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BIODEGRADABILITY

Biodegradability of Ureaformaldehydes and Related Compounds

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A method based upon the principles of the BOD test was used to study the degradation of ureaforms. By making the nitrogen content the limiting factor in the growth media, a relationship between turbidity and nitrogen content allowed for measurement of the degree of degradation of ureaforms. The results correlated with previously reported nitrification studies. An additional parameter, the biodegradability index, was suggested to define more clearly the agronomic utility of ureaforms and processed plastic scrap.

UREAFORMS were biodegraded to the extent of 50 to 55% as measured by a Warburg respirometer technique (*7*). Separation of the undegraded residue and reincubation under the same test conditions showed no growth. While the residue was not degraded under the test conditions, nitrification and turf studies indicated that higher molecular weight fractions degraded to some extent (*3–5*). Evidently, the degradation of the higher molecular weight components of the ureaform was so slow that the growth of the organisms could not be supported.

Since the Warburg respirometer procedure used in the earlier study (*7*) apparently did not achieve the objective of measuring the total potentially available nitrogen in ureaforms, modification of the biodegradability methodology was required. The procedure used in this study did not alter the basic principles of the biological oxygen demand (BOD) test (*1*). All nutrients except the nitrogen source were provided for organism growth. Nitrogen available from the material to be studied was the limiting factor for growth. As the organisms degraded the nitrogen source and utilized it for growth, their numbers increased. As the microorganism population increased, cell protoplasm caused the media to become turbid. By developing a relationship between nitrogen content and the intensity of the turbidity, measurement of nitrogen utilization was possible.

The modified biodegradability procedure described herein offers a simple and rapid method of evaluating ureaforms and similar materials which can be correlated to the more commonly used nitrification studies. This method is useful, since the activity index determination is of limited value as an indication of quality for slow release nitrogen sources such as button scrap.

Method

Apparatus. The shaking apparatus was a Gyrorotary water bath shaker capable of maintaining a temperature of 25° ± 0.5° C., New Brunswick Scientific Co., Model GR 76, equipped with cooling coils to maintain temperature, or its equivalent.

Bausch & Lomb Spectronic 20 colorimeter with 1/2-inch cells, or its equivalent.

Reagents. A. Phosphate buffer solution, 8.5 grams of KH_2PO_4, 21.7 grams of K_2HPO_4, and 33.4 grams of $Na_2HPO_4 \cdot 7H_2O$ dissolved in 1 liter of distilled water (*1*).

B. Magnesium sulfate solution, 22.5 grams of $MgSO_4 \cdot 7H_2O$ dissolved in 1 liter of distilled water (*1*).

C. Calcium chloride solution, 27.5 grams of anhydrous calcium chloride dissolved in 1 liter of distilled water (*1*).

D. Ferric chloride solution, 0.25 gram of $FeCl_3 \cdot 7H_2O$ dissolved in 1 liter of distilled water (*1*).

E. Seed organisms, obtained from domestic sewage, collected, and stored for 24 hours in a refrigerator. Twenty-five milliliters of the supernatant is used as the inoculum.

F. Dilution Water. Add 10 ml. of solution A, 1 ml. each of solutions B, C, and D, 1 gram of glucose, and 25 ml. of seed organisms to a 1-liter volumetric flask and bring to volume with distilled water.

G. Adapted Seed Cultures. Add 50 mg. of the nitrogenous material to be studied to a 250-ml. Erlenmeyer flask. Add 100 ml. of the seeded dilution water; stopper with a cotton plug, and place on the shaking apparatus for 1 week. Turbidity in the media is indicative of the growth of organisms capable of

utilizing the source of nitrogen under study. Allow the contents of the flask to settle for 1 hour. Prepare dilution water using 25 ml. of the supernatant liquid as the inoculum.

Preparation of Standard Curve. Prepare a standard solution of ammonium sulfate so that 1 ml. contains 0.1 mg. of nitrogen per ml. Pipet aliquots ranging from 0.1 to 1.5 mg. of nitrogen into several 250-ml. Erlenmeyer flasks. Bring the volume of solution in all Erlenmeyer flasks to 100 ml. with seeded dilution water; stopper with a cotton plug, and place on the shaking apparatus. Daily measure the intensity of the turbidity at 580 mμ in ½-inch cells by means of a Spectronic 20 spectrophotometer. To obtain a calibration curve, plot the maximum absorbance reading obtained with each concentration against nitrogen content.

Determination of Biodegradability of Ureaforms. If required, grind the material to be studied with a mortar and pestle, so that the maximum particle size is 35-mesh.

Prepare the water-soluble fraction of the ureaforms by washing a 1.0-gram sample with 250 ml. of distilled water and collect the washings in a 250-ml. volumetric flask. Use aliquots containing 0.2 to 1.0 mg. of nitrogen.

Place the cold water–insoluble residue in a beaker containing 250 ml. of boiling water and stir with a magnetic stirrer for 30 minutes. Filter, using a rapid filter paper such as Whatman No. 7. Wash the residue on the filter paper with 100 ml. of boiling water. Place the insoluble residue on the filter paper in a beaker containing 250 ml. of boiling water for a second time and stir with a magnetic stirrer for 30 minutes. Filter again, using Whatman No. 7 filter paper. Wash the residue on the filter paper with another 100 ml. of boiling water. The insoluble residue on the filter paper is the hot water–insoluble fraction.

Combine the washings and filtrates and cool to room temperature. Filter the precipitated solid material, using a Whatman No. 7 filter paper. Air-dry the solid material. This is the cold water–insoluble, hot water–soluble fraction or intermediate molecular weight fraction.

To obtain the total water-soluble fraction place 1.0 gram of ureaform in 250 ml. of boiling water and stir with a magnetic stirrer for 30 minutes. Filter, using a Whatman No. 7 filter paper. Wash the residue with 100 ml. of boiling water. Repeat the extraction of the residue as previously described. Combine the filtrates and washings and evaporate to dryness with the aid of a hair dryer.

Weigh accurately 4.0 to 5.0 mg. of the solid material to be studied into a 250-ml. Erlenmeyer flask. Add 100 ml. of seeded dilution water, stopper with a cotton plug, and place on a shaker. Measure the absorbance as previously described. Determine milligrams of nitrogen from a standard curve. Calculate per cent nitrogen utilized at the time interval desired.

Results and Discussion

Samples of methyleneureas, methylenediurea, dimethylenetriurea, and trimethylenetetraurea were studied for insight into the relationship between increasing chain length and biodegradability. Figure 1 shows the biodegradability of these three methyleneureas. When the per cent of these compounds degraded is plotted against the urea groups per molecule, a straight-line relationship (Figure 2) is noted. In this small range, increases in chain length result in decreased biodegradability. More polymethyleneureas were not available for the experimental determination of the degree of availability beyond four ureas per molecule. The methyleneureas beyond two ureas per molecule are not well characterized. These are mixtures which analyze correctly but undoubtedly contain higher and lower molecular weight compounds.

Ureaform was fractionated into four solubility classes, the cold water–soluble or low molecular weight fraction; the cold water–insoluble, hot water–soluble or intermediate molecular weight fraction; the hot water–insoluble residue or the high molecular weight fraction; and the total water-soluble fraction. The biodegradability curves for these various fractions are shown in Figure 3. The cold water–soluble fraction was completely degraded, the cold water–insoluble, hot water–soluble fraction was biodegraded to the extent of 75%, and the hot water–insoluble fraction was 15% degraded. The total water–soluble fraction degraded to the extent of 66%. The total water–soluble fraction degraded below what would be expected—namely, a value intermediate between the cold water–soluble fraction and the cold water–insoluble, hot water–soluble fraction. This is not totally unexpected in a solution degradability test that has as a limiting factor materials of differing degrees of availability.

Organisms capable of utilizing the low molecular weight fraction increase in numbers more rapidly than those capable of utilizing the intermediate fraction and become the dominant population. The growth of the population utilizing the low molecular weight fraction reaches a maximum and begins to die. The organisms capable of utilizing the intermediate fraction reach a rate of growth equivalent to the death rate. The sum of this results in what can be termed a stationary phase, where numbers remain relatively constant. Eventually, the organisms capable of utilizing the intermediate fraction become dominant and continue to increase in numbers until the nitrogen source is utilized. The death rate exceeds growth and the intensity of the turbidity decreases. The curve of the total water–soluble fraction (Figure 3*c*) follows this pattern. In the stationary phase of 3½ days prior to an increase in turbidity (numbers), considerable nitrogen is undoubtedly utilized, accounting for the lower than expected result.

A comparison of the nitrification of the fractions of ureaform with similar fractions reported by Hays and coworkers (*6*) shows that agreement exists between the results of biodegradability studies and nitrification. Hays *et al.* (*6*) re-

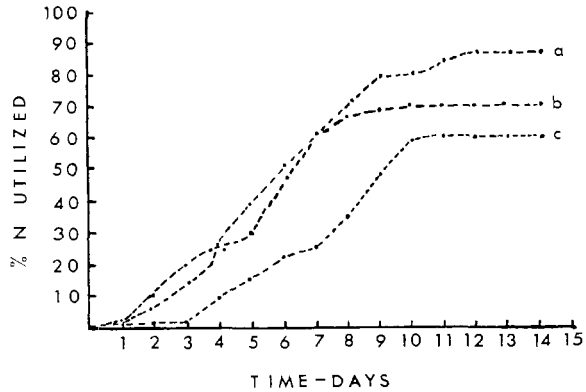

Figure 1. Biodegradability of methyleneureas

a. Methylenediurea
b. Dimethylenetriurea
c. Trimethylenetetraurea

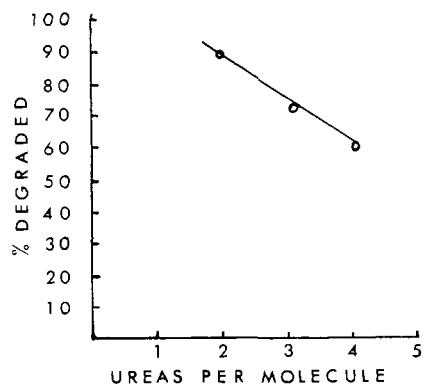

Figure 2. Linear degradability of methyleneureas

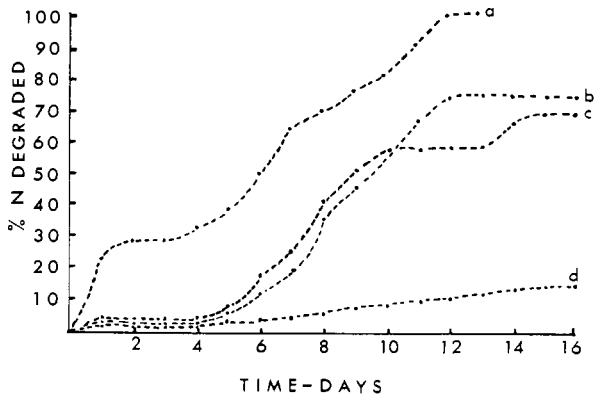

Figure 3. Biodegradability of ureaform fractions

a. Water-soluble nitrogen
b. Cold water–insoluble, hot water-soluble nitrogen
c. Total water-soluble nitrogen
d. Hot water–insoluble nitrogen

ported 80% of the cold water–soluble fraction nitrified, 60% of the cold water–insoluble, hot water–soluble fraction, and 10% of the high molecular weight fraction. Since ammonium sulfate nitrified in these studies to the extent of 90% of the theoretical value, the results would tend to reflect slightly lower conversions than those found by biodegradation. Converting all nitrification data to a 100% conversion of ammonium sulfate, the cold water–soluble fraction was 89% converted as compared to 100% determined by degradation, the intermediate fraction 67% compared to 73% by degradation, and the high molecular weight fraction 11% compared to 15% by biodegradation. The results agree reasonably well.

Standard ureaform products have an excellent correlation among nitrification, solubility, and molecular weight (*3–5, 7, 8, 10*). Standard ureaforms have a solubility pattern of approximately 1/3 cold water–soluble, 1/3 cold water–insoluble, hot water–soluble, and 1/3 hot water–insoluble. Activity index is generally used as a parameter of the quality, although the water-insoluble nitrogen fraction must be specified to define the solubility characteristics of a ureaform. The activity index is defined as:

$$\frac{\text{WIN} - \text{HWIN} \times 100}{\text{WIN}} = \text{A. I.} \quad (2)$$

where WIN is the per cent cold water–insoluble nitrogen and HWIN is the per cent hot water–insoluble nitrogen.

Table I presents the composition of three standard ureaform products, two button scraps, and a low solubility ureaform according to solubility, activity index, and amount of nitrogen degraded. The total water-soluble nitrogen fraction for all six products ranges from 22.9 to 29.1%. The biodegradable nitrogen for the standard ureaforms and the low solubility ureaform is relatively constant, ranging from 24.0 to 25.9% while being extremely low for the button scraps. For the standard ureaforms there is good agreement between water solubility and bioavailability. However, for the button scrap, water solubility and availability are not synonymous.

The activity index is in good agreement with the biodegradable nitrogen for the three standard ureaforms and the low solubility ureaform but not for the button scrap. Although the activity index does not include the consideration of the biodegradable cold water–soluble fraction, the availability of this fraction is assumed. The solubility of the water–insoluble fraction is essentially equivalent to the bioavailability of this fraction. Agreement between activity index and biodegradability is not unreasonable, but the close agreement noted with the three standard products would not necessarily hold consistently with the variations in composition of commercial ureaforms.

With materials such as button scrap, which is not a standard product, these relationships are not valid. Another parameter is suggested to define more clearly the agronomic utility of ureaform and ureaform related products. This parameter could be called the "biodegradability index" and can be defined as the amount of nitrogen bioavailable under the previously described procedure and having a minimum value of 40%. A similar parameter based upon nitrification data would yield equivalent values and can be stipulated as the "nitrification index." This concurs with Volk's thought (*9*) that the activity index alone was not a consistent criterion of quality and could be misleading, especially for processed plastic wastes.

It is extremely doubtful that the approximately 2/3 of the standard ureaform is all the ureaform that is degradable. In laboratory studies of degradability sample size is obviously the limiting factor. Organisms in the solution biodegradability method require a constant supply of nitrogen. In order to reflect the very slow rate of degradation expected of the high molecular weight fraction of ureaform, it will be necessary to increase the sample size in the turbimetric procedure. Sufficiently large samples should reflect more accurately the total amount of ureaform potentially available.

Table I. Comparison of Biodegradable Nitrogen and Solubility Patterns of Ureaforms

Product	Total Nitrogen, %	CWSN, %	WIN, %	CWI-HWSN, %	HWIN, %	TWSN, %	BN, %	AI	N Biodegraded, % of Total
Ureaform A	37.4	10.6	26.8	16.1	10.7	26.7	24.0	60.1	64.2
Ureaform B	37.7	13.3	24.4	15.8	8.6	29.1	25.9	63.8	68.7
Ureaform C	38.7	12.0	26.7	16.0	10.7	28.0	24.2	59.5	62.5
Button scrap A	30.9	9.8	21.1	13.1	8.0	22.9	4.4	38.0	14.3
Button scrap B	31.8	17.5	14.3	10.3	4.1	27.7	6.1	71.9	19.1
Low solubility ureaform	37.5	1.6	35.9	22.4	13.5	25.0	24.4	65.2	65.1

CWSN. Cold water–soluble nitrogen
WIN. Water-insoluble nitrogen
CWI-HWSN. Cold water–insoluble but hot water–soluble nitrogen
HWIN. Hot water–insoluble nitrogen
TWSN. Total water-soluble nitrogen
BN. Biodegradable nitrogen
A.I. Activity index

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UREAFORMS

Soluble Fraction of Ureaforms—Nitrification, Leaching, and Burning Properties

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The large water-insoluble portion of ureaforms is responsible for their slow nitrogen release properties. However, the lower methyleneureas present are somewhat soluble in water. In the activity index procedure, 25 to 35% of the ureaform nitrogen dissolves. Such solubility, under the conditions of this procedure, does not justify attributing properties of quick availability, rapid leaching, and burning to the water-soluble fraction of ureaforms. Over 90% of the nitrogen in ureaform is combined urea nitrogen. The chemical combination of urea, even in the lowest condensates with formaldehyde, results in decreased rates of nitrification and of leaching, as well as in greatly reduced burning properties.

THE large water-insoluble portion of ureaforms is responsible for their useful slow release properties in comparison with the rapid release from soluble fertilizer materials. In the continuous series of methyleneurea polymers which make up a ureaform, the lowest polymers are the more soluble in water (*6*). The lowest member of this series, methylenediurea ($NH_2CONHCH_2NHCONH_2$), is 2.5% soluble in water at 25° C.; dimethylenetriurea, the next member, is only 0.1% soluble in water at 25° C.

The activity index procedure, which offers the simplest direct characterization of ureaforms, involves treatment of a finely ground 1-gram sample with 250 ml. of water at room temperature. Under these conditions, 25 to 35% of the ureaform nitrogen dissolves; the widespread use of the activity index has led to characterization of ureaforms as about "one-third water-soluble." Solubilities under less drastic conditions are of a lower order, as indicated by the values stated in the first paragraph. This limited solubility has led to association of the agronomic properties of the low molecular weight portion of ureaforms with the almost immediate availability, leaching tendencies, and burning properties characteristic of soluble nitrogen sources such as urea and ammonium nitrate (*12*). Such properties would not be expected, since over 90% of the nitrogen in ureaforms is combined urea nitrogen (commercial ureaforms may contain 6 to 8% free urea) (*6*). It was of interest, therefore, to determine rates of nitrification and of leaching as well as to consider the burning properties of the soluble fraction of ureaforms and of the lowest condensates of urea with formaldehyde.

Materials

Methylenediurea. This compound was prepared by a modification of the method of Kadowaki (*8*). Urea (12 moles) and formaldehyde (1.77 moles) yielded 133 grams of crude product, m.p. 185° to 250° C. This product was slurried with 3 liters of hot methanol, cooled to 40° C., and filtered warm. The filtrate was held at 0° C. for 16 hours and the crystalline product filtered off, m.p. 213° C. (Because of the heat sensitivity of methylenediurea and dimethylenetriurea, the sample had to be dropped onto a melting point block heated to within about 5° C. of the final melting point observed to get complete melting. The melting points quoted were taken in this manner.) The extraction procedure was repeated using fresh methanol and re-using the filtrates until the melting point of the product began to increase. In this way, 70% of the crude product was isolated as sharp melting crystals (m.p. 212–214° C.). The material insoluble in methanol melted at about 265° C.

A composite sample of the recrystallized product was analyzed.

ANALYSIS. Calcd. for $C_3H_8N_4O_2$: N, 42.4%; CH_2O, 22.7%. Found: N, 41.6%; CH_2O, 22.2%.

SOLUBILITY IN WATER. 2.5 grams per 100 ml. at 25° C.; 7.0 grams per 100 ml. at 50° C.

SOLUBILITY IN METHANOL. 0.03 gram per 100 ml. at 25° C.; 0.35 gram per 100 ml. at 60° C.

Dimethylenetriurea. This compound was prepared by the method of Winsor and Long (*14*). Dimethylolurea (1.0 mole) and urea (4.0 moles) yielded 62 grams of product, m.p. 275–280° C. (with decomposition). The crude material (50 grams) was recrystallized by slurrying in 2 liters of water at 70° C., filtering hot, and storing the filtrate for 16 hours at 0° C. The crystalline