being investigated in our laboratories and will be reported at a later date.

Summary

An apparatus has been developed which employs U-V energy as an effective agent against *Escherichia coli* and *Salmonella typhosa* when these organisms constitute at least a part of the population in contaminated waters. The apparatus provides sufficient agitation to insure adequate exposure for all areas of a bacterial suspension over a four-fold range of flow rates.

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Effects of Mercapto Compounds on Soil Nitrification

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It has been observed (Quastel and Scholefield, 1949) that DL-methionine is highly effective in bringing about an inhibition of nitrification in soil. Its presence produces a lengthy lag period before the nitrification of ammonium ions commences, after which nitrification of both the added ammonia and that derived from methionine itself proceeds. The problems arose as to whether the inhibition of nitrification is due to a toxic metabolite from methionine and whether a process of adaptation of nitrifying organisms to methionine takes place. The formation of methionine sulphoxide from methionine was suggested as a possible mechanism of inhibition (Quastel and Scholefield, 1951). This compound is a competitive inhibitor of the substrate in glutamic and glutamine transformation (Borek, Miller, Scheiness and Waelsch, 1946) and is as effective as methionine itself in inhibiting nitrification in soil perfusion experiments. Lees (1951) has, however, shown that no inhibition by methionine obtains with isolated nitrifying organisms. In order to throw further light on the nature of this inhibition of soil nitrification the present work was undertaken.

Methods

The technique adopted for soil perfusion is that fully described in previous publications (Lees and Quastel, 1946; Quastel and Scholefield, 1951). Briefly, 30 g air-dried crumbs (2–4 mm diam) of garden soil are continuously percolated with 200 ml solution at 21 C in the dark. When enriched or bacterially saturated soils are required the perfusate is removed, the soils washed with 250 ml distilled water and a fresh perfusate added. This process is repeated several times until analysis of the perfusate indicates that a constant and rapid rate of metabolism of the perfused material is occurring.

The alkyl S-homocysteine derivatives used in this work were kindly supplied by Dr. H. Waelsch. The alkyl mercapto-propionic and -acetic acids were synthesized by condensation of the sodium alkylmercaptides with the sodium salts of β-bromopropionic or monochloracetic acids. They were characterized by their M.P. or B.P. and neutralization equivalents.

The benzylmercapto compounds interfered with the estimation of nitrate but not with that of nitrite. All experiments on the oxidation in soil of ammonium chloride in the presence of these compounds were, therefore, conducted in the presence of sodium chlorate

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which inhibits further oxidation of nitrite to nitrate (Lees and Quastel, 1945).

RESULTS

The Action of Various Sulphur-Containing Amino Acids in Soil

As the addition of DL-methionine produces an inhibition of nitrification in soil, the action of various other S-substituted homocysteine derivatives as possible inhibitors was tested. These derivatives were perfused through soil at a concentration of 2×10^{-3} M in the presence of 10^{-3} M sodium chlorate, with and without 10^{-2} M ammonium chloride. The values recorded in table 1 show the times taken for 50 per cent of the nitrogen of the amino acid (in the absence of added NH_4^+) to appear as nitrite, and the estimated increases in lag period before nitrification of ammonium chloride began in the presence of the amino acids.

Nearly all the mercapto amino acids investigated bring about a diminution of the rate of soil nitrification. The mercapto compounds cause a far greater inhibition than the sulphoxides which, in turn, are somewhat more effective than the sulphones. The highest activity in the mercapto series lies between S-methyl- and S-hexyl-homocysteine. The S-ethyl compounds seem to cause the greatest inhibition of compounds in the sulphoxide and sulphone series. Borek and Waelsch (1949) have found, however, that ethionine sulphoxide is the least effective of the sulphoxides tested for inhibition of the glutamic and glutamine transformation and of bacterial growth. When DL-methionine is perfused and its disappearance followed chromatographically, no methionine sulphoxide or sulphone is detected in the perfusate. In addition, the increase in the lag period before nitrification begins, brought about by the addition of DL-methionine, is equal to the time during which the methionine remains in the perfusate. This observation indicates that methionine may be the active agent in securing the inhibition of soil nitrification. It is, however, possible that DL-methionine may give rise to an inhibitory intermediate which is rapidly metabolized.

TABLE 1. *The effect of* 2×10^{-3} M $R \cdot S(O)_x \cdot (CH_2)_2CH(NH_2)COOH$ *on soil nitrification*

R	FORM OF SULPHUR PRESENT	TIME IN DAYS FOR NITRIFICATION OF 50 PER CENT OF THE AMINO ACID (IN THE ABSENCE OF ADDED NH_4^+)	INCREASE IN LENGTH OF LAG PERIOD* PRECEDING NITRIFICATION, IN PRESENCE OF AMMONIUM CHLORIDE (0.01 M)
Methyl	Mercapto	14	11.5
	Sulphoxide	12	9
	Sulphone	10.5	5
Ethyl	Mercapto	>20	>30
	Sulphoxide	15	13.5
	Sulphone	6	10
Propyl	Mercapto	>20	>30
	Sulphone†	3	5.5
Butyl	Mercapto	>20	>30
	Sulphoxide	4	9.5
	Sulphone	4	5.5
Amyl	Mercapto	>20	>30
	Sulphoxide†	3	7
	Sulphone†	3	4
Hexyl	Mercapto	>20	21
	Sulphone†	3	4.5
Benzyl	Mercapto	4	6.5
	Sulphone‡	3	3.5
Lauryl	Mercapto	3	3
	Sulphone	5	6.5

* Lag period in days estimated as shown in figure 1.

† 10^{-3} M.

‡ 6.7×10^{-4} M.

10^{-3} M sodium chlorate added to all units and nitrification followed by estimation of nitrite production.

The Effect of Alkylmercapto Acids and Mercaptans on Soil Nitrification

Some of the likely intermediates in methionine metabolism have been studied as possible inhibitors of soil nitrification. One such intermediate is α-keto-γ-methylmercaptobutyric acid, which could be formed by the action of amino acid oxidases, attacking either the D or the L form of methionine. This keto acid would yield, on further oxidation, the corresponding β-methyl mercaptopropionic acid and subsequently methyl mercaptan.

The effects of various mercaptoacetic acids, mercaptopropionic acids, mercaptans and α-keto-γ-methylmercaptobutyric acid have, therefore, been investigated.

a) Effects of mercapto compounds on the nitrification of ammonium chloride in fresh soil. The results given in figure 1 illustrate the inhibitory action of the addition of 0.01 M sodium ethyl mercaptoacetate to fresh soil on the rate of nitrification of ammonium chloride to nitrate. Since the two curves shown are approximately parallel, it is concluded that the effect of this compound on nitrate formation in soil is the production of an initial lag period. The effect is similar to that brought about by DL-methionine. The most convenient method of estimating the lag period is by measurement of the increase in time (ΔL) required for nitrification of 50 per cent of the added ammonium chloride (that is, for the value for μg NO_3'-N/ml perfusate to reach 70). However, during the lag period some nitrite is detectable in the perfusate. The addition of sodium chlorate eliminates the further oxidation of nitrite and permits the study of the

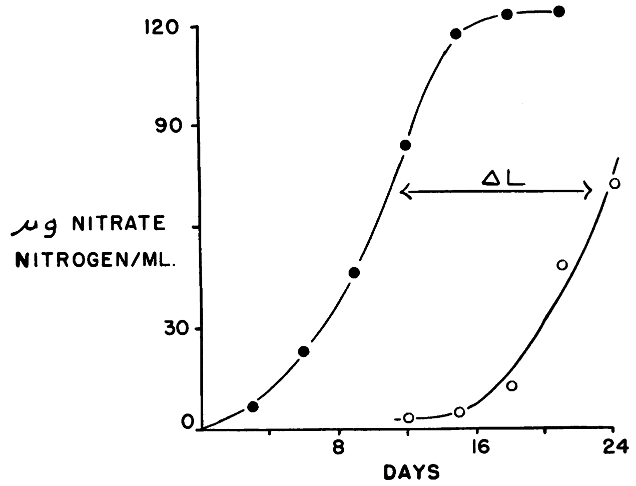

FIG. 1. Effect of 0.01 M sodium ethyl mercaptoacetate on the rate of nitrification of 0.01 M ammonium chloride to nitrate. ●, 0.01 M Ammonium chloride perfused. ○, 0.01 M Ammonium chloride + 0.01 M sodium ethyl mercaptoacetate perfused. ΔL, Increase in time required to produce 70 μg nitrate-N per ml.

oxidation of ammonia in soil to nitrite only. This technique has therefore been used in the following studies on ammonium chloride oxidation.

With β-benzyl and β-ethyl mercaptopropionic acids, which bring about an effective inhibition of soil nitrification, there is a linear relationship between ΔL (the increase in lag period) and the logarithm of the concentration of mercaptopropionic acid used (figure 2).

The inhibition is far less with the alkyl mercapto acetic acids than with the corresponding propionic acid derivatives. Results given in table 2 show the values of ΔL produced by 5×10^{-3} M alkyl mercapto-acids and benzylmercaptan. The inhibitory effects of the addition of alkyl mercaptopropionic acids on nitrification in soil are unlikely to be due to breakdown to the corresponding alkyl mercaptoacetic acids or mercaptans, as the latter compounds produce less inhibition at equivalent concentrations. Further, the alkyl mercaptopropionic acid which might be derived from methionine by oxidative metabolism shows less inhibitory action than methionine at equimolar concentrations. The addition of α-keto-γ-methylmercaptobutyric acid and the corresponding α-hydroxy compound inhibits ammonium chloride oxidation in soil but not as effectively as the addition of DL-methionine. For example, in one experiment, the value of ΔL produced by 5×10^{-3} M α-keto-γ-methylmercaptobutyric acid was 6 days, in contrast to the value of 13 days for DL-methionine and 17 days for DL-ethionine.

Repeated perfusion of sodium ethylmercaptopropionate eventually enriches the soil with organisms attacking this substrate. Mercaptan may be detected on the third perfusion after two hours by 1) characteristic odor, 2) positive nitroprusside reaction of the perfusate, and 3) formation of an insoluble yellow precipitate on adding lead acetate to a sample of the perfusate. After 4 hours all these tests are negative. Mercaptan production during perfusion of alkyl mercaptoacetic acids does not seem to occur. Sodium methoxyacetate and sodium ethoxyacetate were investigated for inhibitory action, these being structural analogues of methylmercaptoacetate. They were found to be far less active in causing the inhibition of the oxidation of ammonia (to nitrite) than the mercapto compounds (table 3).

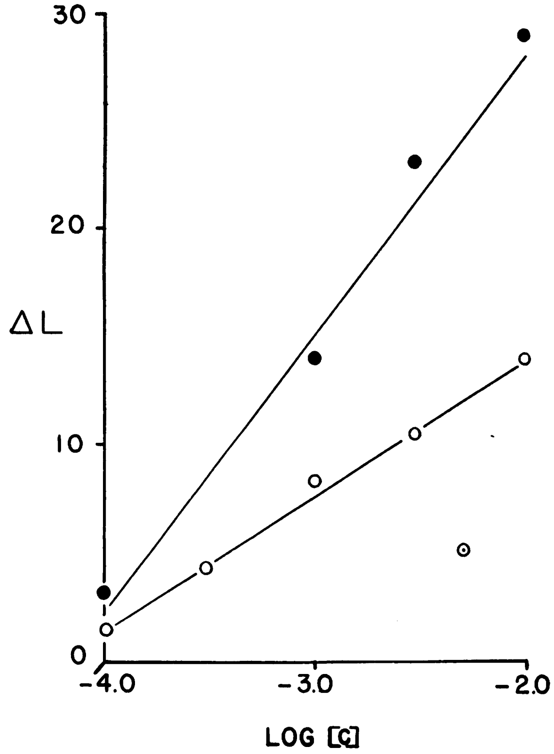

FIG. 2. Relationship between ΔL and log concentration of inhibitor. ●, β-Benzyl mercaptopropionic acid. ○, β-Ethyl mercaptopropionic acid. ⊙, Result obtained with β-methyl mercaptopropionic acid.

It is of interest that 2:4-dichlorophenoxyacetic acid has been shown (Smith, Dawson and Wenzel, 1945) to inhibit nitrite and nitrate formation in soil at a concentration of 100 ppm (5×10^{-4} M). Recovery

TABLE 2. *The increase in lag periods* preceding ammonium chloride oxidation in soil in the presence of various alkyl mercapto compounds*

ALKYL GROUP	MERCAPTOPROPIONIC ACID	MERCAPTOACETIC ACID	MERCAPTAN
Methyl	5	2	—
Ethyl	12	3	—
Benzyl	24	2	10

* All figures are values for ΔL in days.

The concentration of ammonium chloride was 10^{-2} M and the concentration of the alkyl mercapto compounds 5×10^{-3} M.

TABLE 3. *The effect of alkyl mercapto and alkoxy acids on the oxidation of 0.01 M ammonium chloride in soil*

INHIBITOR	MEAN VALUE OF μg NITRATE NITROGEN/ML PERFUSATE AFTER 10 DAYS
None	89
0.01 M Methoxyacetic acid	73
0.01 M Ethoxyacetic acid	76
0.01 M Methylmercaptoacetic acid	48
0.01 M Ethylmercaptoacetic acid	43
0.01 M Methylmercaptopropionic acid	24
0.01 M Ethylmercaptopropionic acid	12

was observed to take place in 10 to 40 days. Similar phenoxy compounds have also been shown to inhibit the nitrifying processes (Newman, 1947) although at higher concentrations.

b) The effect of mercapto compounds on the oxidation of sodium nitrite in fresh soil. Mercapto acids in soil inhibit the oxidation of sodium nitrite in a manner similar to their action on the oxidation of ammonium chloride. They increase the lag period but have no apparent effect on the proliferation constant once proliferation is initiated. The values given for ΔL are the increases in the time required for 50 per cent of the added nitrite to be oxidized. Typical results are shown in table 4. There seems to be little difference between the two series of acids as inhibitors of nitrite oxidation but again the ethyl derivatives are the most effective.

From another series of experiments the values of ΔL for 5×10^{-3} M ethyl mercaptan, β-ethylmercaptopropionate and DL-ethionine were estimated to be 3, 13 and 27 days, respectively, with sodium nitrite as substrate. Again, it seems as if the effective agent when the alkylmercapto amino acids inhibit nitrification is neither the corresponding mercaptan nor the corresponding mercaptopropionate.

c) The effect of mercapto compounds on nitrification in enriched soils. To decide whether the effects reported above are concerned with the enzymes involved in the oxidation of ammonium or nitrite ions, or whether they affect specific systems concerned only with proliferation, these mercapto acids were tested for their effects on nitrification in enriched soils.

TABLE 4. *Increase in lag periods* before nitrite oxidation in soil in the presence of alkyl mercapto acids*

ALKYL GROUP	MERCAPTOACETIC ACID	MERCAPTOPROPIONIC ACID
Methyl	2	4
Ethyl	18	13
Benzyl	6	—

* The values quoted are ΔL in days.

The concentration of nitrite was 10^{-2} M and that of the mercaptoacids 5×10^{-3} M in all cases.

For the experiment, seven perfusion units were perfused many times with 10^{-2} M ammonium chloride and seven with 10^{-2} M sodium nitrite until constant rates of nitrate production were observed in all soils. Finally, one unit in each set was reperfused with 10^{-2} M substrate and the remainder with 5×10^{-3} M methyl, ethyl, and benzyl mercaptoacetates and mercaptopropionates, respectively, plus 10^{-2} M substrate.

In no case was oxidation of sodium nitrite with these enriched soils inhibited by the mercapto compounds. Methyl- and ethyl-mercaptoacetates produced, however, 25 per cent inhibition of the rate of nitrate formation from ammonium chloride while the corresponding mercaptopropionates gave variable inhibitions with a mean of 50 per cent. The two benzyl derivatives gave inhibitions of about 80 per cent, the mercaptopropionate being somewhat more effective.

The rate of oxidation of ammonium ions is inhibited on the addition of both alkyl mercaptopropionates and alkyl mercaptoacetates to a greater extent in fresh soils than in enriched soils. Nitrite oxidation is inhibited by the addition of mercapto acids to fresh soils but not to enriched soils. It appears, therefore, that the inhibitions observed in fresh soils are due to a retardation of the onset of proliferation of the responsible organisms. These effects on proliferation are similar to those obtained in the presence of DL-methionine.

DISCUSSION

Among the mercapto compounds tested as inhibitors of the oxidation of ammonium chloride and of sodium nitrite by soil organisms, the most effective are the mercapto amino acids of the S-alkyl homocysteine series. Both the corresponding sulphoxides and sulphones are less inhibitory than the simple mercapto amino acids.

A conversion of the alkyl mercapto α-amino acids to the alkyl mercaptopropionic acids cannot wholly account for their inhibition of soil nitrification, although the mercapto acids are themselves good inhibitors of this process. Moreover, the α-hydroxy and α-keto compounds corresponding to methionine inhibit ammonium chloride oxidation in fresh soil less effectively than methionine itself. The evidence would indicate that the inhibitory effects of methionine and homologous amino acids on the process of nitrification cannot be ascribed to the effects of their oxidation products.

There can be no doubt, however, that the alkyl mercaptopropionic and acetic acids inhibit *per se* some mechanism concerned in the proliferation of the nitrifying organisms and that they inhibit the oxidation of ammonia itself. None of these compounds, however, inhibits the production of nitrite from pyruvic oxime by heterotrophic organisms (Quastel, Scholefield and Stevenson, 1952). Experiments are now in progress to ascertain what enzymic mechanisms are inhibited by the mercapto acids.

The production of mercaptan from the mercapto propionates but not from the mercapto acetates indicates that there is not a direct cleavage of the C-S bond to give a mercaptan but rather an oxidation of the propionic acid moiety.

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SUMMARY

The effects of a number of S-alkyl homocysteine compounds on the process of soil nitrification have been investigated. All compounds, whether of the mercapto, sulphoxide or sulphone series, when added to soil, inhibit the conversion of NH_4^+ or NO_2^- into NO_3^-. The largest inhibitory effect results from the addition of the mercapto compounds and the ethyl derivatives in each series.

The addition of alkylmercapto derivatives of propionic and acetic acids inhibits the process of nitrification in soil by prolongation of the period before nitrification begins. Using the increase in lag period as a measure of inhibitory action, it is found that the effect of DL-ethionine exceeds that of β-ethylmercaptopropionic acid and the effect of the latter exceeds that of ethyl mercaptan. The α-hydroxy and α-keto compounds corresponding to methionine are inhibitors but are not as effective as DL-methionine itself.

Studies with fresh and enriched soils have shown that the effect of the mercapto acids on ammonia oxidation is largely concerned with the process of proliferation. With nitrite oxidation, however, *only* the proliferation of the responsible organisms is affected.

None of the sulphur compounds studied inhibits nitrite production from pyruvic-oxime by heterotrophic organisms.

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