less. For the substrates with doubling times of more than 6 hours, the percentages of substrate-C converted to CO_2 varied between 25 and 45% at the time that the CO_2 production was maximal. After reaching the maximum activity the rate of CO_2 production generally declined rapidly when NH_4NO_3 had been added initially, while without the addition of NH_4NO_3 the decline was generally less rapid.

In the presence of NH_4NO_3 the percentages of carbon of glucose, vanillic acid, *p*-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid and 2,4-dihydroxybenzoic acid converted to CO_2 at 250 hours amounted to 61, 59, 61, 60 and 65%, respectively (Fig. 3). These percentages were lower for gallic acid, 3,4-dihydroxybenzoic acid, ferulic acid and *p*-hydroxycinnamic acid, *viz* 33, 48, 40 and 50%, respectively.

Addition of NH_4NO_3 at 186 hours to samples which had not initially been supplied with nitrogen generally enhanced the CO_2 evolution rate, but the percentages of substrate-C converted to CO_2 at 250 hours were considerably lower than those where samples had been supplied with nitrogen at the start of the experiment. The pH values for all the soil samples at the end of the incubation period varied from 6.5 to 7.0.

The decomposition of glucose and phenolic compounds in soil at 13°C

The rate of CO_2 evolution in soil supplied with gallic acid and vanillin scarcely exceeded 1 μg CO_2–C.g $soil^{-1}.h^{-1}$ (Fig. 4). During the exponential stage of the decomposition of glucose, 3,4-dihydroxybenzoic acid and vanillic acid the time required for doubling the CO_2 evolution rate was equal to about 8, 8 and 15 hours, respectively, being about twice as long as those at 20°C. The percentages of the carbon of glucose, gallic acid, 3,4-dihydroxybenzoic acid, vanillic acid and vanillin converted to CO_2 in the presence of NH_4NO_3 at 160 hours amounted to 39, 19, 29, 30 and 20%, respectively (Fig. 5). The pH values at the end of the incubation period varied from 6.0 to 6.5.

The decomposition of glucose, casamino acids, casein and dead microbial cells at 13°C

The decomposition of casamino acids became exponential almost immediately after its addition, while the exponential phase for glucose, sterilized bacterial cells

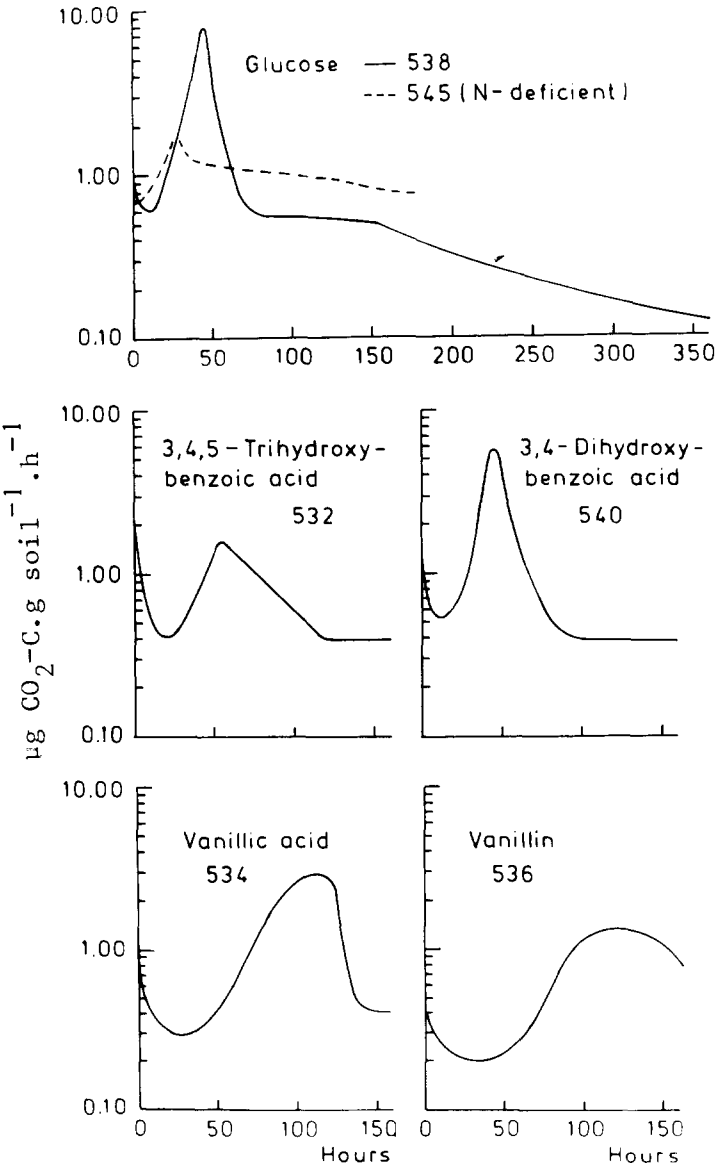

Fig. 4. The CO_2 evolution rates of arable soil at 13°C after addition of glucose or phenolic compounds. The figures in the graphs represent the amounts of substrate added (μg C.g soil^{-1}). The soil samples received 450 μg KH_2PO_4.g soil^{-1} supplied as a neutral solution. The sample size amounted to 50 g of soil. ——: Nitrogen was added at the start of the experiment (100 μg NH_4NO_3–N.g soil^{-1}); – – – –: No NH_4NO_3 was added.

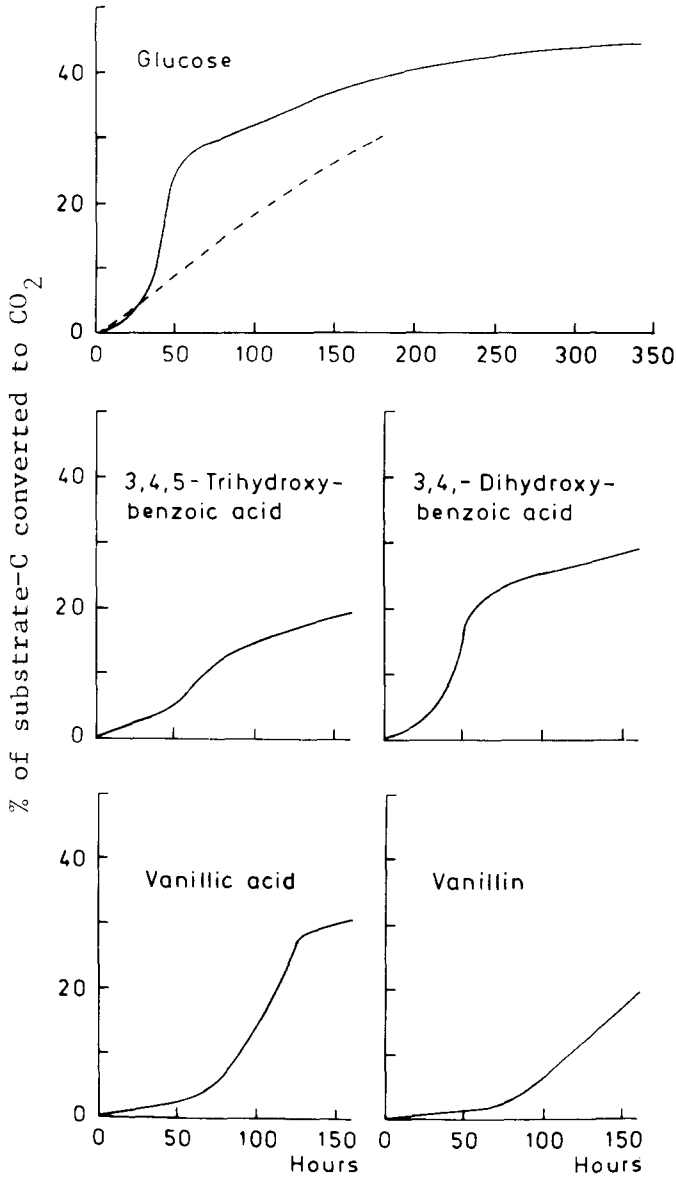

Fig. 5. The cumulative recovery of substrate-C as CO_2 at 13°C. Values of the produced CO_2 measured in variants without substrate have been subtracted. Further details are in subscript of Fig. 4.

and casein was reached 12, 12 and 30 hours later, respectively (Fig. 6). At 140 hours the percentage of casamino acid-C converted to CO_2 amounted to 47%, those of glucose and casein to 41%, while that of bacterial cells amounted to only 29%.

The effect of water content on the course of decomposition of glucose in soil samples

This experiment followed the observation that only a small part of the substrate had been converted to CO_2 when the CO_2 evolution rate was maximal. Soil samples supplied with glucose were subjected to 6 different water contents: 8.9, 10.2, 11.6, 14.3 (being 60% of waterholding capacity), 16.9 and 19.6%. The course of the respiration rate was the same for all water contents during the first 33 hours (Fig. 7). After this period the respiration rate at the highest water content remained more or less constant for 33 hours (probably due to O_2 deficiency), while the respiration rates at the other water contents reached a sharp peak at 43 hours after start of the experiment. A slightly positive effect of the increase of water content from 8.9 to 16.9% was only obtained after a period of 30 hours since the maximum respiration rate was reached. At 140 hours the percentage of glucose converted to CO_2 was the highest at the highest soil water content.

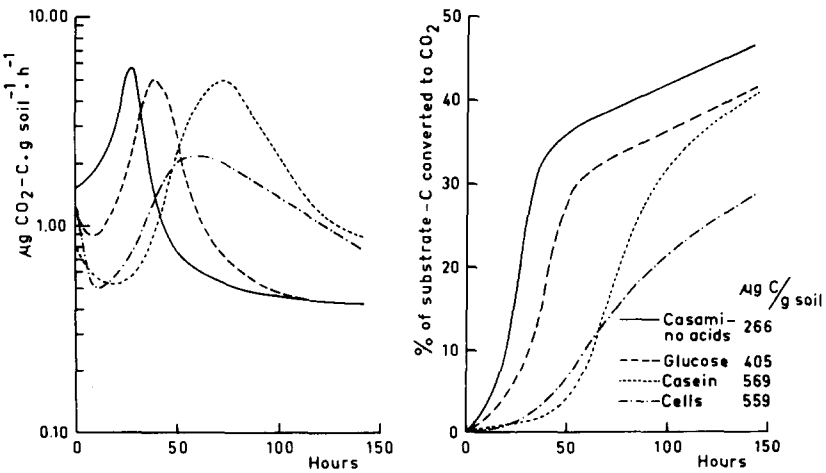

Fig. 6. The decomposition of glucose, casamino acids, casein and dead bacterial cells at 13°C. The soil samples received 450 µg KH_2PO_4.g soil^{-1} supplied as a neutral solution. The sample size amounted to 10 g of soil.

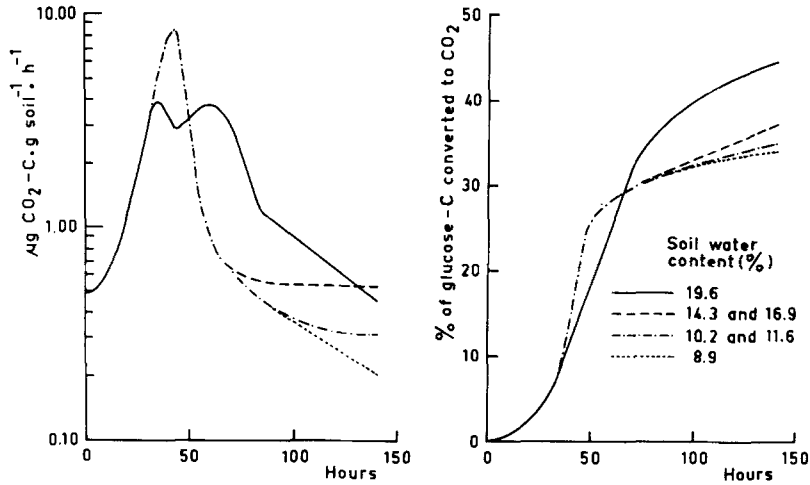

Fig. 7. The influence of the soil water content on the course of the decomposition of glucose at 13°C. The soil samples were supplied with 500 µg glucose-C, 450 µg KH_2PO_4, and 100 µg NH_4NO_3–N per g of soil. The sample size amounted to 40 g of soil. The data of the left figure have been corrected for CO_2 evolved from soil without glucose added.

The effect of re-inoculation of sterile soil

The CO_2 evolution rates of the unsterilized soil samples without added glucose decreased, while those of the re-inoculated samples increased initially (Fig. 8). The presence of glucose in the re-inoculated samples had no influence on the CO_2 evolution rate during the first part of the incubation period. When the CO_2 evolution rates of the fresh soil and of the sterile soil samples amended with 4, 1 and 0.1 g of fresh soil reached maximum values, 17, 18, 23 and 21%, respectively, of the applied glucose had been converted to CO_2 (when a possible priming effect of glucose is not taken into consideration). These results are in contrast to the expectation that diffusion processes of substrates to the colonies are responsible for the low percentage of substrate converted to CO_2 at the time of maximal respiration rate (see Introduction). At 140 hours 11.5, 62, 64 and 58 µg CO_2–C.g $soil^{-1}$, respectively, had been evolved from the soil organic matter and 37, 46, 44 and 43%, respectively, of the added C had been lost as CO_2.

The relation between the course of the CO_2 evolution rate and the glucose concentration in soil samples at 20°C

When the CO_2 evolution rate in the soil samples treated with 200 µg glucose-C.g $soil^{-1}$ was maximal, 22% of the glucose-C had been converted to CO_2, while still

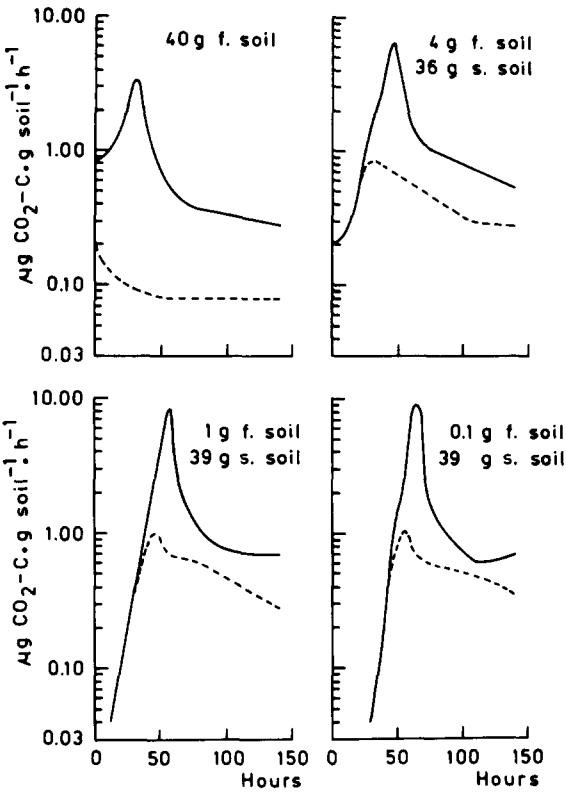

Fig. 8. The influence of re-inoculation of arable soil on the CO_2 evolution rate at 13°C with (——) and without (– – –) 270 µg glucose-C. The soil samples were supplied with 1100 µg KH_2PO_4 and 50 µg NH_4NO_3–N per g of soil (f = fresh soil, s = sterile soil).

only 15% of the added glucose was present as such (Fig. 9). Thus 85% of the added glucose had been used and only about one fourth part of this consumed amount had been converted to CO_2. The remaining 15% of the added glucose was utilized with a decreasing rate.

The relation between the CO_2 evolution rate and the glucose concentration in a water culture of an Arthrobacter *sp.*

The temperature of the liquid culture was 26°C. The rate of the CO_2 evolution doubled in 1.5 hour in the presence of glucose. When the CO_2 evolution was maximal, 21% of the glucose-C had been converted to CO_2, while still only 25%

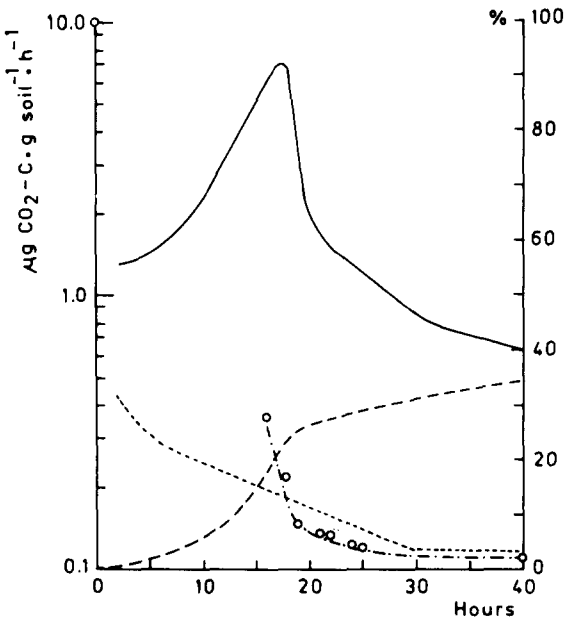

Fig. 9. The relation between the course of the CO_2 evolution and the glucose consumption at 20°C in samples of arable soil supplied with 200 µg glucose-C, 50 µg NH_4NO_3–N and 450 µg KH_2PO_4 per g of soil. ——: respiration rate in the presence of glucose; ·····: respiration rate in the absence of glucose; – – – –: % of glucose converted to CO_2; -·-·-: % of added glucose still present in the soil.

of the added glucose was present as such (Fig. 10). Thus 75% of the added glucose had been used and of this amount something more than a quarter had been converted to CO_2. These results are similar to those obtained in the previous experiment.

DISCUSSION

The rapid decreasing respiration rate of the untreated soil samples during the first 20 hours of the incubation period are probably due to removal of the soil samples from their environment and to the mixing of the samples. The respiration rates decreased slowly after this period and remained more or less constant after 300 hours. It is interesting to compare the values for the respiration rates after 300 hours with those obtained by Chahal and Wagner[11] who recorded average respiration rates of 0.3 µg CO_2–C.g $soil^{-1}.h^{-1}$ at 25°C. When the CO_2 evolution rates at 13°C and at 300 hours are used for the calculation of the percentage of soil organic matter carbon decomposed to CO_2 during 200 days, these percentages

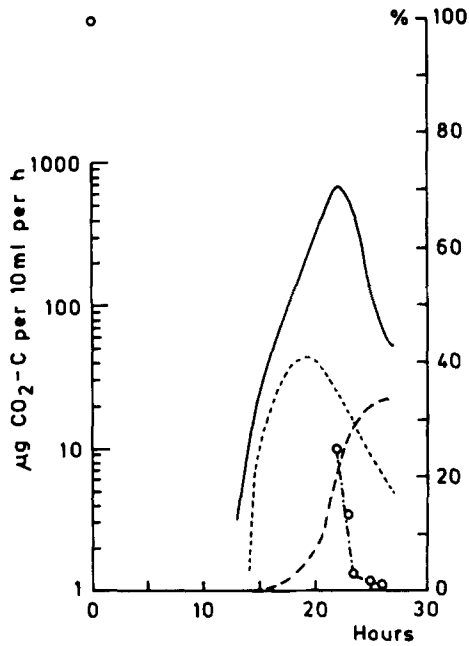

Fig. 10. The relation between the course of the CO_2 evolution and the glucose consumption by an *Arthrobacter* sp. in a liquid medium at 26°C. ——: respiration rate in the presence of 7670 µg glucose-C per 10 ml; ·····: respiration rate in the absence of glucose; – – –: % of glucose-C converted to CO_2; -·-·-: % of added glucose still present.

for the pasture soil amounted to 3.5% and for the arable soil to 1.6%. Figures of the same order of magnitude have been recorded elsewhere[1].

The initial reduction of the CO_2 evolution rate of soil samples treated with organic compounds by NH_4NO_3 is remarkable. The same kind of reduction was noticed previously by other investigators using the Warburg technique[3]. Here the reduction of CO_2 evolution was ascribed to the production of gases resulting from denitrification and consequently suggesting no real reduction of the CO_2 evolution. In the present investigation where the CO_2 evolution was measured directly it has been shown that the respiration is reduced by NH_4NO_3 during the initial stage of incubation. Reduction of CO_2 evolution from field plots by N fertilization has been recorded recently[7]. The negative influence of NH_4NO_3 on the CO_2 evolution might be a salt effect, although the concentration of added NH_4NO_3 was low, *viz* 57 µg.g soil^{-1}. However, McCormick and Wolf[9] recorded that 250 µg NaCl.g soil^{-1} significantly reduced CO_2 evolution. An explanation for the observed phenomenon might be that a part of the soil microflora is

inhibited by a low salt concentration, so that the decomposition of glucose starts with a lower number of microorganisms.

Greater percentages of the organic substrates were lost as CO_2 at 20°C than at 13°C, indicating a higher efficiency at lower temperature. This is not always realized, as recently[8] the same efficiency factors (0.3 and 0.4) have been used for modelling the growth of bacteria during glucose decomposition in soil under different temperatures.

The percentages of carbon lost as CO_2 were low for the phenolic compounds tested with OH groups in the ortho position (gallic acid and 3,4-dihydroxybenzoic acid. Replacement of one of these groups by a methoxyl group (vanillic acid) resulted in a greater percentage of the C lost as CO_2. The replacement of the COOH group by a C=C–COOH group had a decreasing effect on the decomposition of the phenolic acids tested. The decomposition of vanillic acid, *p*-hydroxybenzoic acid and of the benzoic acids with OH groups in the meta position was similar to that of glucose, amino acids or casein. The phenolic compounds with a low percentage of decomposition are probably in part stabilized by incorporation into humic compounds during oxidation[4].

No evidence could be obtained that the low percentage of substrate converted to CO_2 at the time of maximal respiration rate was due to the decreasing diffusion rates of substrate to the microbial colonies in the soil during the consumption of substrate. At that time the larger part of the substrate was probably converted to biosynthetic intermediates and not yet to microbial cells or CO_2.

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