FURTHER OBSERVATIONS ON THE METABOLIC ACTIVITY OF THE SOIL MICROFLORA[1]

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ABSTRACT

The respiratory activity of the microflora of intact, untreated soil has been studied manometrically. Sugars, amino acids, aromatic compounds and tricarboxylic acid cycle intermediates were tested in four soils varying markedly in fertility and organic matter content. The effects of soil reaction and substrate concentration were also investigated. The results are discussed in relation to the use of metabolic or enzymatic activity of soil as an index of fertility.

INTRODUCTION

The use of manometric methods for the study of the metabolic behaviour of soil as an ecological unit has been described previously (Katznelson and Stevenson, 1956). It was found that intact, untreated soil could metabolize mixtures of various substrates such as amino acids, sugars and organic acids. The most striking effect was obtained with casamino acids which appeared to be oxidized adaptively; this adaptation was inhibited by 2, 4-dinitrophenol. Other mixtures of substrates were oxidized directly at a fairly constant rate. Stevenson and Katznelson (1958) have recently reported on the direct oxidation of ethanol and acetate in soil, and studies have been continued in an effort to find other single compounds rather than mixtures, whose oxidation might be used as an index of microbial activity in soils.

The following studies include a general survey of the oxidation of numerous substrates in a number of different soils. In addition the effects of substrate concentration and soil *p*H on oxidation were investigated.

EXPERIMENTAL

Manometric Techniques

A 4 g. sample of the 0.5–2.0 mm. fraction of a soil was placed in the main chamber of a conventional Warburg vessel. The soil was brought to 60 per cent holding capacity by the direct addition of water or substrate. "Accordion-pleated" pieces of filter paper were inserted in the centre well and 0.2 ml. of 20 per cent, potassium hydroxide added. The vessels were then attached to their respective manometers and placed in the water bath at 30°C. Manometers remained static during the experimental period. Unless otherwise stated results are presented as accumulated oxygen uptake with the appropriate endogenous values subtracted. Inasmuch as soils arriving from the field exhibited considerable variation in moisture content, they were air-dried for a short period before sieving and weighing.

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Plating Techniques

Numbers of bacteria and fungi were determined by plating appropriate soil dilutions with Soil Extract Agar and Rose Bengal-Streptomycin Agar.

Results

Initial studies were undertaken with a variety of substrates in different concentration in one soil. Accumulative oxygen uptake values for a period of six hours are given in Table 1.

Table I

Oxidation of a Variety of Substrates in Soil

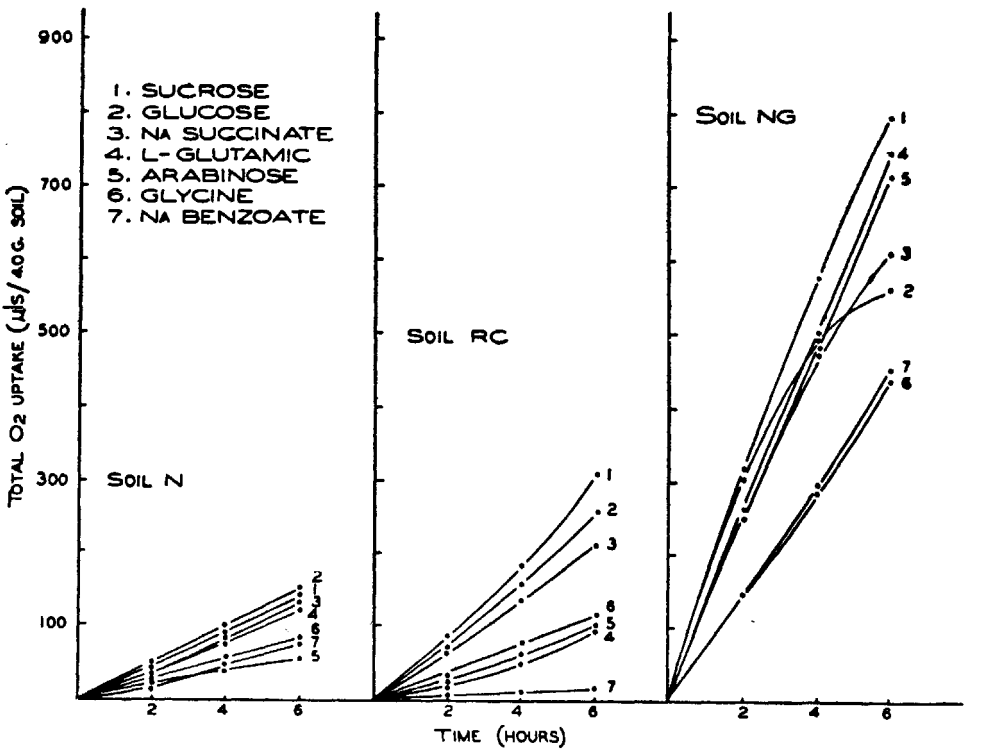
Substrate	Conc.* mgms.	O_2 uptake	Substrate	Conc.* μM	O_2 uptake
Sucrose	5	288	Na Benzoate	25	66A
	10	389		50	22A
	20	252	Catechol	25	19A
Lactose	5	13A**		50	42A
	10	33A	Guiacol	25	0
	20	6A		50	0
Maltose	5	89	Vanillin	25	13A
	10	123		50	0A
	20	74	l-Alanine	25	3
Fructose	5	72		50	17
	10	41	Arginine	25	27
	20	65		50	73
Glucose	5	133	Glycine	25	58
	10	124		50	59
	20	89			
Galactose	5	0	Na Pyruvate	25	50
	10	0	Na Citrate	25	20A
	20	20	α-ketoglutarate	25	20A
Xylose	5	0	Na Succinate	25	158
	10	6A	Na Fumarate	25	120
	20	19A	Na Malate	25	141
Arabinose	5	12	Na Acetate	25	30
	10	23	Na Cis-Aconitate	25	28
	20	19	Na Iso-citrate	25	0

*Concentrations of substrates per 4.0 gms. soil.
**Adaptive oxidation.

Sucrose proved to be the most readily oxidizable substrate used and it is noteworthy that the oxygen uptake for this compound was greater than the sum of the oxygen values for fructose and glucose in equivalent concentrations. Maltose was utilized at a moderate rate whereas lactose oxidation was very slow. Glucose was the most readily available of the three hexose sugars tested while little activity was noted with galactose. Pertose sugars were also oxidized very slowly with a slow adaptation observed in the case of xylose. With the amino acids studied, glycine was found to be oxidized at a steady rate while the oxidation of l-alanine and arginine proceeded more slowly. Little oxygen uptake was noted with the aromatic compounds and this usually occurred after a fairly long lag period varying from two hours for benzoate and catechol to four hours for vanillin. A distinct inhibition of respiration occurred with guiacol and with the higher concentration of benzoate and vanillin. Of the tricarboxylic acid cycle intermediates the dicarboxylic acids were oxidized most rapidly and directly. Pyruvate and acetate were

utilized more slowly. The remaining acids were oxidized very slowly with a gradual adaptation occurring.

A comparison of the oxidation of seven selected substrates by three other soils of varying fertility and organic matter contents are presented in Figure 1.



TEXT-FIG. 1

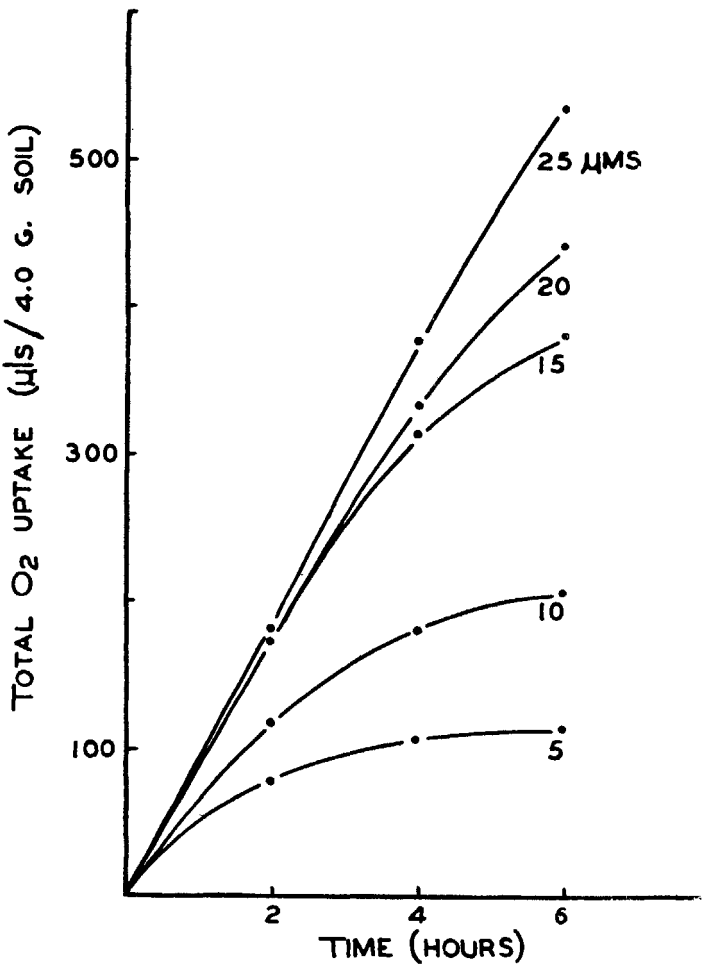
Oxidation of seven selected substrates in three soils. Warburg vessels contained 4.0 gms. of soil supplemented with 25μ Ms of substrate.

A distinct gradation in overall activity is observed among the three soils illustrated. All substrates are metabolized extremely rapidly in soil NG. This soil is the highest in organic matter content (Stevenson, 1956) as well as in metabolic activity (Katznelson and Stevenson, 1956). Intermediary rates of oxidation are noted in the case of the Rideau Clay (RC) soil whereas least activity is observed in the relatively infertile soil N. It is of interest to note that the substrates most actively oxidized by soil X (Table 1), namely, sucrose, glucose and succinate are also oxidized most readily by soils N and RC. Rates of oxidation of these substrates are also among the highest with soil NG although substrate concentrations may have become limiting in the case of succinate and glucose.

The effect of substrate concentration on the oxidation of succinate in soil NG is illustrated in Fig. 2.

With extremely low concentration (5-10μ M) the initial oxidation rates are somewhat lower than those of the higher concentrations. In all cases the duration of this intial direct oxidation increases as substrate concentration increases, to be followed by a rapid decline in oxidation rates.

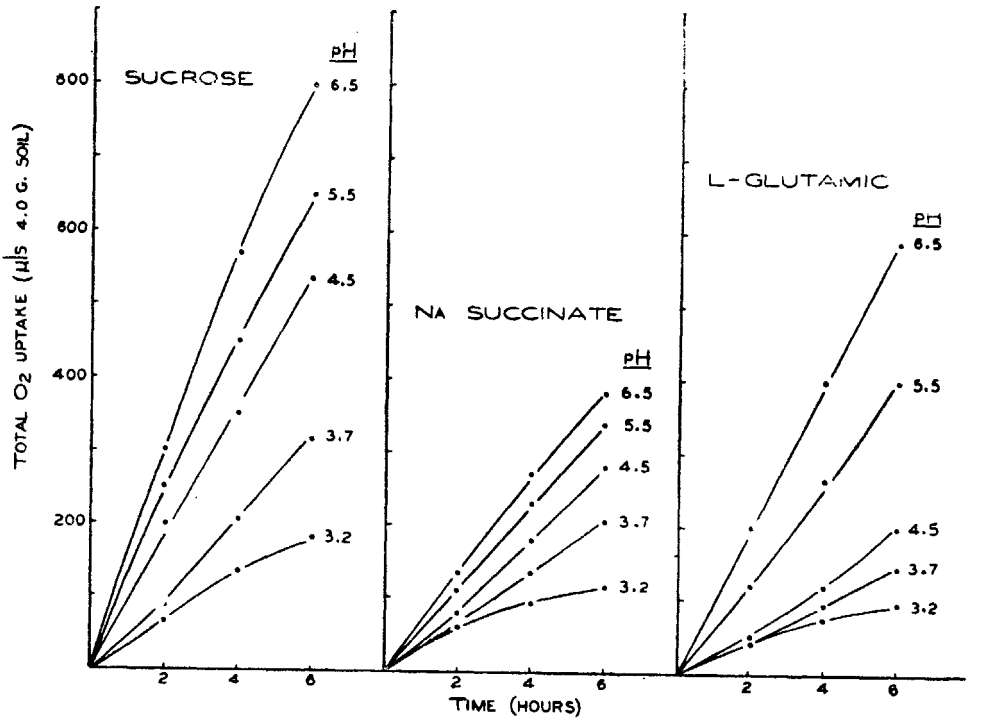
In order to study the effect of *p*H on substrate oxidation soil NG was treated with varying amounts of HCl to provide a series of samples of *p*H 3.2, 3.7, 4.5, 5.5 and 6.5. The oxidation data for sucrose, succinate and l-glutamic acid in these acidified soils are presented in Figure 3.



TEXT-FIG. 2

The effect of different concentrations of succinate on oxidation in soil NG.

A marked decrease in activity is noted with all substrates as the soil *p*H is lowered though some oxidation is still evident even at *p*H 3.2. Numbers of bacteria and fungi were also determined in acidified soils after a 6 hour incubation period. These data are given in Table II.



TEXT-FIG. 3

The oxidation of sucrose, succinate and l-glutamic acid in soil NG at different pH's. Soil supplemented with 25μ Ms substrate.

TABLE II

*Number of Bacteria and Fungi in Soil at Different pH's**

Soil	Bacteria** $\times 10^6$	Fungi*** $\times 10^6$
Control Unadjusted	33.0	13.6
*p*H 6.5	35.6	14.0
*p*H 5.5	32.0	29.6
*p*H 4.5	20.3	11.0
*p*H 3.2	6.3	8.0

*Soils adjusted to appropriate *p*H and allowed to stand 6 hours prior to plating.
**Plate count in Soil Extract agar.
***Plate count in Rose Bengal-Streptomycin agar.

It is evident that the reduction of respiration with increasing acidity occurs before a noticeable reduction in plate counts takes place. Below *p*H 5.5 there

is a significant drop in bacteria and this is especially noticeable at *p*H 3.2. Numbers of fungi do not decrease to the same extent.

The rapid oxidation of sucrose in the various soils suggested further investigation as to its breakdown products. Twenty gram samples of soil NG were brought to 60 per cent water holding capacity with a 1 per cent sucrose solution so that the final concentration was equal to 10μ M sucrose per gram of soil. Samples were incubated at 30°C and extracted with water at intervals of 2, 5, 24 and 48 hours. Aliquots of the aqueous extracts were spotted on filter paper and chromatographed with n-butanol : acetic acid : water (4 : 1 : 5); the papers were sprayed with solutions of silver nitrate or 2,-aminobiphenyl. Little, if any, hexose sugars could be detected at the 2 and 5 hour extraction periods though sucrose was still present in relatively high concentrations. By 24 hours glucose and fructose spots were distinct while the concentration of sucrose had decreased. At 48 hours no evidence of sucrose was found whereas traces of the hexose sugars were still evident. It is of interest to note that these data like the respiration data show the rapid disappearance of sucrose with a slower oxidation of the constituent hexoses.

Discussion

The pattern of substrate oxidation by the four soils used in this study in which sucrose, glucose and succinate were favoured may be of general significance in relation to the metabolic activity of the soil microflora. Sucrose and glucose occur in both the free and combined state in plant residues and are readily attacked by a wide variety of soil micro-organisms. Succinate is a common respiratory intermediate and it is not inconceivable that soils would contain a microflora well adapted to utilize this compound. Sugars such as lactose or galactose not usually found in plant debris are oxidized very slowly or after adaptation as in the case of lactose (Table 1). Pentoses are not usually present as free sugars in plant material and are only liberated through hydrolysis of more complex molecules such as gums, hemicelluloses and nucleic acids (Bonner, 1950). It might be expected therefore that the soil microflora would not be adapted to utilize these substrates directly. It is of interest to note that the highly organic soil NG appears to contain a population which is able to oxidize arabinose very rapidly.

Aromatic substances are not found in abundance in soil. They originate from the slow decomposition of substances such as lignins and do not accumulate to any extent. In consequence a relatively small population is present capable of direct oxidation although the soil population appears to be able to adapt itself slowly to these compounds.

The oxidation of amino acids varies considerably with the different soils. Since these compounds are liberated regularly from plants and plant constituents it was expected that the soil population would utilize them as rapidly as they were produced. Oxygen uptake values for these subtrates were found to be relatively low but in view of the numerous non-oxidative conversions amino acids can undergo this is not surprising.

The intermediates of the tricarboxylic acid cycle are widely distributed in plants and appear readily available to soil micro-organisms. Four-carbon acids such as succinic, fumaric and malic acids are the most rapidly oxidized. Acids such as citric, iso-citric and cis-aconitic are not readily oxidizable ; this may well be due to permeability barriers.

An attempt was also made during these studies to determine the groups of soil organisms concerned in the overall metabolic activity observed. Experiments with selective antibiotics such as actidione and streptomycin or chloromycetin gave inconclusive results (unpublished data). The antifungal agent (actidione) did not reduce soil respiration in the presence of substrate whereas the antibacterial agents caused only partial reduction in activity; however, lowering the *p*H of the soil

caused a consistent and marked decrease in oxygen uptake with the three substrates used. There was no correlation between numbers of bacteria and fungi and respiration between the *p*H range of 5.5 and 6.5 and it may be expected that the decrease in respiration was due to a general inhibition of the enzymes concerned. At *p*H 4.5 and especially at *p*H 3.2 there was a marked reduction in numbers of bacteria. The fungal count was less severely affected by the acid environment though at *p*H 3.2 there was a forty per cent reduction in numbers. The reduction of both bacteria and fungi at the lowest *p*H values may account for the low metabolic activity of the soils with emphasis on the severe reduction in bacterial numbers. The possibility of a direct or indirect *p*H effect on other types of soil organisms, such as protozoa, can not be disregarded, however.

A number of investigators have studied invertase activity in soil and have attempted to relate the results of overall microbial activity and soil fertility (Kiss, 1957; Seegerer, 1953). The data obtained in the present investigations through respiratory and chromatographic studies support the reports of active invertase in soils. Variation in activity in different soils as indicated in Fig. 2 suggests the possibility of using 'invertase activity' as a criterion for assessing microbial activity or fertility of soils.

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