

Table I. Interferences Due to Reaction of Soluble Soil Extract Components with Sulfuric Acid, Expressed as Equivalent Increases in Soil Nitrate Values

Soil	Organic C Content of Soil, %	Interference, P.P.M. NO ₃ -N
Urrbrae loam (red-brown earth)	2.2	0.20
Claremont clay (Wiesenboden)	4.2	0.04
Wollongbar clay loam (Krasnozern)	5.8	0.47

less than 20 p.p.m. by adding 0.01% sulfamic acid to extractants and standard nitrate solutions. Also, to ensure rapid removal of nitrite the extracting solution should be acidified to pH 1 with sulfuric acid. At higher nitrite levels, which require larger additions of sulfamic acid, some adjustment of technique may be needed to maintain a desired level of sensitivity in the nitrate estimation.

NITROGEN AVAILABILITY

Nitrification of Fractions from Commercial Ureaforms

Three commercial ureaforms have been fractionated by use of the cold and hot water solubility procedures used in the activity index determination. Approximately equal fractions—cold water-soluble, cold water-insoluble but hot water-soluble, and hot water-insoluble—were nitrified at rapid, intermediate, and slow rates, respectively. Good agreement was obtained in comparing nitrification rates of these ureaforms with the values obtained by recombination of their fractions, showing that the fractions were not greatly changed by the fractionation procedure and had no significant effect on each other in nitrification. The effect of granule size on nitrification rate is much less for commercial ureaforms than for other insoluble nitrogen sources such as oxamide or magnesium ammonium phosphate. While affected by granule size to some extent, ureaforms show their characteristic nitrogen release pattern, even when finely divided.

UREAFORM fertilizers are generally considered to consist of a continuous series of polymethyleneureas, as shown by the increase in solubility and in rate of nitrification observed as the urea-formaldehyde mole ratio is increased and the average molecular weight is decreased (6, 20). Rates of nitrification of ureaforms were originally related to solubility through the activity index (AI) (9, 17), and the general nitrification pattern for ureaforms has been confirmed by subsequent work (3, 5).

While it has not been possible to separate the individual components from a ureaform, there have been several studies on nitrification of the lower methyleneureas (mono-, di-, and tri-)

Table II. Recovery of Nitrate Nitrogen Added to Sterile Soil

Soil	Nitrate N, P.P.M.	
	Added	Recovered
Urrbrae loam	35.4	101.2
	42.0	100.1
	66.3	100.0
	92.4	98.2
Wanbi sand	100.2	99.9
	16.3	99.7
	20.0	98.8
	40.0	101.5
	50.4	97.7

Recovery of Nitrate Added to Soil. Known amounts of nitrate added to soil which had been leached and sterilized by γ -irradiation, were recovered quantitatively (Table II).

Chromotropic Acid-Nitrate Reaction

The nature of this reaction is not known with certainty. West and Lyles (6) believed that a nitro derivative of chromotropic acid was formed, in a manner similar to the nitration of phenoldisulfonic acid. More than one

product may be formed with some samples of chromotropic acid. Thus the West and Lyles reagent gave absorption spectra with a pronounced "hump" at wavelengths of 400 to 450 m μ in addition to a peak at 357 m μ , whereas with the BDH reagent this second maximum was lacking and the main peak was displaced to approximately 350 m μ (Figure 1). The role of chloride ions in increasing the intensity of color development is also unknown.

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(4). Both of these insoluble nitrogen sources are quickly available in finely divided form, but function as slow-release fertilizers in larger granules. A ureaform, on the other hand, showed much smaller changes in availability with changes in granule size (17). Similarly, the original report (17) on methods for available nitrogen in ureaforms showed that changes in the particle size (from 20-mesh to 100-mesh) had only slight effects on the activity index, which, in turn, was correlated with the rate of nitrification.

Since commercial ureaforms are available in different granule sizes, including a finely powdered form, we have studied the effect of particle size on the nitrification of such products.

Materials

Ureaforms. Four commercial ureaforms were used in this work. They were characterized as shown:

	% WIN ^a	% HWIN ^b	AI	% N	% Free Urea
A (Nitroform) (produced by Hercules Powder Co. in 1962)	26.3	13.1	50	38.7	6.8
B (Nitroform) (produced by Hercules Powder Co. in 1963)	25.6	13.8	46	38.1	6.5
C (Uramite) (purchased August 1961)	25.4	10.1	60	37.8	8.0
D (Nitroform) (produced by Hercules Powder Co. in 1963)	26.8	13.2	51	38.6	...

^a WIN, water-insoluble nitrogen. ^b HWIN, hot water-insoluble nitrogen.

Ureaform Fractions. Ureaform A was fractionated by the Association of Official Agricultural Chemists (AOAC) activity index method (7) using the specific quantities and procedures described, except that the phosphate buffer was omitted in the hot water treatment to simplify recovery. Separate experiments showed that this had no significant effect on the solubilities of these samples. The procedure was repeated 15 to 20 times, and the corresponding fractions from the various runs were combined. The cold water-soluble fraction (CWS) and the hot water-soluble portion of the cold water-insoluble fraction (HWS-CWS) were obtained as solids by evaporation of the filtrates at room temperature by placing the filtrates in large pans lined with polyethylene film and blowing air over them. The pans were covered with cheesecloth to protect the system from dust. All fractions were dried in a vacuum desiccator over magnesium perchlorate for 24 hours at room temperature.

Analytical values for these fractions are shown in Table I. In addition, the following analyses were obtained:

CWS
20.6% free urea (by urease)
5.2% ash as NaCl

HWS
11.0% free urea (by urease)
2.6% ash as NaCl

Ureaforms B and C were separated into hot water-soluble and -insoluble

(HWI) fractions by scaling up the AI procedure (7). Samples of about 7.5 grams each were mixed with 1875 ml. of boiling, distilled water in a large beaker. As with Ureaform A, the phosphate buffer was omitted. The mixture was then treated according to the AI procedure. A 12.5-cm. Büchner funnel with No. 50 Whatman filter paper was used for filtration. One liter of almost boiling water was used to wash the residue. The filtrates were evaporated as described for Ureaform A fractions. The solids obtained and the insoluble fractions were dried in a vacuum desiccator over magnesium perchlorate for 24 hours at room temperature.

Analytical values are shown in Table I. The following additional analyses were obtained:

Ureaform B, HWS
10.0% free urea (by urease)
1.3% ash as Na₂SO₄

Ureaform C, HWS
11.5% free urea (by urease)
3.2% sulfate ash

Methods

Activity Index. The official AOAC activity index procedure was used (7). Samples for both cold and hot water solubility tests were crushed to pass a U. S. No. 40 sieve (35-mesh).

Nitrification Tests. The procedure described by Clark and coworkers (7) was followed closely. In the final filtration, a clear filtrate was found to be readily obtained by use of a glass fiber filter paper (No. 934-AH, 12.5 cm., Hurlbut Paper Co.). In the final nitrate determination, the nitrate is

dissolved in a total of 270 ml. of water, including the 20 ml. added to the soil sample initially.

Samples were taken of the ureaforms, their fractions, and the recombinations of fractions to provide 20 mg. of nitrogen. These samples were crushed to pass a U. S. No. 40 sieve (35-mesh). Results are shown in Figures 1 to 4. These curves show per cent conversion of non-nitrate nitrogen to nitrate with time.

Preparation of Samples of Different Particle Size. A sample of Ureaform D which had been ground to pass a 35-mesh screen for the AI test was further ground to prepare portions which entirely passed through 80-mesh and 200-mesh screens. Samples of Ureaform B used were: unground material (-8 +60) and a sample ground to pass a 35-mesh screen. Ureaform C was ground to give portions which pass through 20-, 35-, 80-, and 200-mesh screens. Nitrification results are shown in Figure 5.

Results and Discussion

Complete fractionation of a commercial ureaform (Ureaform A) as shown in Table I yielded three approximately equal fractions: cold water-soluble (CWS), cold water-insoluble but hot water-soluble (HWS-CWS), and hot water-insoluble (HWI). The cold water-insoluble (CWI) and the total hot water-soluble (HWS) portions were also isolated.

The cold water-soluble fraction contains the lowest molecular weight urea-formaldehyde condensates and also free urea (20.6%) and salts from neutralization of the acid catalyst (5.2% as NaCl). The analyses were corrected for free urea, salts, and moisture to give the nitrogen and formaldehyde values shown in Table I. Of the various analyses available, total formaldehyde is most sensitive to the change in molecular weight, on the basis of the assumption that the condensates consist of series of linear polymethyleneureas. The calculation of de Jong and de Jonge (10)

Table I. Analyses of Fractions of Commercial Ureaforms

Fraction	% of Original	% N	% HCHO	Av. No. of Ureas/Molecule
Ureaform A				
CWS (cold water-sol.)	32.6	40.1 ^a	24.8 ^a	2.4
HWS-CWS (insol. cold, but sol. hot)	32.9	39.5 ^a	32.8 ^a	4.5
HWI (hot water-insol.)	34.5	38.4	35.8	7.7
HWS (total hot water-sol.)	65.5	38.9 ^a	31.9 ^a	4.3
CWI (cold water-insol.)	67.4	38.3	34.8	6.6
Ureaform B				
HWI	35.2	38.6	36.2	8.0
HWS	64.8	39.0 ^a	30.7 ^a	3.8
Ureaform C				
HWI	26.3	38.9	36.6	8.2
HWS	73.7	38.8 ^a	32.0 ^a	4.4

^a Corrected for free urea and salts.

using both total formaldehyde and nitrogen was used to obtain the number of ureas per molecule for each fraction, as shown in the last column of Table I. The average compositions are subject to considerable variation, since normal variations in the nitrogen and formaldehyde determinations could cause a deviation of ± 0.6 urea unit.

The negligible amounts of methylol formaldehyde ($-\text{CH}_2\text{OH}$ groups) generally found in ureaforms indicate that the formaldehyde is bound as methylene. The analyses reported in Table I, however, would not detect small amounts of ether linkages and would not rule out the possibility of minor amounts of cyclic or branched structures. The dependence of solubility on molecular weight is nevertheless clearly shown by these analyses.

Supplementing the complete fractionation and analysis of Ureaform A, two additional commercial ureaforms (B and C) were separated into a total hot water-soluble fraction (HWS) and a hot water-insoluble fraction (HWI). Analyses of these fractions as shown in Table I also demonstrate interdependence of solubility and molecular weight.

Nitrification rates were measured on three commercial ureaforms fractionated, on their fractions, and on recombinations of the fractions. As seen in Figure 1, ammonium sulfate and urea showed their characteristic rapid nitrification, the ureaforms being closely similar to each other at the expected slower rates.

The three fractions of Ureaform A nitrified at rapid, intermediate, and slow rates as seen in Figure 2A. Figure 2B, shows nitrification of additional fractions of Ureaform A. The cold water-insoluble fraction (CWI), free of the soluble portion, gave an almost constant, moderate rate of release. Recombination of the two fractions into which this cold water-insoluble portion had been separated gave satisfactory agreement with the curve for the CWI fraction before separation. Similarly, the total hot water-soluble fraction (HWS), free of the insoluble portion, was largely nitrified, first at a rapid rate and then more gradually.

Figure 2C, shows the good agreement obtained in comparing nitrification rates of Ureaform A itself with the recombination of its two major fractions and with the values obtained by adding the contributions of each fraction at each point.

The nitrification studies were extended to Ureaforms B and C and to the hot water-soluble and insoluble fractions into which they were separated. Results similar to those for Ureaform A were obtained (Figures 3A and 4A). A very slow rate of nitrification was observed for the hot water-insoluble fractions from all three ureaforms (HWI, Figures 2A, 3A, and 4A). While this

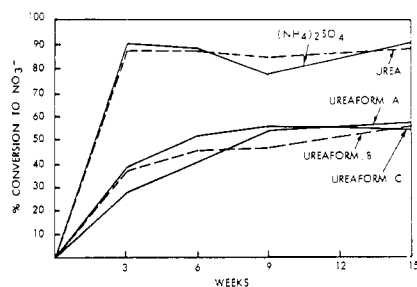


Figure 1. Nitrification of ureaforms

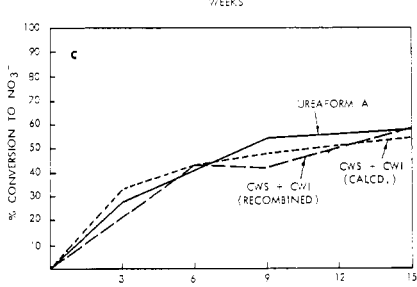
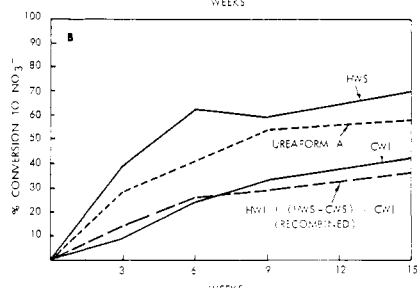
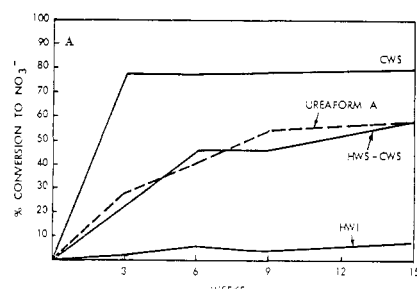


Figure 2. Nitrification of Ureaform A fractions

is consistent with the low solubility and high molecular weight of this fraction, the slight response over the 3- to 15-week period was somewhat unexpected. It was reasoned that this effect might be exaggerated by the absence of the more rapidly nitrified fractions, particularly in view of the suggestion by Pereira and Smith (78) that these fractions enhance the rate of nitrification of the hot water-insoluble fraction. To test this hypothesis, an equal amount of nitrogen as urea was added to the HWI fractions from Ureaforms B and C. While the urea portion nitrified rapidly, the effect on the rate of nitrification of the HWI fractions was almost negligible (Figures 3A and 4A).

Figures 3B and 4B show the remarkably close agreement obtained between the nitrification rates for Ureaforms B and C and the recombinations of the fractions into which they were

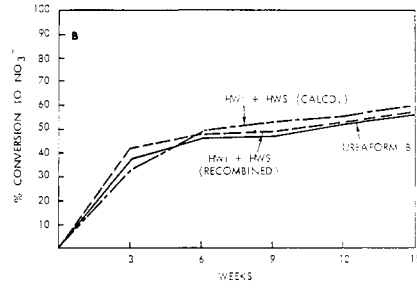
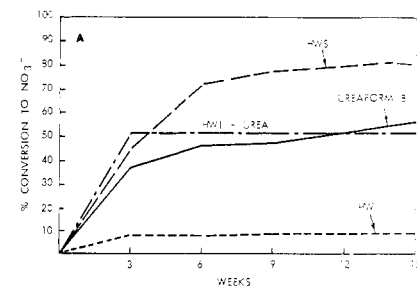


Figure 3. Nitrification of Ureaform B fractions

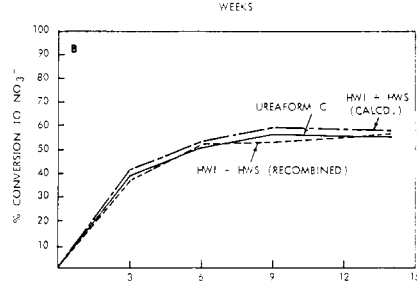
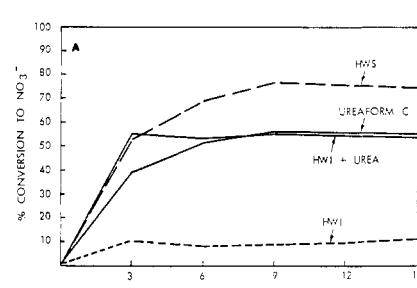


Figure 4. Nitrification of Ureaform C fractions

separated. Similar excellent agreement was obtained by adding the contributions of each fraction at each point. These curves, taken with the data on Figure 2, B and C, indicate clearly not only that the fractions were not greatly changed by the fractionation procedure but also that they had no significant effect on each other in these nitrification experiments. This is contrary to the conclusion reached by Pereira and Smith (78) on the basis of limited nitrification data.

While our results are self-consistent and certainly give the qualitative relations expected, it is generally conceded that the incubation type of nitrification does not accurately show the rate of release of all the available nitrogen. Specifically, in our laboratory nitrifications, the rate curves for the ureaforms tend to level off at 60 to 70% conversion to nitrate, perhaps largely because of the slow rate observed for the hot water-

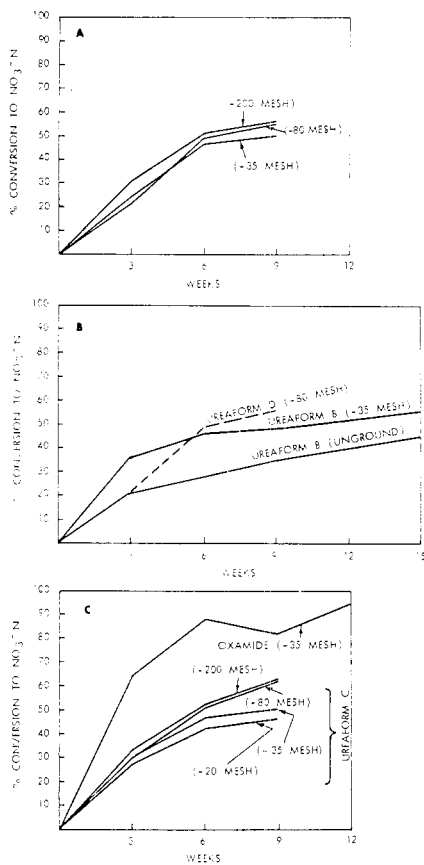


Figure 5. Effect of particle size on nitrification of ureaforms

A. Ureaform D

insoluble fraction. This type of nitrification does not provide information on the frequently observed carry-over of residual nitrogen in the use of ureaforms (13) or on the suggestion of Byrne and Lunt (5) that the rate of nitrification of the resistant fraction tends to increase as it ages. Cropping studies over a sufficiently extended period under natural conditions will be required to obtain quantitative confirmation of the more complete availability of ureaform nitrogen generally observed in actual use. Work of this type is in progress.

The data reported here offer added support for the correlation of nitrification rate with solubility and molecular weight. The most soluble fraction is made up largely of methylenediurea and dimethylenetriurea, in addition to free urea. Completely soluble under the conditions of the activity index procedure, it is nitrified almost as rapidly as urea in the laboratory method (Figure 2.4). Since most of the urea is combined, this fraction is found to be essentially non-burning in fertilizer use (8). The solubility designation and the laboratory nitrification data on this material are somewhat misleading in view of the large excess of water used in the AOAC activity index procedure and the fine particle size to which solids are ground for these tests. Even the lower methyl-

eneureas are relatively insoluble materials. When used in a pelleted or granular fertilizer form, their release, while still relatively rapid, may be expected to be significantly slower than that of very soluble materials such as urea.

The intermediate fraction of ureaform which is soluble in hot water but insoluble in cold (HWS-CWS) approaches ureaform in composition and average molecular weight. Its nitrification in our work was almost identical with that of the ureaform from which it was derived (Figure 2.4). The major component expected in this fraction is trimethylene-tetraurea. Previous workers (15, 16, 19) have found this compound to nitrify rapidly. The next higher methyleneureas thus appear to be responsible for the desirable low solubility and gradual release properties of this fraction as indicated by our analytical and nitrification values.

The hot water-insoluble fraction is the highest in molecular weight and the slowest to release its nitrogen. It is thus largely responsible for the reserve of nitrogen provided by continued use of ureaforms. The rate at which its nitrogen becomes available under use conditions remains to be determined.

The results of this fractionation study are consistent with the concept that ureaforms are made up of a continuous series of low molecular weight polymers which are successively decomposed by soil microorganisms.

Nitrification rates and solubility determinations in the activity index procedure were run on samples ground to pass a 35-mesh screen in the work reported. Grinding to finer sizes appears to have only a small effect, as shown in Figure 5A. This includes the range of the commercial powdered product (Nitroform Powder Blue, Hercules Powder Co.) which passes 60-mesh. On the other hand, going to larger particle size has a more striking effect, as shown in Figure 5B. The rate for the unground commercial product (-8 +60) is noticeably slower than the rate for the ground material (-35) used in the analytical determinations. The nitrification rate for the unground material indicates a more uniform and gradual release than suggested by curves based on ground product. The soluble portion presumably dissolves and nitrifies much more slowly when occluded in a granule than when made immediately available by grinding.

Figure 5C, shows a slightly more pronounced but similar effect of particle size on the rate of nitrification of another commercial ureaform. A nitrification curve for oxamide, ground to pass 35-mesh, is also shown for comparison and confirms the previous observation that it releases its nitrogen rapidly when finely ground.

These nitrification data support the earlier nitrogen recovery experiments (77), which showed ureaforms to be much less sensitive to change in particle size than oxamide. While affected by granular size to some extent, ureaforms show their characteristic nitrogen release pattern, even when finely divided.

Acknowledgment

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