The Effects of Various Organic Materials on Soil Nitrification

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In his early work on the isolation of nitrifying organisms from various soils Winogradsky (1891) found that the organisms could not be cultured on gelatin. Winogradsky & Omeliansky (1899, 1902) showed that various organic materials (glucose, gelatin, asparagine, peptone, etc.) were actually toxic to the nitrifiers in culture. The more recent work on nitrification does not entirely confirm these findings. Kingma Boltjes (1935) observed nitrification in the presence of glucose, and succeeded in showing that the inhibitory effect of peptone was due to the presence of free amino-acids.

Hes (1937) in studying *Nitrosomonas* not only cultured his organisms on agar, but found that the addition of an egg preparation actually stimulated their growth, thus confirming the results of Fred & Davenport (1921) and of Kingma Boltjes (1935). Bömecke (1939), on the basis of the results of Warburg respiration experiments, even claimed to have shown a small but definite heterotrophic dissimulation in nitrifying cultures. Nevertheless, the vague concept that 'organic matter is toxic to nitrification', which arose from the work of Winogradsky & Omeliansky (1899), is still current in the literature on nitrification, and within the last 10 years Pandalai (1946) and Desai & Fazal-ud-Din (1937) have been led to put forward theories of symbiosis to account for the fact that nitrification proceeds in the presence of organic matter. But the situation remains obscure, and the present work was begun in order to determine whether there is in fact any need for a theory of symbiosis to account for soil nitrification and to discover whether the various organic materials reported as being toxic in culture have any direct effect on the nitrification process in soil.

METHODS

The percolation technique previously described (Lees & Quastel, 1946*a*) was used throughout, but the percolation apparatus was the simplified and improved form described by Lees (1947). Measurements of the time taken for ammonium ions, freshly added to the percolate, to reach an equilibrium with the soil exchange complexes have shown that equilibrium is reached within 3 hr. Because this equilibrium is reached so quickly, it has been possible to conduct replicable nitrification experiments of only 5 hr. duration.

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The garden soil containing 0.4% organic N used in previous experiments (Lees & Quastel, 1946*a, b, c*) has been used in all the present work. It is herein described as the allotment soil. Except where otherwise stated experiments were carried out on 10 g. of this soil (air dry and sieved to 4.0–1.0 mm. mesh) and 100 ml. percolate.

Determinations of the various forms of inorganic N were made by methods similar to those described by Lees & Quastel (1946*a*).

Of organic materials 10 ml. at 1% concentration were added to 90 ml. percolate. The gelatin and peptone solutions were dialyzed against 100 vol. distilled water for 24 hr. before use, in order to minimize the possible toxic effect of free amino-acids noted by Kingma Boltjes (1935). The 'humus' was prepared at pH 7 from a Littleport fen soil by a pyrophosphate extraction (Bremner & Lees, in course of preparation).

Calculation of results. The values of ' $\mu\text{g. NO}_3\text{-N formed/g. soil}$ ' given in the tables were calculated from percolate analyses by methods similar to those already described (Lees & Quastel, 1946*a*).

Because the experimental period was necessarily short, the measured formation of $\text{NO}_2\text{-N}$ or $\text{NO}_3\text{-N}$ in the percolates was small (only some 3–4 $\mu\text{g. N/ml.}$). The reagent blank on the phenoldisulphonic acid estimation was equivalent to about 1.0 $\mu\text{g. NO}_3\text{-N/ml.}$ percolate or about 25% of the actually estimated amount of nitrate. This reagent blank seems unavoidable; it appears to be due mainly to the H_2O_2 used for oxidation of nitrite in the sample. Fortunately a reagent blank introduces only a small error into comparative colorimetric readings if all the 'test' readings are close to one another and are read against the same standard, and even this small error has been eliminated by the use of the following easily derived equation in the calculation of the results:

$$U = \frac{S(T - B)}{(S - B)},$$

where S = nominal concentration of standard reference solution; T = colorimetrically observed concentration of test solution X ; B = colorimetrically observed concentration of blank solution; and U = true concentration of test solution X .

Experimental procedure. The design of a valid experimental procedure to test the effect of organic materials on the nitrification of ammonium ions by soil is not simple. The nitrifying organisms have a considerable lag phase in their proliferation curve; nitrification of ammonium ions percolated through the allotment soil is not appreciable during the first 3 days of the experiment and is not well established before the 6th (Lees & Quastel, 1946*a*). On the other hand heterotrophic proliferation in response to the addition of a carbon source to the percolate is likely to result in a relatively enormous immobilization of mineral N during the first 4 days (Fig. 1). This figure shows only the immobilization of $\text{NO}_3\text{-N}$ in the presence of various organic materials,

but similar curves are obtained if $(\text{NH}_4)_2\text{SO}_4$ is substituted for NaNO_3 in the percolate. The gradual reappearance of $\text{NO}_3\text{-N}$ in the percolates after 4 days indicated that all the carbon of the added organic material had been consumed

Fig. 1. The immobilization of nitrate N by organic materials percolated through allotment soil. Allotment soil (10 g.) percolated with 100 ml. 10^{-3}M-NaNO_3 containing: curve 1, sucrose; curve 2, glucose; curve 3, glycerol; all at 0.4 mg. C/ml.

and that lysis of a moribund heterotrophic population was taking place. The difficulty of following nitrification (i.e. the formation of nitrite and subsequently nitrate, from ammonium ions) under these conditions was largely overcome by the use of the 'stimulated soil' technique, already described by Lees & Quastel (1946c), in which soils are stimulated to active nitrification before the experiment proper. Such soils begin to nitrify fresh $(\text{NH}_4)_2\text{SO}_4$ immediately it is percolated; there is no lag phase in the process. It has been found that nitrification can proceed to some measurable extent in these 'stimulated soils' before breakdown of any organic material added to the percolate causes serious immobilization of either ammonium or nitrate ions.

The procedure used to test the effect of organic materials on soil nitrification was therefore as follows. Allotment soil in 10 g. lots was stimulated to active nitrification by percolation with 100 ml. of $2.5 \times 10^{-3}\text{M}-(\text{NH}_4)_2\text{SO}_4$ for 12 days at $18-21^\circ$. The samples were then washed 3 times with 50 ml. lots of distilled water and finally washed by percolation with 100 ml. distilled water for 24 hr.; this percolate and the previous washings were discarded. All detectable nitrite and nitrate were thus removed from the soils, which were then percolated either with 100 ml. of

$2.5 \times 10^{-3}\text{M}-(\text{NH}_4)_2\text{SO}_4$ alone, or with 100 ml. of $2.5 \times 10^{-3}\text{M}-(\text{NH}_4)_2\text{SO}_4 + 0.1\%$ of the organic material under test. Nitrite and nitrate formation by the soils in the presence and absence of organic material were determined by percolate analyses.

RESULTS

The first experiments were of 24 hr. duration. The formation of nitrite N and nitrate N during this period by soils percolated with ammonium sulphate alone and with ammonium sulphate plus glucose (or starch) are given in Table 1. Similar soils percolated with sodium nitrate, and with sodium nitrate plus 0.1% glucose (or starch) served as controls. The virtual absence of any nitrite formation by the control soils demonstrated that the appreciable quantities of nitrite formed by the test soils percolated with ammonium sulphate plus glucose (or starch) could not have arisen by reduction of traces of nitrate left in the soil. The nitrite formation observed in the presence of glucose and starch must have been from ammonium ions. This shows that in the allotment soil the oxidation of ammonium ions to nitrite ions, i.e. the first stage of nitrification, can proceed in the presence of 0.1% glucose or 0.1% starch. Nevertheless, before the 24 hr. were complete the glucose, but not the starch, percolates were showing a cloudiness, indicative of some heterotrophic proliferation that might have accounted for the obviously diminished nitrate-N formation by the glucose percolated soils. The next experiments, performed on a wider range of organic materials, were therefore of only 5 hr. duration (Table 2).

The results in Table 2 are complicated by breakdown of gelatin and peptone, but they agreed with those of Table 1 in showing that the organic materials tested had little effect on soil nitrification. This was checked by an experiment in which only 50 ml. of percolate were used (Table 3), and in which the concentration of nitrate N was reduced from $70 \mu\text{g. NO}_3\text{-N/ml.}$ to $10 \mu\text{g. NO}_3\text{-N/ml.}$ The results obtained agreed satisfactorily with those of Table 2, not only qualitatively but quantitatively. The quantitative agreement indicates that the estimation methods used for nitrite N and nitrate N were reliable, even though the total quantity of nitrogen estimated was only a few $\mu\text{g./ml.}$

Table 1. The formation of nitrite N and nitrate N by 10 g. stimulated allotment soil in the presence of glucose and starch

(Duration of experiment, 24 hr.)

Soil percolated with 100 ml. of	Nitrite N or nitrate N formed/g. soil ($\mu\text{g.}$)		
	Nitrite N	Nitrate N	Nitrite N + nitrate N
$2.5 \times 10^{-3}\text{M}-(\text{NH}_4)_2\text{SO}_4$	20	220	240
$2.5 \times 10^{-3}\text{M}-(\text{NH}_4)_2\text{SO}_4$ with 0.1% glucose	15	130	145
$2.5 \times 10^{-3}\text{M}-(\text{NH}_4)_2\text{SO}_4$ with 0.1% starch	12	200	212
$5 \times 10^{-3}\text{M-NaNO}_3$	0.1	—	—
$5 \times 10^{-3}\text{M-NaNO}_3$ with 0.1% glucose	0.1	—	—
$5 \times 10^{-3}\text{M-NaNO}_3$ with 0.1% starch	0.1	—	—

Table 2. *The formation of nitrite N and nitrate N by 10 g. stimulated allotment soil in the presence of various organic materials*

(Duration of experiment, 5 hr.)

Soil percolated with 100 ml. of	Nitrite N or nitrate N formed/g. soil (μ g.)		
	Nitrite N	Nitrate N	Nitrite N + nitrate N
2.5×10^{-3} M-(NH ₄) ₂ SO ₄	11, 16	31, 20	42, 36
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % glucose	7, 15	32, 20	39, 35
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % agar	7, 13	31, 19	38, 32
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % gelatin	17	18	35
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % peptone	31, 13	0, 22	31, 35
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % humus	16	14	30
5×10^{-3} M-NaNO ₃ and 0.1 % glucose	0.1, 0.1	—	—
5×10^{-3} M-NaNO ₃ and 0.1 % agar	0.1, 0.1	—	—
5×10^{-3} M-NaNO ₃ and 0.1 % gelatin	5	—	—
5×10^{-3} M-NaNO ₃ and 0.1 % peptone	22, 5	—	—
5×10^{-3} M-NaNO ₃ and 0.1 % humus	0.1	—	—
Water alone	0.1	c. 1	c. 1

Table 3. *The formation of nitrite N and nitrate N by 10 g. stimulated allotment soil in the presence of various organic materials*

(Duration of experiment, 5 hr.)

Soil percolated with 50 ml. of	Nitrite N or nitrate N formed/g. soil (μ g.)		
	Nitrite N	Nitrate N	Nitrite N + nitrate N
2.5×10^{-3} M-(NH ₄) ₂ SO ₄	22	14	36
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % glucose	22	15	37
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % agar	22	8	30
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % gelatin	18	12	30
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % peptone	26	4	30
2.5×10^{-3} M-(NH ₄) ₂ SO ₄ and 0.1 % humus	20	10	30
5×10^{-4} M-NaNO ₃	0	0	0
5×10^{-4} M-NaNO ₃ and 0.1 % glucose	0	0	0
5×10^{-4} M-NaNO ₃ and 0.1 % agar	0	0	0
5×10^{-4} M-NaNO ₃ and 0.1 % gelatin	8	3	11
5×10^{-4} M-NaNO ₃ and 0.1 % peptone	11	3	14
5×10^{-4} M-NaNO ₃ and 0.1 % humus	0	0	0

DISCUSSION

The results reported show that if the organic materials that are supposed to inhibit nitrification in culture do so in soil, the inhibitions by 0.1 % of the materials tested are small. Moreover, if the lack of inhibition is due to a symbiosis between heterotrophs and nitrifiers, the symbiosis is either established with startling rapidity, or exists preformed in a soil not previously treated with organic material under test. Although results show reasonably clearly that 0.1 % gelatin has little effect on nitrification in soil, this does not mean that gelatin could have no effect on nitrification in culture at a similar concentration. Gelatins always contain 5–50 p.p.m. of lead, zinc and copper, and the actual balance of at least the last two of these trace elements may profoundly affect the speed of nitrification in culture (Meiklejohn & Lees, 1948). If, however, the reported inhibitions of culture nitrification were in fact due to trace-element disturbances, there is no possibility of demonstrating the same effect in a normal soil because in it the

trace-element balance is strongly buffered by soil-complex reserves of these elements.

The present work seems to contradict much published work on the inhibition of culture nitrification by organic materials, but it should be remembered that the inhibitions observed by Winogradsky & Omeliansky (1899, 1902) and other subsequent workers may have been inhibitions not of nitrification, but of the growth of a young, and therefore sensitive, culture of nitrifying bacteria. Most of the experiments on the action of organic materials on culture nitrification have consisted in inoculating a sterile medium containing the organic material with a small amount of actively nitrifying culture, and following the subsequent formation, in the medium, of nitrite and nitrate. It is clear that any inhibition observed by this technique is as likely to be due to bacteriostasis as to inhibition of the characteristic activities of the bacteria. Meyerhof (1916 a, b, 1917) recognized the distinction, but unfortunately his results have not been given the attention they deserve. It should be mentioned

however that Nelson (1931) evaluated the glucose inhibition of both nitrite formation and nitrate formation in culture by adding glucose to actively nitrifying cultures, and found that 10% glucose inhibited the nitrification by only 4%. It is precisely this method of evaluating inhibition of nitrification, which seems the only valid one, that has been used in the present work.

SUMMARY

1. The effects of various organic materials on the oxidative nitrification process in soil have been tested by a new short-term technique.

2. None of the materials, all of which were tested at 0.1% concentration, markedly inhibited the process.

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The Immobilization of Mineral Nitrogen in Soils by Different Organic Materials

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When an organic carbon source, such as glucose, is percolated through soil together with a source of mineral N, such as nitrate, a heterotrophic population quickly builds up at the expense of both compounds, and the amount of mineral N in the percolate decreases. When the supply of one compound is used up, the heterotrophic proliferation stops and the population begins to decline. The lysis of the dead cells produces ammonium ions, which are nitrified by the soil to nitrate ions, and the nitrate-N concentration begins to rise again. Not all the N initially immobilized is released again, because a certain amount is lost, as a result of its conversion to gaseous nitrogen by the denitrifying bacteria, some of which can function under aerobic conditions (Meiklejohn, 1940).

This nitrogen immobilization cycle in soil has often been studied in pots or in the field, but there seems to have been no detailed study of the biochemical processes involved. The work here reported is a preliminary survey of these processes.

METHODS

The soil percolation technique (Lees & Quastel, 1944) has been used throughout.

Soils were percolated with either NaNO₃ solution alone, or with NaNO₃ solution plus the organic material under test, in the original soil percolation apparatus (Lees, 1946a). Ammonium, nitrite, and nitrate N were determined from time to time in 2 ml. samples of the percolates by the analytical methods already devised (Lees & Quastel, 1946). In fact the nitrite-N and ammonium-N concentrations in the percolates were never found to be more than a few µg./ml., and for simplicity they are excluded from the reported results. When it was necessary to use CO₂-free air, the modified percolation apparatus (Lees, 1946a) was employed, and air was supplied to it through two large KOH bubblers followed by a Sofnolite indicator tube. When extra CO₂ was necessary, it was introduced into the reservoir by means of a bubbler tube coupled to a Microid CO₂ generator. The CO₂ was supplied at the rate of approximately 1 bubble of CO₂/10 bubbles of entrant air.

Two soils have been used: (1) The garden soil (0.4% organic N, pH 7.8) used by Lees & Quastel (1946) (this soil is here described as the allotment soil); (2) Romney Marsh soil (0.2% organic N, pH 8.0) used by Lees (1946b).

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