Sulphur Metabolism in Soil

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INTRODUCTION

Quastel, Hewitt and Nicholas (1948) carried out pot and field experiments to compare the effects of administration of thiosulphates and sulphur on the incidence of manganese deficiency in oat, beet and pea grown in manganese deficient soils. They showed that thiosulphate treatments increased the manganese uptake by the plants, reducing the symptoms of manganese deficiency. This was particularly true with beet, whose growth was stimulated by placement treatment of thiosulphate on a manganese deficient soil. Thiosulphate exerted a similar effect to that of sulphur, but far more rapidly and without appreciable change of pH of the soil. The action of sulphur in liberating divalent manganese in soil was, doubtless, partly due to thiosulphate formation. Peas were severely damaged when thiosulphates were applied as broadcast dressings on soil, showing marked contrast to beet in this respect. Audus and Quastel (1947), studying the effects of low concentrations of sodium thiosulphate on the germination and growth of plants, showed that root growth of pea, cress and cabbage was greatly inhibited by low concentrations of thiosulphate whilst that of carrot, for example, was relatively insensitive. Sodium dithionate and potassium trithionate were much less effective than sodium thiosulphate at equivalent concentrations. These investigations of the effects of sulphur and sulphur compounds on plant growth prompted the present enquiry into the various factors that control thiosulphate breakdown in soil.

Much is becoming known of the biological transformations undergone by sulphur in soil (see reviews by Starkey (1950), and Bunker (1936). Guittoneau (1927) and Roach (1930) have shown that sulphur may undergo biological attack with the formation of thiosulphate, and Guittoneau and Keilling (1932) have found that heterotrophic organisms may transform sulphur into thiosulphate and tetrathionate. The autotrophs *Thiobacillus thiooxidans* and *Thiobacillus thioparus* accomplish the oxidation of thiosulphate to sulphate and sulphur, whilst a variety of heterotrophs oxidise thiosulphate to tetrathionate (Starkey, 1934). Lockett (1914), many years ago, demonstrated that thiosulphate and other polythionates, except dithionate, when passed through sewage sludge are oxidised by microbiological means to sulphate. Vishniac (1952) has recently shown that *Thiobacillus thioparus* oxidises thiosulphate with intermediate formation of tetrathionate and trithionate. He also points out that elemental sulphur may arise in cultures of *T. thioparus* by a nonbiological mechanism, excess thiosulphate catalysing dismutation of tetrathionate to trithionate and pentathionate, the latter breaking down to tetrathionate and sulphur. Tamiya *et al.* (1941) had already suggested that spontaneous decomposition of tetrathionate could give rise to sulphur and trithionate.

The following paper is concerned with studies on metabolism of sulphur, thiosulphate and polythionates, in soil, at 21C using the soil perfusion technique of Lees and Quastel (1944) as modified by Audus (1946). A full description of the technique is given by Quastel and Scholefield (1951). Soils of different origins were used but the results with all soils investigated were consistent.

EXPERIMENTAL TECHNIQUE

Sulphur compounds used in these experiments were colloidal sulphur, sodium thiosulphate $Na_2S_2O_3$, $5H_2O$, sodium tetrathionate $Na_2S_4O_6$, $2H_2O$: sodium dithionate $Na_2S_2O_6$, $2H_2O$ and potassium trithionate $K_2S_3O_6$. The colloidal sulphur preparation was freed from polythionates by dialysis, and centrifuged at 2500 rpm to precipitate the sulphur. This was then washed with distilled water, and resuspended in distilled water. The process was repeated until no polythionates were detectable.

Qualitative analyses of the various polythionic acids were carried out using the methods described by Mellor (1930). Thiosulphate was determined iodometrically (in the presence of acetic acid). Any sulphite present was removed by treatment with formaldehyde. Quantitative analyses of polythionates were made using the methods described by Starkey (1935).

Sulphate was determined by a modification of a method described by Snell and Snell (1936). For analysis, 15 ml of a known dilution of sulphate was added to 5 ml 50 per cent glycerol and shaken. Two ml acid barium chloride solution (100 g $BaCl_2$ $2H_2O$, dissolved in 48 ml concentrated HCl and made up to 1000 ml with water) were then added and the mixture

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was again thoroughly shaken. The precipitate of barium sulphate was held suspended evenly in the mixture, and its absorption of light was determined by a Hilger Spekker Light Absorptiometer. A linear relationship existed between sulphate concentration (between the values of 0–15 ml 0.0004 M Na_2SO_4) and the light absorption.

Since all the relevant compounds, except sulphur, were soluble in the perfusate, serial analyses of the perfusate yielded an accurate picture of the rate of utilisation or formation of the various compounds. Values for elemental sulphur were taken as the difference between the total sulphur concentrations originally present and those found for the various constituents analysed at the end of the experiment. All results are expressed in terms of μM sulphur.

Results

Non-biological Oxidation of Thiosulphate in Soil

Iron is a common mineral constituent of many soils, and it is well known that ferric iron will oxidise thiosulphate to tetrathionate. In soil perfusion experiments, tetrathionate is a common oxidation product of thiosulphate and it was essential to know how far the chemical and microbiological oxidations were interrelated. For experiment, ferric cations were introduced into the soil by perfusion with 1 per cent ferric chloride solution, the cation being taken up by base exchange. The soils were then well washed with water, 0.02 N thiosulphate solution was perfused and its transformation followed. The experimental results show that the soil containing the excessive quantity of absorbed ferric ions will oxidise thiosulphate without a lag phase whereas, in the control soil, to which ferric ions had not been added, thiosulphate is oxidised to tetrathionate and sulphate after an extensive lag period. The difference in the distribution of sulphur after 6 days is shown in table 1.

The oxidation due to ferric cations in the soil is inhibited by the presence of excess disodium hydrogen phosphate in the perfusion solution probably through formation of insoluble ferric phosphate. Sodium azide, on the other hand, has little influence on the oxidation of thiosulphate by iron adsorbed in the soil. It will be shown, however, that azide inhibits the biological oxidation of thiosulphate and in this manner non-biological and biological oxidation of thiosulphate may be differentiated in soil.

The Biological Oxidation of Sulphur in Soil

Colloidal sulphur, after a lag period of several days, is metabolised in soil to sulphate. This oxidation is inhibited by the presence of 0.01 per cent sodium azide. If a soil that has already been perfused with a colloidal sulphur solution is washed free from sulphates and reperfused with the colloidal sulphur preparation, the time taken for the sulphate to reappear is shortened. This is due to the fact that the soil has become enriched with micro-organisms responsible for the oxidation of sulphur. Thiosulphate when perfused through such an enriched soil is metabolised immediately without lag, and at a constant rate to tetrathionate and sulphate. Hence organisms whose growth is stimulated by the presence of sulphur, and which presumably are responsible for the oxidation of sulphur in soil, will also oxidise thiosulphate.

Table 1. *Change in distribution of sulphur on perfusion of 0.02* N $Na_2S_2O_3$ *through soil for six days*

Soil	$S_2O_3^=$-S	$S_4O_6^=$-S	$SO_4^=$-S
Control	−39.3*	+5.0	+33.9
Fe treated	−30.5	+32.1	0.0

* All results as μM S.

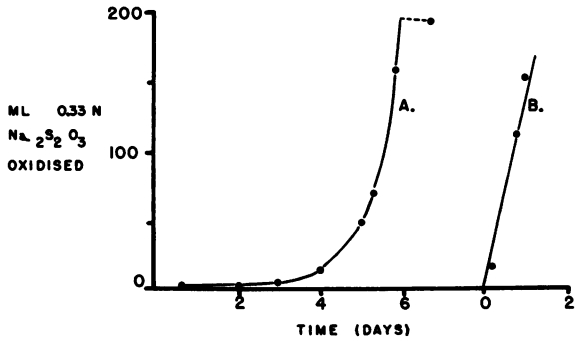

Fig. 1. Oxidation of sodium thiosulphate in normal soil.
A. 30 g soil perfused with 200 ml 0.033N $Na_2S_2O_3$.
B. Same soil as in (A) washed after initial thiosulphate perfusion and reperfused with a second equal amount of sodium thiosulphate solution.
Ordinate: Ml 0.033N $Na_2S_2O_3$ oxidized.
Abscissa: Time in days.

The Biological Oxidation of Thiosulphate in Normal Soil

Thiosulphate is oxidised in soil after an initial lag period at an increasing rate to either sulphate and tetrathionate, or sulphate and sulphur (figure 1). If the perfusate is removed and a new thiosulphate solution is perfused, the latter is metabolised at a constant rate without any lag period (figure 1B). A soil in this condition is known as an "enriched" soil. Lees and Quastel (1944) have described a similar phenomenon with the nitrifying bacteria. An important fact to be noted is that little or no oxidation of thiosulphate occurs in the perfusate free from soil.

Retention of Oxidising Powers of Enriched Soils

Wet "enriched" thiosulphate oxidising soils can be quickly dried in a current of cold air and will retain their high oxidising activities for several months if they are stored between 0–4 C. However, if the soils

are kept at room temperature, this property of rapid oxidation of thiosulphate is lost in a few days. Enrichment of a soil with thiosulphate oxidising organisms may be accelerated by inoculation of a fresh soil with a small quantity of an enriched soil.

The Influence of Thiosulphate Concentration on its Oxidation

The main effect of increasing thiosulphate concentration is to increase the lag phase before oxidation takes place. Thus a lag period of one day, obtained when 0.01 N sodium thiosulphate is perfused through soil,

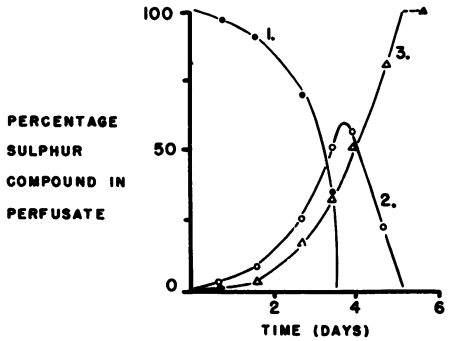

FIG. 2. Thiosulphate oxidation on perfusion through soil. 28 g Garden soil, inoculated with 2 g enriched thiosulphate oxidising soil, perfused with 300 ml 0.005N $Na_2S_2O_3$.
(1) Course of thiosulphate disappearance.
(2) Course of tetrathionate appearance and disappearance.
(3) Course of sulphate appearance.
Ordinate: Percentage of sulphur present in perfusate as thiosulphate, or tetrathionate, or sulphate.
Abscissa: Time in days.

TABLE 2. *Biological oxidation of thiosulphate by an enriched soil in six days*

CONCENTRATION OF $S_2O_3^=$ PERFUSED	μM $S_2O_3^=$-S PER ML	μM $S_4O_6^=$-S PER ML	μM $SO_4^=$-S PER ML
0.01N	−20.5	0	+20.5
0.04N	−75.9	+39.8	+39.8
0.10N	−191	+116	+77.7

is increased to three days when 0.1 N sodium thiosulphate is similarly perfused. Once the lag phase is over, however, the rates of oxidation in soils perfused at a higher concentration are much higher than those perfused at a lower concentration. The course of thiosulphate oxidation follows a logarithmic law, until the soil becomes saturated with the bacteria, when the rate of oxidation becomes constant. The final rate of oxidation of thiosulphate is increased with increase of the initial concentration of thiosulphate. This may be due to a variety of factors: for example, (a) growth of specific variants of the species having a higher enzyme concentration per cell, (b) an increase in thiosulphate oxidising capacity per cell, (see Quastel and Scholefield, (1951) for similar results in their studies of nitrite oxidation in soils).

During conversion of thiosulphate to sulphate, tetrathionate appears as an intermediate. This undergoes further oxidation to sulphate. Typical results showing the rates of thiosulphate disappearance, tetrathionate appearance and removal, and sulphate formation during thiosulphate perfusion through a normal soil are shown in the curves given in figure 2.

Typical balance sheets showing quantitative conversion of thiosulphate to tetrathionate and sulphate during perfusion through an enriched soil are shown in table 2.

Biological Oxidation of Tetrathionate in Soil

Tetrathionate is converted to sulphate during soil perfusion after a short initial lag period (figure 3).

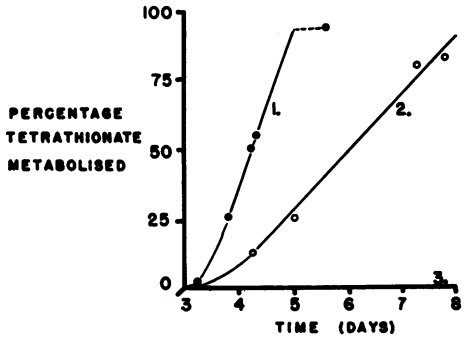

FIG. 3. Effects of change of tetrathionate concentration on its metabolism in soil. 30 g perfused with 200 ml tetrathionate solution.
(1) 0.0025M $Na_2S_4O_6$.
(2) 0.005M $Na_2S_4O_6$.
(3) 0.01M $Na_2S_4O_6$.
Ordinate: Percentage tetrathionate metabolised.
Abscissa: Time in days.

Increase of concentration of the tetrathionate perfused, however, increases the lag period and decreases the rate of oxidation. Thus 0.0025 M is oxidised at a maximum rate of 2.32 μM $S_4O_6^=$-S/g. soil/hr., 0.005 M at 45 per cent of this rate and 0.01 M not at all.

The presence of 0.01 per cent sodium azide completely inhibits the oxidation of tetrathionate, this result indicating the biological nature of the oxidation.

Sodium thiosulphate, when perfused through a soil enriched with tetrathionate oxidising organisms, is oxidised at a constant rate, without lag, to sulphate and tetrathionate. This result demonstrates that the tetrathionate oxidising organisms can also oxidise thiosulphate; otherwise a lag period would have appeared. It seems likely, therefore, that one organism is concerned with both the oxidation of thiosulphate and tetrathionate. This may be contrasted with the behaviour of nitrifying organisms in soil. In this case, enrichment of the soil with organisms oxidising nitrite

does not ensure immediate oxidation of ammonium ions when the latter are perfused, showing the necessity for two organisms oxidising nitrite and ammonium ions respectively (Quastel and Scholefield, 1951).

Influence of Tetrathionate Concentration on Thiosulphate Oxidation in Soil

When a mixture of low concentrations of thiosulphate and tetrathionate is perfused through a soil, inoculated with thiosulphate oxidising organisms, the initial rate of sulphate formation does not exceed the rate of sulphate formation found when the same thiosulphate concentration, in the absence of added tetrathionate, is perfused through the soil. This result is consistent with the conclusion that tetrathionate is an intermediate in the oxidation of thiosulphate to sulphate.

The Oxidation of Potassium Trithionate and Sodium Dithionate in Soil

Potassium trithionate is oxidised to sulphate at a constant rate by a thiosulphate-enriched soil and its oxidation is inhibited by 0.01 per cent sodium azide. Sodium dithionate is apparently very resistant to such oxidation in both normal and thiosulphate-enriched soils. However, sodium dithionate has no inhibitive effect on thiosulphate oxidation.

The Influence of Biological Inhibitors on Thiosulphate Oxidation

Sodium azide (0.01 per cent) and sulphanilamide (0.1 per cent) both inhibit thiosulphate oxidation in fresh soil. However, while azide inhibits thiosulphate oxidation in an enriched soil, sulphanilamide has no such effect. This difference can be explained by the fact that azide is a respiratory poison, whilst sulphanilamide exerts its effects by retarding the proliferation of the thiosulphate oxidising organisms. A soil that has been perfused with sodium azide will regain its ability to oxidise thiosulphate after being washed with water. Typical results, illustrating this reversibility of action, are shown in figure 4.

Chloretone, a narcotic, which is known to inhibit nitrification (Lees and Quastel, 1944) also inhibits thiosulphate metabolism in both a normal and an enriched soil.

The presence of 2:4-dinitro-o-cresol and 2:4-dinitro-o-phenol brings about an inhibition of thiosulphate metabolism in normal soil. Out of a series of nitro-compounds examined as possible inhibitors of thiosulphate oxidation in enriched soils, only dinitro-o-phenol and dinitro-o-cresol showed activity. Representative results are given in table 3. The inhibitive effects of dinitrophenol and dinitrocresol are of considerable interest in view of the well-known effects of these substances on phosphorylation mechanisms of the cell (Clowes and Krahl, 1937). The fact that dinitrophenol dissociates phosphorylations from certain respiratory activities (Loomis and Lipmann, 1948, Hotchkiss, 1944) suggests that bacterial thiosulphate oxidation depends on the integrity of phosphorylation mechanisms which are affected by dinitrophenol. It is important in this connection to recall the observations of Vogler and Umbreit (1942) on the esterification of inorganic phosphate during the oxidation of sulphur by *Thiobacillus thiooxidans*.

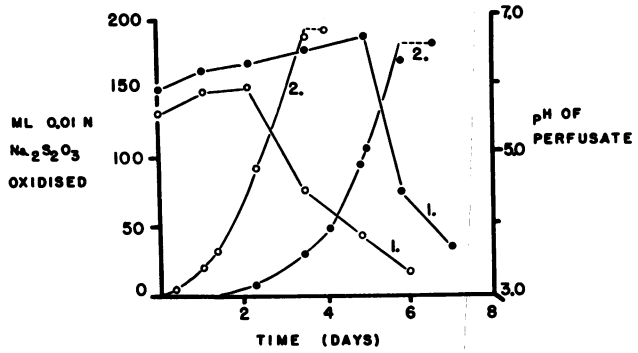

FIG. 4. Effects of azide treatment of soil on thiosulphate oxidation. 30 g Soil, inoculated with 2 g soil enriched with thiosulphate oxidising organisms, perfused for 7 days either with 200 ml water, or with 200 ml 0.01 per cent sodium azide solution. After this period both soils were washed with water and then each was reperfused with 200 ml 0.01N $Na_2S_2O_3$.

○ Normal Soil
● Azide perfused soil
(1) Change of pH of perfusate.
(2) Change of thiosulphate concentration in perfusate.

Ordinates: Left. Ml 0.01N $Na_2S_2O_3$ metabolised.
Right. pH of perfusate.
Abscissa: Time in days.

TABLE 3. *Effects of organic nitro compounds on thiosulphate oxidation in soil*

UNIT	CONTENTS OF PERFUSION FLASK IN ADDITION TO 0.04N $Na_2S_2O_3$	μM $S_2O_3^-$-S OXIDISED PER ML PERFUSATE AFTER 4 DAYS PERFUSION
1	Nil	8.0
2	0.01% nitranilic acid	7.5
3	0.005% 2:4 dinitro-o-cresol	2.9
4	0.01% 2:4 dinitro-o-phenol	2.7
5	0.01% 2:4 dinitro-1-naphthol-sulphonic acid	7.0
6	0.01% 3:5 dinitro-salicylic acid	7.0
7	0.01% picric acid	7.3

Arsenites, selenites and tellurites inhibit thiosulphate oxidation, but the corresponding arsenates, selenates and tellurates have no effect.

Biological Oxidation of Thiosulphate to Sulphur and Sulphate, or to Tetrathionate

Sulphate may be produced in soil by an oxidation of thiosulphate not apparently involving tetrathionate as a necessary intermediate, for it may be shown, that, in presence of a concentration of tetrathionate suffi-

ciently high to inhibit sulphate formation from tetrathionate itself, sulphate is still formed from thiosulphate. Either perfusion of a relatively high thiosulphate concentration, or perfusion of thiosulphate together with a relatively high phosphate concentration, above pH 7.0, seems to secure such conditions. It is important, however, to note (Vishniac, 1952) that thiosulphate catalyses the nonbiological dismutation of tetrathionate and this phenomenon may partly explain the results described.

In the experiments where thiosulphate perfusion led only to the formation of sulphur and sulphate, the resulting soils were used for inoculation of fresh soils. When thiosulphate solutions were perfused for six days through 28 g soil "inoculated" with 2 g of such an enriched thiosulphate oxidising soil, no tetrathionate

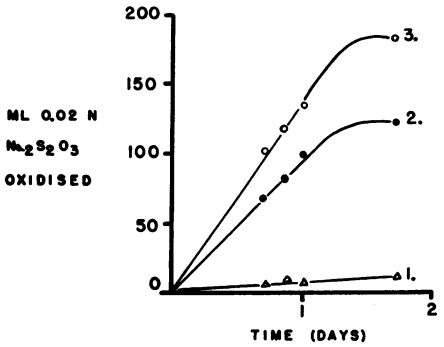

FIG. 5. Effects of presence of soil on metabolism of thiosulphate by a suspension of heterotrophic bacteria isolated from soil. All units run with 200 ml 0.02N $Na_2S_2O_3$, containing equal quantities of a suspension of bacteria (probably *P. fluorescens*) isolated from soil, in presence of 0.1 per cent sulphanilamide.

(1) No soil present.
(2) 30 g normal soil present.
(3) 30 g garden soil, previously heated at 170 C for one hour, present.

Ordinate: Ml 0.02N $Na_2S_2O_3$ oxidised.
Abscissa: Time in days.

was found in the perfusate and 60 per cent of the thiosulphate sulphur was converted into sulphate, the remainder presumably appearing as sulphur which was visibly precipitated over the soil crumbs.

The presence of ammonium chloride or sodium nitrate has but little influence on the course of thiosulphate metabolism in the soils that have been investigated. The presence of urea, however, which undergoes rapid conversion to ammonia in the soil, inhibits sulphate formation. The three compounds do not influence the course of oxidation of thiosulphate in enriched soils.

The presence of amino acids, for example, sodium aspartate or glutamate, and glycine, or of the sodium salts of succinic and fumaric acids in the presence of ammonium ions, apparently stimulates the proliferation of heterotrophic microflora that can accomplish a rapid oxidation of thiosulphate to tetrathionate only. This effect is not seen in the absence of added ammonium ions.

It has been found that substances present in the water extract of a heated soil will also stimulate the type of oxidation of thiosulphate that leads to a high yield of tetrathionate. This oxidation may be inhibited by the presence of sodium azide. Eventually, however, the tetrathionate disappears and sulphate is formed. If thiosulphate solution is perfused at 21 C through a soil that has been heated at 170 C for one hour, an initial high rate of thiosulphate conversion to tetrathionate takes place. Presumably this is due to the proliferation of relatively heat resistant heterotrophic organisms developing on the soluble organic matter released from soil during heat treatment.

Effect of a Heterotrophic Organism Isolated from Soil on Thiosulphate Oxidation

From a soil accomplishing a rapid transformation of the thiosulphate into tetrathionate, a heterotroph was isolated in pure culture. It was coccoid, and fluorescent when grown on nutrient agar. This organism, which had the cultural and morphological characteristic of *Pseudomonas fluorescens*, (see Starkey, 1934), was grown in bulk on nutrient agar plates and after 24 hours' growth it was removed from the plates and a heavy suspension made up in 0.85 per cent saline. The effect of this suspension of organisms on thiosulphate oxidation in the presence of heated and normal soil was investigated. An aliquot of this suspension was added to 200 ml 0.02 N $Na_2S_2O_3$ solution and this was perfused through soil which had been heated at 120 C for 30 minutes. An equal quantity of the suspension in 200 ml 0.02 N $Na_2S_2O_3$ solution was perfused through normal soil. Sulphanilamide (0.1 per cent) was also added to the perfusion fluid to inhibit the growth of any organisms in the soils themselves that would accomplish the oxidation. The results, given in figure 5, show that thiosulphate conversion to tetrathionate takes place in the presence of normal soil, but that a quicker rate of oxidation occurs in the presence of heated soil, under conditions where, when the suspension alone was perfused in the absence of any soil, only a feeble rate of conversion of thiosulphate to tetrathionate occurred.

The Influence of Carbohydrates on Thiosulphate Metabolism in Soil

When a solution of a carbohydrate such as glucose, sucrose or mannitol is perfused through soil, it promotes the growth of a heavy heterotrophic microflora in the soil and perfusate. Thiosulphate under these conditions, after a slight initial oxidation to tetrathionate, remains quite stable, and in some cases a reduction of the tetrathionate to thiosulphate occurs.

When the soil is partially sterilised by heat treatment,

thiosulphate is quickly oxidised to tetrathionate when it is perfused in the presence of carbohydrate, a heavy growth appearing in the perfusate and in the soil.

Thus there appears to be two groups of microorganisms present initially in the soil, a heat sensitive group which can reduce tetrathionate to thiosulphate, and a less heat sensitive group which oxidises thiosulphate to tetrathionate. The reduction of tetrathionate to thiosulphate when perfused through soil in the presence of 1 per cent sucrose or 1 per cent glucose is shown by the results given in figure 6. This reduction does not take place in a heated soil. The results conform with the observations by Pollock and Knox, (1943) on tetrathionate reduction by bacteria. It is clear that the thiosulphate-tetrathionate equilibrium in soil is determined by the chemical factors, present in soil, which control the growths of the relevant specific microflora.

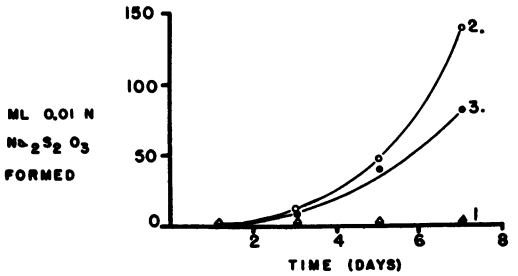

FIG. 6. Reduction of tetrathionate to thiosulphate in soil. 30 g Soil perfused with 200 ml 0.005M $Na_2S_4O_6$.
(1) 200 ml 0.005M $Na_2S_4O_6$ alone.
(2) 200 ml 0.005M $Na_2S_4O_6$ + 1% sucrose.
(3) 200 ml 0.005M $Na_2S_4O_6$ + 1% glucose.
Ordinate: Ml 0.01N Thiosulphate formed.
Abscissa: Time in days.

With soils enriched with thiosulphate oxidising organisms, normal oxidation of thiosulphate to sulphate occurs in the presence of carbohydrates, and even after several days few, or no, heterotrophic microflora are seen proliferating in the soil perfusate. This is possibly due to the fact that during the initial perfusion with thiosulphate, the carbohydrate utilising microflora of the soil are greatly suppressed, due to the unfavourable acid conditions which develop during sulphate formation.

MANOMETRIC STUDIES

Using the technique already described by Ellinger and Quastel (1948) and by Quastel and Scholefield (1951), it is possible to study the respiratory activities of soils enriched with thiosulphate oxidising organisms.

Typical curves showing the rates of oxygen uptake in presence of thiosulphate by an enriched soil are given in figure 7, which indicates the results obtained when two different concentrations of thiosulphate are added to the soil. The difference found between the quantities of oxygen required to oxidise the two amounts of thiosulphate is that calculated from the equation:

$$Na_2S_2O_3 + 2O_2 + H_2O = 2NaHSO_4$$

The thiosulphate oxidation is considered complete when the rate of oxygen uptake of the soil containing the thiosulphate falls to the same value as that of the soil to which water alone was added. Thus, in the experimental results quoted in figure 7, the total oxygen uptake, after correction for the control, for the oxidation of 1.5 ml $\frac{N}{150}$ $Na_2S_2O_3$ was 240 μl and that for the oxidation of 1.5 ml $\frac{N}{75}$ $Na_2S_2O_3$ was 685 μl, the difference being 445 μl. The calculated difference was

$$\frac{44.8 \times 1000 \times 1.5}{150} \mu l. = 448 \mu l.$$

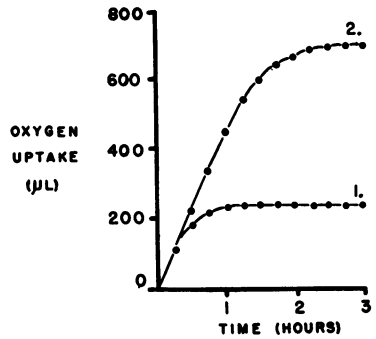

FIG. 7. Oxygen uptakes of air dried "enriched" soil in presence of thiosulphate.
(1) 4 g "enriched" soil + 1.5 ml N/150 $Na_2S_2O_3$.
(2) 4 g "enriched" soil + 1.5 ml N/75 $Na_2S_2O_3$.
Ordinate: μl oxygen uptake.
Abscissa: Time in hours.

It is evident that complete oxidation of thiosulphate (probably via tetrathionate) takes place.

The presence of 0.01 per cent sodium azide greatly suppresses the rate of oxygen uptake of an enriched soil in presence of sodium thiosulphate. Thus the rate of oxygen uptake of a soil in presence of 1.5 ml 0.01 N $Na_2S_2O_3$ was 52.5 μl per gram soil per hour. In presence of the sodium azide, this figure was reduced to 8.7 μl O_2 uptake per gram soil per hour.

Effects of Changes of Polythionate Concentration on Oxygen Uptake of a Soil Enriched with Thiosulphate Oxidising Organisms

The rate of oxygen uptake of thiosulphate in an enriched soil increases gradually with increase of concentration of thiosulphate added to the soil.

Thiosulphate, trithionate and tetrathionate all yield similar rates of oxygen uptake in an enriched soil at a concentration of approximately 20 μM S/l. With

increase of concentration, the rate of oxygen uptake with thiosulphate increases to a maximum at 200 μM S/l., while both the rates obtained with trithionate and tetrathionate fall off rapidly as the concentrations are increased (figure 8). Dithionate, or colloidal sulphur, is not apparently oxidised and has no influence on the oxidation of thiosulphate in these enriched soils.

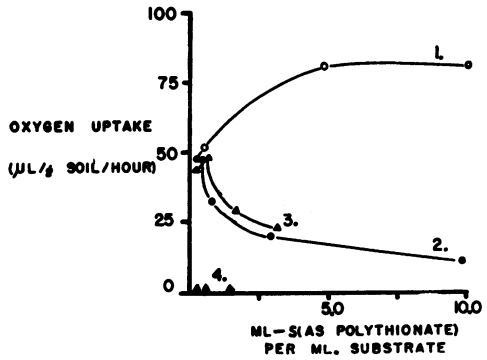

FIG. 8. Effects of polythionates on rates of oxygen uptake. 4 g Soil enriched with thiosulphate oxidising organisms in each vessel.

(1) 1.5 ml $Na_2S_2O_3$ solution.
(2) 1.5 ml $Na_2S_4O_6$ solution.
(3) 1.5 ml $K_2S_3O_6$ solution.
(4) 1.5 ml $Na_2S_2O_6$ solution.
1 ml S = 44.6 μM S
Ordinate: Oxygen uptake (μl/g/soil/hour).
Abscissa: ml S, as polythionate, per ml substrate solution.

TABLE 4. *Influence of glucose and of sodium pyruvate on the oxygen uptakes of enriched soils in presence of polythionates*

EXPERIMENT	SUBSTRATES ADDED	μl O_2 UPTAKE PER GRAM SOIL PER HOUR
1	0.05M glucose	2.0
	0.01N $Na_2S_2O_3$	62.0
	0.01N $Na_2S_2O_3$ + 0.05M glucose	63.0
2	0.066N $Na_2S_2O_3$	55.0
	0.066N $Na_2S_2O_3$ + 0.033M sodium pyruvate	19.0
3	0.033M sodium pyruvate	1.0
	0.0066M potassium trithionate	40.0
	0.0066M potassium trithionate + 0.033M sodium pyruvate	8.5

Influence of Glucose and of Sodium Pyruvate on Thiosulphate Oxidation

The presence of glucose has no influence on the oxygen uptake of enriched soils in presence of thiosulphate. On the other hand, the presence of pyruvate inhibits the rate of oxygen uptake due to thiosulphate and trithionate in an enriched thiosulphate oxidising soil (see results given in table 4). This may be due to a direct inhibition of the enzyme responsible for the polythionate oxidation or to complex formations between pyruvate and thio groups involved in the oxidation. The inhibitory influence of pyruvate on the oxygen uptake due to *Thiobacillus thiooxidans* in presence of sulphur has been noted by Vogler *et al.* (1942) Vogler and Umbreit (1942) and Vogler (1942).

Influence of Some Enzyme Inhibitors on Thiosulphate Oxidation

The results given in table 5 demonstrate the effects of several enzyme inhibitors on the rate of oxygen uptake of enriched soils in the presence of sodium thiosulphate. 2:4-Dinitro-o-cresol, arsenite, selenite and tellurite are inhibitors of thiosulphate oxidation whereas sulphanilamide, arsenate, selenate, and tellurate appear to have no adverse effect. These results are in accordance with those obtained in the perfusion work.

TABLE 5. *The influence of some enzyme inhibitors on the rate of oxygen uptake due to thiosulphate in air dried garden soil enriched with thiosulphate oxidising organisms*

SUBSTRATES ADDED WITH 0.005N $Na_2S_2O_3$	O_2 UPTAKE IN μl/G SOIL PER HOUR	PERCENTAGE INHIBITION
Nil	45.0	—
0.0003% 2.4 dinitro-o-cresol	13.1	71
0.1% sodium arsenite	22.5	50
0.1% sodium tellurite	35.5	26
0.1% sodium selenite	21.5	52
0.1% sulphanilamide	45.0	0
0.1% sodium arsenate	45.0	0
0.1% sodium tellurate	45.0	0
0.1% sodium selenate	45.0	0

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SUMMARY

1. Perfusion of sodium thiosulphate solution through air-dried garden soil under aerobic conditions leads to an enrichment of the soil with thiosulphate oxidising organisms. The transformation of thiosulphate is logarithmic such as is to be expected if the metabolism is due to proliferating organisms. When the soil is enriched, or saturated, with the organisms, the rate of metabolism of thiosulphate becomes constant.

2. Transformation of thiosulphate in soil is greatly suppressed by the presence of sodium azide which may be used to discriminate between biological and non-biological oxidation of thiosulphate in soil. The latter process is mainly accomplished by relatively large quantities of ferric ions in the soil, and is inhibited by the presence of sodium phosphate but not sodium azide.

3. Thiosulphate is transformed to either sulphate and tetrathionate, or sulphate and sulphur, the former products being more commonly occurring. The presence

of relatively high concentrations of phosphates or thiosulphate tends to favour the production of sulphur and sulphate.

4. Soils enriched with thiosulphate oxidising organisms will retain their oxidising activity for several months if they are dried and stored at 0 C.

5. Tetrathionate is converted to sulphate during soil perfusion. A soil enriched with tetrathionate oxidising organisms will oxidise thiosulphate at a constant rate, without initial lag, pointing to the probability that such organisms are also capable of oxidising thiosulphate.

6. Tetrathionate is a normal intermediate in the conversion of thiosulphate to sulphate in soil.

7. With increase of concentration of tetrathionate there is an increase of the initial lag time and a diminution of the rate of tetrathionate breakdown. Increase of concentration of thiosulphate perfused also leads to a lengthening of the initial lag, but after this phase is over the rate of thiosulphate breakdown increases with increase of thiosulphate concentration.

8. Thiosulphate metabolism in soil is inhibited by the presence of low concentrations of 2:4-dinitrophenol and 2:4-dinitro-o-cresol. This result points to the likelihood that phosphorylations are involved in this metabolism. Other nitro compounds such as picric acid, etc., at similar concentrations are without effect.

9. The presence of amino acids, or of sodium succinate or sodium fumarate in presence of ammonium ions, increases the rate of oxidation of thiosulphate to tetrathionate.

10. The presence of glucose, sucrose, or mannitol favours the reduction of tetrathionate to thiosulphate in soil. This reduction does not take place in a soil that has been partially sterilised by heat treatment.

11. Whereas thiosulphate, trithionate, and tetrathionate are all broken down in soil, dithionate is not.

12. Sulphanilamide inhibits thiosulphate metabolism in a fresh soil but has no effect in a soil enriched with thiosulphate oxidising organisms.

13. Chloretone, arsenite, selenite, and tellurite inhibit transformation of thiosulphate in soil. No effects of arsenate, selenate, and tellurate have been noted.

14. Manometric studies with soils enriched with thiosulphate oxidising organisms show that thiosulphate is quantitatively oxidised to sulphate. The process is inhibited by 2:4-dinitrophenol and 2:4-dinitro-o-cresol and by respiratory poisons as expected from the results of the perfusion studies. The presence of glucose has no effect, but that of sodium pyruvate is very inhibitory to the process of thiosulphate oxidation. Sodium pyruvate is also inhibitory to trithionate oxidation.

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