Soil Biol. Biochem. Vol. 8, pp. 423 to 427. Pergamon Press 1976. Printed in Great Britain.

USE OF RESPIROMETRY TO EVALUATE SULPHUR OXIDATION IN SOILS

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(*Accepted* 16 *January* 1976)

Summary—Conventional Warburg respirometric techniques were used to evaluate sulphur-oxidizing activity in soils derived from sediments deposited in brackish environments. Experiments were carried at 30° C on fresh samples dried under vacuum or a warm draught, and enriched either with thiosulphate or with elemental S. By difference from the respiration of the non-enriched sample, a rate of S oxidation was calculated. This rate was higher when the soil was moistened by a 1% solution (v/v) of Tween 80 than when water alone was used. Sublimed elemental S was a better substrate than thiosulphate for the measurement of S oxidizing activity. In all samples, the rate of S oxidation was correlated with counts of autotrophic S oxidizing microorganisms, (*Thiobacillus* sp) on selective agar.

INTRODUCTION

Microbial oxidation of reduced inorganic S compounds in soils is mediated by one of three different ways: (i) dissimilatory oxidation by the colourless S bacteria; (ii) oxidation by the photosynthetic S bacteria, and (iii) incidental oxidation by various heterotrophic microorganisms. Although there is no means for measuring *in situ* the actual contribution of each process in S oxidation in soils (Postgate, 1966), it is generally agreed that the chemoautotrophic S oxidizers of the genus *Thiobacillus* are of greatest importance (Vishniac and Santer, 1957; Starkey, 1966; Freney, 1967). However the microbial contribution to the oxidation of reduced S compounds in soils cannot be assessed by total counts of the microorganisms responsible: incidental oxidation would not be accounted for, and although selective media are available for the colourless and the photosynthetic S bacteria, numeration techniques need further refinement (Postgate, 1966).

In this study the total microbial contribution to S oxidation in soils was estimated by respirometry.

MATERIALS AND METHODS

Samples of three mangrove soils, forming part of a soil sequence described by Vieillefon (1969), were collected from the estuary of the River Casamance. Three samples were collected from different basins in the estuary of the River Senegal, these acid sulphate soils are former mangrove soils, no longer reached by the tide. Soil samples G0, G1 and G3 were collected from experimental plots maintained to study the desalinization of acid sulphate soils in the estuary of the River Senegal: G0 was from the check plot, G1 was enriched with 20 tonnes.ha^{-1} of gypsum, and G3 enriched with 4 tonnes.ha^{-1} of gypsum. In all plots a system of drain pipes was placed at 1.5 m depth. Gypsum was used in order to increase the permeability of the soils to drainage water.

All soils were collected during the dry season at 0–20 cm depth. Principal physical and chemical characteristics and analysis of the S fractions of the samples are summarized in Table 1.

Plate counts of thiobacilli

Thiobacilli were counted by spreading various dilutions of soils on thiosulphate agar plates (Postgate, 1966).

Respirometric methods

Measurements were carried out at 30° C using a Warburg apparatus (model V 166 B. Braun Melsungen, W. Germany). If not otherwise specified, 10 g of dried soil ground and sieved to 2 mm were introduced into a 130 ml Warburg vessel, and moistened to the appropriate moisture content either with water or with 1% solution (v/v) of Tween 80 (Prolabo, France). Immediately the vessels were put into a desiccator and evacuated three times (details are given below). When elemental S was used as a substrate for measurement, the appropriate amount of sublimed S (Merck, W. Germany) was mixed with the dry soil before it was introduced into the vessel. When thiosulphate was the substrate, a solution of thiosulphate calculated to obtain a known concentration and correct moisture was placed in the side arms of the Warburg vessel and tipped into the soil at zero time after thermal equilibration. Coefficients of the vessels were calculated from the dry weight, density and final moisture of the samples. The results are the means of three replicates.

To absorb the CO_2 released, 1 ml 40% (w/v) KOH was placed in the central well of the vessel. This quantity of KOH can theoretically absorb 400 ml CO_2, and it is considered that absorption is complete for the first 40 ml produced. As the quantity of CO_2 released is about equivalent to the quantity of O_2 consumed; it is possible to measure the consumption of 40 ml O_2 before changing KOH in the central well.

N-ethyl maleimide (NEM) was used in some experiments as a selective inhibitor of microbial S oxidation.

Table 1. Physico-chemical characteristics of soils

Soil Sample	pH in situ	Moisture (%d.w.)	Clay (%d.w.)	C (%d.w.)	Total S (%d.w.)	Sulphate (%d.w.)	Sulphur (%d.w.)	Sulphide (parts/10^6)
57	6.7	250	90	ND	0.80	0.52	0.120	300
55	6.8	150	80	13	3.40	0.08	0.040	30
53	5.5	60	75	1.5	0.40	0.05	0.100	10
RT	5.5	41	24	1.3	0.06	0.02	0.004	ND
BN	5.5	67	48	ND	0.10	0.05	0.004	ND
KS	6.4	48	58	3.3	0.08	0.03	0.002	ND
G 0	6.5	40	45	ND	0.25	0.20	0.003	1
G 1	6.0	45	48	ND	0.26	0.21	0.002	2
G 3	5.5	45	46	ND	0.20	0.17	0.015	15

ND : not determined

RESULTS

Influence of the treatment of the sample

The respiration rate of control samples (without added S compound) is influenced by the drying treatment. These effects are more important when the total S content of the soil is high. The respiration rate of soil 55, containing 3.5% total S, and moistened to 40% with water, is respectively 0.89 μmole $O_2.g^{-1}.h^{-1}$ for air dried soil, 1.11 μmole if the soil is dried under a draught and i.r. lamps and 2.14 μmole if the soil is dried under vacuum.

Respiration is reduced according to the fall of pH during drying. With an initial pH of 6.8, the final pH of soil 55 was (measured as a 1/2.5 water paste) air dried sample pH 3.8, dried under a draught and i.r. lamps pH 4.4, and dried under vacuum pH 5.0.

In all acid sulphate soils the oxidation of an important fraction of the reduced S compounds occurs during the drying treatment. But it can be seen from Fig. 1 that if the total respiration is affected by the drying treatment and the decrease of pH, the respiration due to the S compound added was only reduced by 10%. Thus the simplest drying method was preferred, and all samples for further experiments were obtained by drying a freshly collected soil under i.r. lamps, with a draught to maintain the temperature below 40°C.

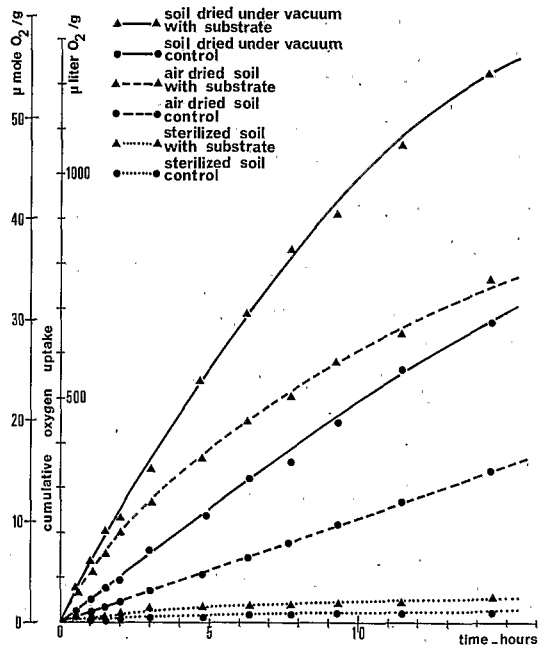

Fig. 1. Influence of drying treatment on respiration of soils.

Procedure used to wet soils in the vessels

Immediately after the wetting solutions were added, vessels containing the samples were put into a desiccator and evacuated three times. This operation allowed better wetting, O_2 consumption was enhanced and the results were more reproducible. The respiration rate of soil 55 was 0.7 μmole $O_2.g^{-1}.h^{-1}$ if the sample was moistened at atmospheric pressure, and 1.1 μmole if moistened under vacuum to a final moisture of 40%. A possible explanation may be that acid sulphate soils acquired hydrophobic characters during drying due to their high organic matter content. For this reason, all samples were placed under vacuum before mounting in the manometers.

Influence of final moisture of the soil

Experiments were carried out with soil G3 to determine the optimum moisture for respirometric measurement of S oxidation. Samples (10g dry weight) were enriched with 5 mmole S° or with 0.5 mmole thiosulphate, and brought to a final moisture content of 30%, 40%, 50% and 60% on a dry weight basis. In Figs. 2 and 3 are shown the cumulative differences between the O_2 consumption of enriched samples and the controls. It can be seen that the optimum moisture for high respiration rate and reproducible results was about 40%, which is close to the "moisture equivalent" for acid sulphate soils from Senegal. The respiration rate was higher at 30% moisture, but reproducibility was poor. Measurement performed with soil suspensions (20%, w/v) in agitated vessels showed that total O_2 uptake was much lower in this treatment and that no significant difference between enriched and control samples could be detected. Thus further experiments were carried out at 40% moisture.

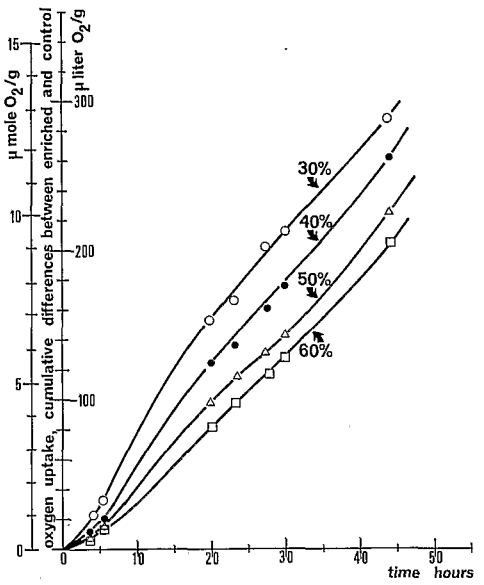

Fig. 2. Influence of moiture on respiration of soils enriched with thiosulphate.

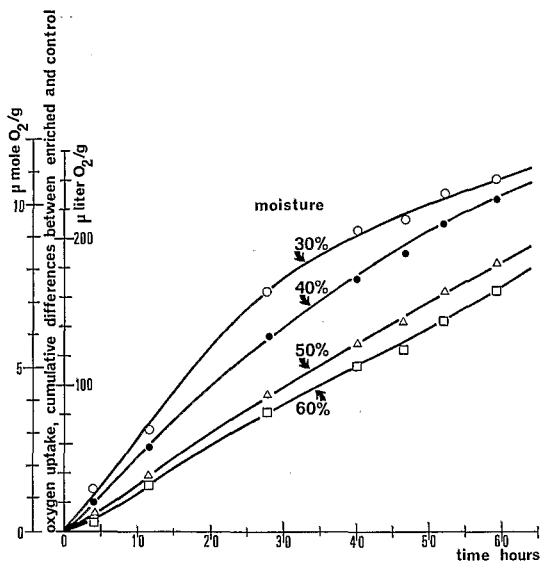

Fig. 3. Influence of moiture on respiration of soils enriched with elemental sulphur.

Oxidation of thiosulphate

In this experiment the dry sample was moistened with water to a final moisture content of 40%, with an allowance for the solution of thiosulphate placed in the vessel side arm. Addition of 0.5 mmol thiosulphate was shown to be necessary to obtain maximum O_2 consumption.

Summation curves of the differences between enriched soil and the controls are seen in Fig. 4. The respiration rate due to the thiosulphate is higher for soil 55 (mangrove soil) than for soil 57 (primitive material) or soil 53 (acid sulphate soil). O_2 uptake due to the thiosulphate stopped after 24 h for the three soils of the sequence from the estuary of Casamance and lasted for 48 h for acid sulphate soils from the estuary of Senegal. In Table 2 are summarized the rates of O_2 consumption during the linear phase, the final efficiency calculated as the percentage of thiosulphate oxidized to sulphate and the counts of thiobacilli for each sample.

Oxidation of elemental S

Use of Tween 80 as a wetting agent. It has been reported by several authors (Cook, 1964; Kodama and Mori, 1968; Taylor, 1968) that the oxidation rate of S° by thiobacilli was improved by the use of Tween 80 as a wetting agent. This result was confirmed by our experiments, and the respiration rate due to the S° added increased from 0.24 to 0.56 µmole $O_2.g^{-1}.h^{-1}$ when the sample was moistened with 1% (v/v) solution of Tween 80. In Fig. 5 are shown the O_2 uptakes by soil BN subjected to various treatments. Results were also more reproducible when Tween 80 was employed as wetting agent.

Effect of quantity of elemental S added. Soils sample G3 was enriched with different amounts of S° (0.1, 0.5, 2.5, and 5 mmol $S°.g^{-1}$ dry soil) and moistened with 1% solution (v/v) of Tween 80 under vacuum to a moisture content of 40%. In Fig. 6 are shown O_2 consumptions corresponding to each quantity of S° added. It can be seen that the respiration rate during the linear phase was not increased if the quantity of S added exceeded 0.5 mmol $S°.g^{-1}$ soil and reached 0.26 µmole $O_2.g^{-1}.h^{-1}$ for soil G3. After 30 h a second phase begins, corresponding probably to a multiplication of the S oxidizing bacteria and the curves diverge from one another. We may assume that the rate of O_2 uptake of the first linear phase reflects the initial population of S oxidizers as no augmentation of this rate occurs if the amount of S added is increased.

As the initial concentration of elemental S in soil G3 was 0.015%, an enrichment of 0.5 mmol of S

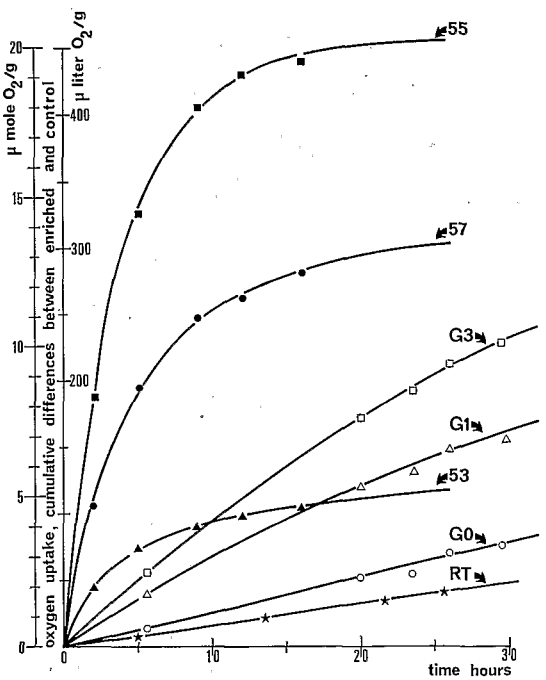

Fig. 4. Oxygen uptake due to enrichment of 0.05 mmol thiosulphate g^{-1} dry soil.

Table 2. Respirometric measurements and *Thiobacilli* counts

Soil Sample	Respiration rate due to substrate µmole O_2/ g h^{-1} $S_2O_3^=$	S°	Final efficiency as % of $S_2O_3^=$ oxidized to $SO_4^=$	thiobacilli / g dry soil aerobic	anaerobic
57	2.0	0.28	14	$2x10^7$	$5x10^5$
55	4.5	0.58	20	10^{11}	$8x10^8$
53	1.0	0.06	7.5	$< 10^6$	$4x10^3$
RT	0.08	0.05	3	$< 10^6$	$< 10^3$
BN	ND	0.25	ND	$4x10^8$	$4x10^3$
KS	ND	0.13	ND	$< 10^7$	$< 10^3$
G O	0.13	0.07	8	$< 10^8$	$< 10^3$
G1	0.29	0.18	10	$< 10^8$	$< 10^3$
G 3	0.42	0.26	12	$2x10^8$	$7x10^7$

ND : not determined

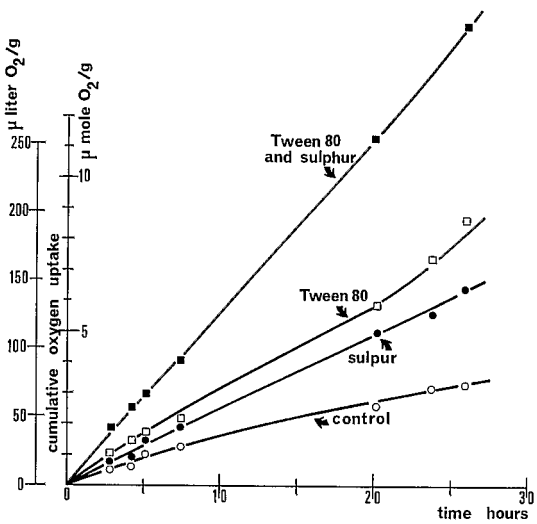

Fig. 5. Influence of Tween 80 on O_2 uptake.

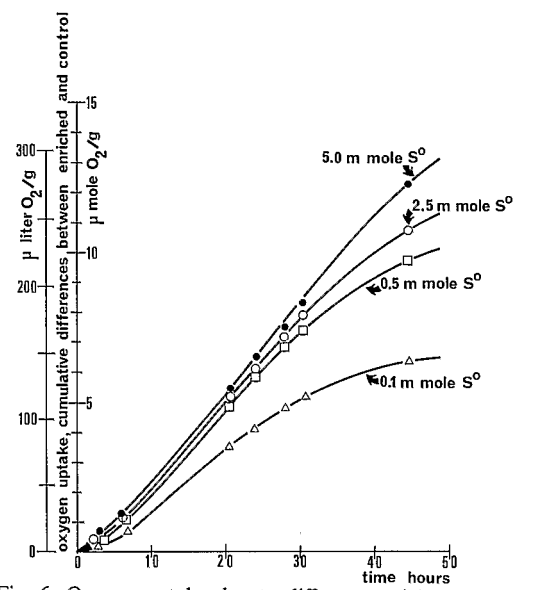

Fig. 6. Oxygen uptake due to different enrichments with elemental sulphur.

corresponds to a final concentration of 1.6% S in the sample, i.e. 100 × the initial concentration. Even in mangrove soils from Casamance where the initial concentration was 0.12% S°.g^{-1}, addition of 0.5 mmol S°.g^{-1} was found to be adequate for the measurement of S oxidizing activity.

Effect of a specific inhibitor of S oxidation. According to Duncan *et al.* (1967) and to Kodama and Mori (1968), N-ethyl maleimide (NEM) is a specific inhibitor of S oxidation by thiobacilli. It was not possible to exceed a final concentration of 1 mmol NEM in the wetting solution due to the low solubility of NEM in the pH and temperature conditions of our experiments. Results are shown in Fig. 7. The percentage inhibition of O_2 uptake due to the substrate added was approximately 60% for both thiosulphate and S°, while 13% inhibition of respiration was noticed in the controls.

Respirometric measurement of S oxidation in different soils. The O_2 consumption rates due to the addition of thiosulphate or elemental S are summarized in Table 2, together with counts of aerobic and anaerobic thiobacilli. Mangrove soil 55 is noteworthy

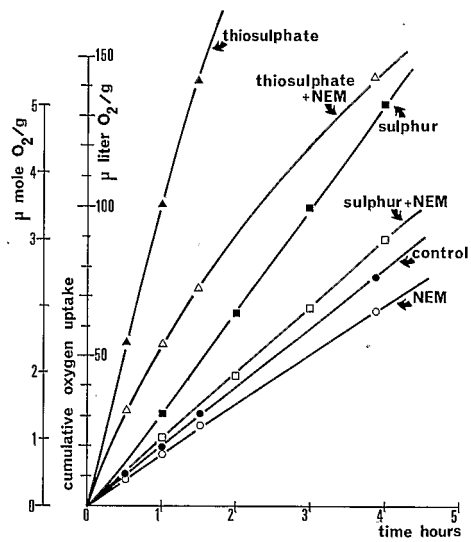

Fig. 7. Inhibition of respiration of soil by NEM.

because of its high S oxidation rate compared to all other samples, and this correlates with the population of aerobic thiobacilli.

DISCUSSION

The respirometric response of soils to enrichment with S° was on the whole more rapid than the response to thiosulphate. This observation may be because S° is a substrate present naturally in soils, while thiosulphate could not be detected in the samples by chemical analysis. Thus oxidation of S° added required little or no adaptative phase for the responsible organisms, while an adaptation was necessary for thiosulphate.

It was possible that the O_2 consumption that took place with the addition of thiosulphate was the result of a chemical reaction. As seen in Fig. 1 no response to thiosulphate was observed with soil sterilized for 1 h on 3 consecutive days. As oxidation of S° cannot occur chemically at the conditions of temperature and pressure of our experiments, the response to S° may be ascribed to the activity of S oxidizing organisms in the sample.

Evaluation of S oxidation by respirometry was found to be in agreement with the plate counts of thiobacilli (Table 2). In spite of the fact that this method is lengthy and requires specialized apparatus, significant information on the S oxidizing activities of soils was obtained even when chemical analysis did not suggest differences.

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