

FORMS OF ORGANIC NITROGEN IN DOMESTIC WASTEWATER

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Although there is a need to trace organic nitrogen degradation pathways during wastewater treatment, the procedure presented in "Standard Methods"¹ for organic nitrogen does not differentiate the various forms; instead organic nitrogen compounds are determined collectively. Differentiation determinations usually require sophisticated instrumentation and complex procedures. Furthermore, interpretation of nitrogen determinations of a particular treatment process can only remain obscure until all forms of nitrogen can be determined specifically. With increased emphasis on secondary treatment, it has become essential to determine those forms of nitrogen most resistant to oxidation. Following tertiary treatment, the residual nitrogen species entering the receiving stream should be known in order to evaluate their potential effects on aquatic plant growth.

Moreover, a variety of industrial wastes are deficient in nitrogen, and, consequently, require supplementary nutrients for efficient treatment. The most readily assimilated form of nitrogen should be used, but very little information is available concerning this particular point. Ammonium salts have been widely used, but losses through volatilization and the possibility of toxicity suggest that other

species of nitrogen may be more efficient. Urea has been used as a nitrogen supplement for crop production and may, therefore, be applicable to waste treatment processes requiring nitrogen enrichment. Regardless of whether or not urea is an efficient nitrogen source for enrichment of industrial wastes, analytical procedures that sort the various forms of nitrogen would be helpful to evaluate the most efficient species for nitrogen supplementation.

In this investigation, an attempt has been made to determine organic nitrogen more specifically than "Standard Methods" does without employing sophisticated instrumentation that is usually not available to the average treatment plant control laboratory.

Domestic Wastewater

Domestic wastewater is essentially urine, feces, and cellulose dispersed in relatively large volumes of water, although additional waste materials may also be present. Table I indicates the organic constituents of normal urine, and Table II shows the chemical constituents of feces. Daily per capita excretion of urine is 1,500 ml, and daily per capita excretion of feces is 150 to 200 g (wet) and 25 to 50 g (dry).

Approximately 80 percent of total nitrogen is introduced into wastewater as urea. Tables I and II indicate the precursor of most of the free ammonia in wastewater is urea. Urea is readily hydrolyzed to ammonia by the specific enzyme urease.

From Tables I and II and assuming 100 gpd/cap (380 l/day/cap) waste-

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TABLE I.—Organic Constituents of Normal Urine, Miller²

Constituents	Concentration (g/l)
Urea	25.0
Uric acid	0.6
Creatinine	1.5
Ammonia	0.6
Undetermined-N	0.6
Sugar	Trace
Ketone bodies	Trace
Pigments	Present
Mucin and mucinlike substances	Present

water, the amount of nitrogen introduced into domestic wastes is approximately 53 mg/l, of which 80 percent may be attributed to urea. Substantial variations from these values indicate larger or smaller volumes of dilution water or additional sources of nitrogen. Previous work has established that urea nitrogen is recovered in the "Standard Methods" procedure for organic nitrogen.⁴

Description of Sampling

The main consideration in the selection of sampling points was convenience. Catch samples were collected from the Madison, Wis., Nine Springs wastewater treatment plant and the Cross Plains, Wis., plant between 8 and 9 AM each morning. Cross Plains is located approximately 10 miles (16.1 km) west of Madison. Only influent samples were collected. This type of sampling permitted analytical work to be started almost immediately. Enzy-

TABLE II.—Chemical Constituents of Feces, Kolmer³

Constituents	Amount
Calcium	0.4 to 0.8 g/day
Total fat	7.3 to 27.6% dry feces
Neutral fat	2.5 to 11.8% dry feces
Free fatty acids	1.1 to 10.0% dry feces
Combined fatty acids (soaps)	0.5 to 11.5% dry feces
Total nitrogen	0.5 to 1.5 g/day (average 1.3 g/day)
pH	6.9 to 7.2

matic and biochemical reactions were terminated with the addition of reagents. Analytical results were intended to be representative of the wastewater at the time of sampling.

The Madison Nine Springs plant receives wastewater from a population of 200,000, while the Cross Plains plant serves only 950 people.

A meat-packing plant, which pre-treats its waste before discharge to the Nine Springs plant, contributes about 3 mgd (11,355 cu m/day), or 11 percent of total flow. A metal-working plant and a battery producer are the next largest waste contributors to flow volume. The organic load of these two sources is quite low.

At Cross Plains a creamery contributes about 18 percent of the volumetric flow to the village. A flow-equalization tank permits flow to be apportioned over a 24-hr period. No other industrial waste enters the sewers of Cross Plains.

Procedures Used

The microsteam distillation procedures that have been used extensively for nitrogen determinations in soils seemed to be adaptable to wastewater nitrogen determinations. However, when serious foaming developed in the urea-N determination, a macro-version of the micro-procedure was developed, which required that several "Standard Methods" determinations be used for comparable results.

Procedures finally evolved were as follows. Free ammonia and organic-N were those of "Standard Methods." A micro-procedure for urea-N was scaled up to fit into wastewater laboratory procedures. The sample was incubated for 2 hr with urease at 35°C. The sample was distilled using the "Standard Methods" buffer for ammonia-N. Because results included both free ammonia-N and urea-N, the free ammonia-N was subtracted from the combined-N to calculate urea-N values.

TABLE III.—Summation of Ammonia and Urea Nitrogen as Percent of Total Nitrogen in Madison, Wis., Raw Wastewater, 1968

Date	NH ₃ -N (mg/l)	Urea-N (mg/l)	Total-N (mg/l)	NH ₃ -N + Urea-N (% of Total-N)
July 5	11.9	0.0	19.2	62.5
July 9	12.6	4.9	31.2	56.1
July 10	13.2	1.4	18.9	77.3
July 12	12.8	1.3	20.3	69.5
July 17	9.8	0.6	16.3	63.8
July 19	11.0	2.3	16.8	79.2
July 24	9.9	2.3	18.2	67.0
July 29	14.4	1.3	21.1	74.4
August 2	12.8	1.4	18.7	75.7
August 7	6.6	0.4	11.7	59.8
August 12	13.1	0.9	19.0	73.3
August 16	9.6	0.3	15.3	64.7
August 21	7.4	0.7	12.9	62.8

Free α amino acid-N was determined by the microsteam distillation of soils analysis using ninhydrin. Bremner⁵ found that the condensation that occurs between ninhydrin and free α amino acid can be suppressed at pH 2.5 and that quantitative recovery of α amino-N can be achieved by treatment of amino acids with ninhydrin at pH 2.5 followed by steam distillation of the reaction products at pH 11.2.

Total α amino acid-N was determined in the same manner as free α amino acid-N except that the sample was hydrolyzed in hydrochloric acid (6 N HCl) for 12 hr to release free α amino acids from proteins before the reaction with ninhydrin.

Particulate-N may be defined as the nitrogen retained on a membrane filter. A wastewater sample was concentrated fivefold by vacuum evaporation. The concentrate was filtered through a membrane filter. Total-N was determined on a preconcentrated unfiltered sample as well as on the filtrate. The difference in N between the unfiltered and filtered sample represented particulate-N. Particulates were transferred from the membrane to a

TABLE IV.—Percentage of Total α Amino Acid Nitrogen Relative to Organic Nitrogen in Madison, Wis., Raw Wastewater, 1968

Date	Total α Amino Acid-N (mg/l)	Organic-N (mg/l)	Organic-N (%)
July 5	2.5	7.2	34.7
July 9	7.4	18.7	39.6
July 10	2.5	5.7	43.9
July 12	2.1	7.5	28.0
July 17	1.4	6.5	21.5
July 19	2.4	5.8	41.4
July 24	2.3	8.3	28.0
July 29	1.6	6.7	23.8
August 2	2.2	5.9	37.3
August 7	1.3	5.1	25.5
August 12	1.6	5.9	27.1
August 16	2.0	5.7	34.2
August 21	0.8	5.5	14.5

receiving flask, and the solids were hydrolyzed under reflux for 12 hr to release free α amino acid-N from the particulates. Total α amino acid-N was determined on the solids retained by the membrane filter following acid hydrolysis.

Presentation of Results

Table III shows recoveries of (NH₃-N + urea-N) expressed as percent of total-N on raw wastewater at the Nine Springs plant. The partial nitro-

TABLE V.—Proportion of Organic Nitrogen Represented by Total α Amino Acid and Urea Nitrogen in Madison, Wis., Raw Wastewater, 1968

Date	Total α Amino Acid-N (mg/l)	Urea-N (mg/l)	Organic-N (mg/l)	Urea-N + TAA-N (% of Organic-N)
July 5	2.5	0.0	7.2	34.7
July 9	7.4	4.9	18.7	65.8
July 10	2.5	1.4	5.7	68.4
July 12	2.1	1.3	7.5	45.3
July 17	1.4	0.6	6.5	30.8
July 19	2.4	2.3	5.8	81.0
July 24	2.3	2.3	8.3	55.4
July 29	1.6	1.3	6.7	43.3
August 2	2.2	1.4	5.9	61.0
August 7	1.3	0.4	5.1	33.3
August 12	1.6	0.9	5.9	42.4
August 16	2.0	0.3	5.7	39.5
August 21	0.8	0.7	5.5	27.3

TABLE VI.—Proportion of Total Nitrogen Represented by the Sum of Ammonia, Urea, and Total α Amino Acid Nitrogen in Madison, Wis., Raw Wastewater, 1968

Date	NH ₃ -N (mg/l)	Urea-N (mg/l)	TAA-N (mg/l)	Total-N (mg/l)	NH ₃ -N + Urea-N + TAA-N (% of Total-N)
July 5	11.9	0.0	2.5	19.2	75.1
July 9	12.6	4.9	7.4	31.2	79.8
July 10	13.2	1.4	2.5	18.9	90.5
July 12	12.8	1.3	2.1	20.3	79.8
July 17	9.8	0.6	1.4	16.3	72.4
July 19	11.0	2.3	2.4	16.8	93.5
July 24	9.9	2.3	2.3	18.2	79.7
July 29	14.4	1.3	1.6	21.1	82.0
August 2	12.8	1.4	2.2	18.7	87.7
August 7	6.6	0.4	1.3	11.7	70.9
August 12	13.1	0.9	1.6	19.0	82.1
August 16	9.6	0.3	2.0	15.3	77.9
August 21	7.4	0.7	0.8	12.9	68.9

gen balance ranged from 56.1 to 79.2 percent; the median was 67.0 percent. Table IV shows total α amino acid-N expressed as percentage of organic-N. The minimum was 14.5, and the maximum was 43.9, with a median of 28.0 percent. In Table V the sum of urea-N and total α amino acid-N are shown as percent of organic-N. The range was from 27.3 to 81.0 percent; the median was 43.3. Table VI compares (NH₃-H + urea-N + total α amino acid-N) to total-N for Nine Springs raw wastewater. Recoveries varied from 68.9 to 93.5, with a median of 79.8 percent.

TABLE VII.—Summation of Ammonia and Urea Nitrogen as Percent of Total Nitrogen in Cross Plains, Wis., Raw Wastewater, 1968

Date	NH ₃ -N (mg/l)	Urea-N (mg/l)	Total-N (mg/l)	NH ₃ -N + Urea-N (% of Total-N)
July 15	28.4	4.5	46.4	70.9
July 22	28.1	6.6	49.9	69.5
July 26	16.8	4.0	27.7	75.0
July 31	24.8	10.3	38.9	90.0
August 5	27.3	8.9	47.4	76.4
August 9	24.9	4.8	39.1	75.9
August 14	27.0	3.9	43.3	71.3
August 19	30.0	5.6	43.2	82.4

Table VII shows the results of a preliminary nitrogen balance (NH₃-N + urea-N) expressed as percent of total-N of Cross Plains raw wastewater. Recoveries varied from 69.5 to 90.0 percent, with a median of 75.5. Table VIII shows the percent of total amino acid-N relative to organic-N for Cross Plains raw wastewater. Results varied from 13.4 to 26.0 percent, and the median was 18.45. Table IX shows the sum of urea-N and the total α amino acid-N as percent of organic-N. The minimum was 40.0, and maximum was 98.5; median percent was 54.2. Table X lists the results of (NH₃-N + urea-N + total α amino acid-N) expressed as percent of total-N for Cross Plains raw wastewater. The minimum percentage was 76.7, and the maximum was 99.5, with a median of 82.2.

A total of 20 samples from both Madison and Cross Plains were examined for free α amino acid-N, but only four exceeded the minimum reportable concentration of 0.3 mg/l. In the Madison area, little if any free α amino acid-N was found.

Total α amino acid-N varied from 21.7 to 58.7 percent of particulate-N. If substantially all particulate-N had

TABLE VIII.—Proportion of Organic Nitrogen Represented by Total α Amino Acid Nitrogen in Cross Plains, Wis., Raw Wastewater, 1968

Date	Organic-N (mg/l)	Total α Amino Acid-N	
		(mg/l)	% of Organic-N
July 15	18.0	2.7	15.0
July 22	21.8	4.3	20.0
July 26	10.9	2.8	26.0
July 31	14.1	3.6	25.5
August 5	20.1	2.7	13.4
August 9	14.2	2.4	16.9
August 15	16.3	2.7	16.6
August 19	13.2	2.9	22.0

been total amino acid-N, then the particulate-N determination could have served as a control test for protein-N because the determination required no special instrumentation and could be completed within 1 to 2 hr. Unfortunately, particulate-N could not function as a control test for protein-N because recoveries were variable and did not exceed 60 percent. Nevertheless, a protein-N procedure that would fit into normal wastewater laboratory operations appeared to be useful. The procedure as developed cannot serve as a control test because it requires 12 hr for hydrolysis.

The micro-procedure for total α amino acid or protein-N was scaled up, and a comparison of results by the two procedures is shown in Table XI. Although average values of the 11

TABLE IX.—Proportion of Organic Nitrogen Represented by Total α Amino Acid and Urea Nitrogen in Cross Plains, Wis., Raw Wastewater, 1968

Date	TAA-N (mg/l)	Urea-N (mg/l)	Organic-N (mg/l)	Urea-N + TAA-N (% of Organic-N)
July 15	2.7	4.5	18.0	40.0
July 22	4.3	6.6	21.8	50.0
July 26	2.8	4.0	10.9	62.4
July 31	3.6	10.3	14.1	98.5
August 5	2.7	8.9	20.1	57.7
August 9	2.4	4.8	14.2	50.7
August 15	2.7	3.9	16.3	40.5
August 19	2.9	5.6	13.2	64.4

determinations by the two procedures were quite similar—2.2 mg/l-N, and 2.3 mg/l-N, respectively—individual values differed by as much as 1.4, with a minimum of 0.3 mg/l-N. The two methods differed significantly in the following steps. In the micro-procedure after hydrolysis, the sample was neutralized and made up to 100 ml. Then 5.0 ml was taken for analysis. An additional 5.0 ml withdrawn from the same 100-ml volumetric flask served as a blank. In contrast, the scaled up version required a separate blank plus evaporation under vacuum to remove excess acid after hydrolysis, and the entire sample concentrated to 5.0 ml was used for the analysis. In view of these differences in procedures, particularly the possibility of slight differences of hydrolysis conditions between sample and blank, agreement between the two methods was reasonably satisfactory.

TABLE X.—Proportion of Total Nitrogen Represented by the Sum of Ammonia, Urea, and Total α Amino Acid Nitrogen in Cross Plains, Wis., Raw Wastewater, 1968

Date	NH ₃ -N (mg/l)	Urea-N (mg/l)	TAA-N (mg/l)	Total-N (mg/l)	NH ₃ -N + Urea-N + TAA-N (% of Total-N)
July 15	28.4	4.5	2.7	46.4	76.7
July 22	28.1	6.6	4.3	49.9	78.5
July 26	16.8	4.0	2.8	27.7	82.3
July 31	24.8	10.3	3.6	38.9	99.5
August 5	27.3	8.9	2.7	47.4	82.1
August 9	24.9	4.8	2.4	39.1	84.6
August 15	27.0	3.9	2.7	43.3	77.6
August 19	30.0	5.6	2.9	43.2	89.6

TABLE XI.—Comparison of Results from Macro- and Micro-Procedures for the Determination of Total α Amino Acid Nitrogen in Madison, Wis., Area Raw Wastewater, 1968

Date	Source	Total α Amino-Acid-N	
		Micro-Method (mg/l as N)	Macro-Method (mg/l asN)
July 29	Nine Springs	1.6	2.2
July 31	Cross Plains	3.6	4.3
August 2	Nine Springs	2.2	2.7
August 5	Cross Plains	2.7	4.1
August 7	Nine Springs	1.3	0.7
August 9	Cross Plains	2.4	1.3
August 12	Nine Springs	1.6	2.4
August 14	Cross Plains	2.7	1.7
August 16	Nine Springs	2.0	1.7
August 19	Cross Plains	2.9	2.0
August 21	Nine Springs	0.8	1.3
Average		2.2	2.3

Discussion of Results

Generally, ammonia, urea, and total-N values of the Cross Plains plant were almost twice the values at the Madison Nine Springs plant. One reason for these differences is the size of the collection system. The Nine Springs plant has a tributary population of almost 200,000 in contrast to 950 for Cross Plains. Consequently, the Madison collection system is very much larger than that of Cross Plains. Even a tight collection system allows some groundwater infiltration, and the nitrogen concentrations of groundwater are considerably lower than wastewater nitrogen concentrations, so the dilution factor would be very much greater in the larger system. The longer time required for wastewater to reach the Madison plant would permit urea to hydrolyze to ammonia and could explain the relatively low levels of urea-N at Madison. The partial nitrogen balance $[(\text{NH}_3\text{-N} + \text{urea-N}) \text{ expressed as percent of total-N}]$ was higher at Cross Plains. This higher balance could be predicted from greater ammonium and urea-N concentrations at Cross Plains.

Industrial wastes high in nitrogen content add to the wastewater strength of both communities. On balance, however, the greater contribution to nitrogen content of Cross Plains wastewater must be attributed to untreated milk-processing wastes. These wastes amounted to 18 percent of the Cross Plains volumetric flow, but the meat-processing wastes were only 11 percent of the Madison flow. In addition, pretreatment of the meat-processing wastes by trickling filters would remove substantial amounts of nitrogen.

The proportion of organic-N represented by total α amino acid-N ranged from 14.5 to 43.9 percent at Madison, with a median of 28.0 percent. At Cross Plains the median was 18.4 percent. Median absolute values were nearly the same—2.1 at Madison and 2.75 mg/l at Cross Plains. Because total α amino acid-N values were nearly the same at both plants, the percentage of organic-N represented by α amino acid-N would be lower at the plant where higher urea N concentrations prevailed, that is, Cross Plains.

The sum of urea-N and total α amino acid-N values as percent of organic-N fluctuated from 27.3 to 81 percent, with a median of 43.3 percent at Madison. At Cross Plains the minimum was 40.0 percent and the maximum was 98.5 percent, with a median of 54.2 percent.

If the sum of ammonia, urea, and total α amino acid-N is compared to total N, the median percentages for both communities were quite close—79.8 percent at Madison and 82.2 percent at Cross Plains.

Literature Values

Hunter and Heukelekian⁶ reported that amino acid-N averaged 55 percent of total organic-N. Amino acid-N is equivalent to total α amino acid-N because the sample was hydrolyzed. Total organic-N was determined on solid samples and would not, therefore, include urea-N.

From Table V, if urea-N is subtracted from organic-N and total α -amino acid-N is compared to the remaining organic-N, the average value becomes 38.7 percent. Similarly, from Table VII, for Cross Plains the mean value becomes 36.4 percent. These results are considerably lower than the percentage reported by Hunter and Heukelekian, but they are not strictly comparable because these authors worked only with particulates. Painter *et al.*⁷ reported 37 to 38 percent of total organic-N was in solution.

If the particulate-N content is subtracted from the total organic-N content, the average value for soluble organic-N is 43.0 percent. This is in satisfactory agreement with results obtained by Painter *et al.* The same authors indicated that 45 to 65 percent of Kjeldahl-N reacted in the ninhydrin test for amino acids. It is not clear whether Kjeldahl nitrogen includes free ammonia-N, but in any event, results of this investigation are closer to 45 than to 65 percent.

Observations by Kahn and Wayman⁸ that 41 percent of amino acids are present in the free state receive no support from this study. Quite the opposite was observed. Nearly all the amino acids, with only four exceptions, were present in the combined state.

Unaccounted Nitrogen

The addition of urea and total α -amino acid-N to "Standard Methods" procedures for total organic-N permitted accounting for only 80 percent of total-N. Obviously, other species of nitrogen remain to be determined.

There is a parallel between unaccounted nitrogen in soils and wastewater. In soils, α -amino acid-N ranged from 20 to 50 percent of hydrolyzed-N. In this study total α -amino acid-N ranged from 14 to 41 percent of organic-N. There are also differences between soils and wastewater-N. Hexosamine-N amounts to 5 to 10 percent of total-N in soils, but a few explora-

tory analyses did not indicate that hexosamine-N was significant in wastewater. Similarly, in fresh wastewater as much as 25 percent of total-N may be present as urea, while in soils results are expressed in micrograms per gram.¹² Also, in the investigation free ammonia-N averaged 60 percent of total-N, while in soils the average is 22 to 25 percent of total-N.

For both wastewater and soils some of the nitrogen released by acid hydrolysis is probably in the form of purine and pyrimidine bases and other organic nitrogen compounds. However, there is no evidence that these compounds account for a significant amount of nitrogen.

There is considerable speculation about the nature of the nonhydrolyzable nitrogen. In soils, early workers assumed most of the nitrogen was in acid insoluble humin produced during acid hydrolysis of proteins. When purified proteins are hydrolyzed with acids, the amount of nitrogen converted to acid insoluble humin N is usually very small, but humin formation is greatly increased when carbohydrates are present. Probably amino acids (particularly tryptophan) and furfural released by acid hydrolysis of protein-carbohydrate mixtures condense to form humins.

Some of the nonhydrolyzable nitrogen may be heterocyclic nitrogen compounds or in complexes formed by reaction of phenolic substances or their derivatives (lignins, tannins, quinones) with inorganic forms of nitrogen or with amino compounds such as amino acids and amines, but there is little evidence to support this hypothesis.⁹

The detection of α , ϵ , diamino-pimelic acid, orinithine, β alanine, α -amino-n-butyric acid, and 3:4 dihydroxyphenylalanine in soil hydrolyzates is of unusual interest because these amino acids do not occur in proteins. Some of these nonprotein amino acids may be formed from decomposition of other amino acids during acid hydrolysis.

The presence of α , ϵ , diamino-pimelic acid in acid hydrolyzates is very significant because this amino acid is confined almost exclusively to bacteria.⁹ In wastewater, as in soils combined amino acid-N is probably in mucopeptides and teichoic acids. These substances are important constituents of bacterial cell walls. Mucopeptides are amino acid complexes containing an amino sugar and muramic acid. The latter may be involved in the linkage of carbohydrate to the amino acid moiety in cell walls. More importantly, muramic acid has been detected in wastewater.⁷

Some nitrogen may be present as chitin, a polymer of N-acetyl glucosamine. This substance occurs in most fungi. Adenine, guanine, cytosine, thymine, and uracil are components of nucleic acids. Xanthine and hypoxanthine are formed by microbial decomposition of nucleic acids, xanthine being produced by enzymatic deamination of guanine, and hypoxanthine by enzymatic deamination of adenine.⁹

Histamine, creatinene, indole, and skatole have been either detected in wastewater or have been enumerated as nitrogen compounds discharged to sewers, but individually and collectively they account for only a small percentage of total-N.

The Browning Reaction

Bremner⁹ has presented a partial explanation for the low recoveries of amino acid-N from acid hydrolysis of soils. He suggested that amino compounds react with carbohydrates to form brown unsaturated polymers or copolymers of nitrogen. This reaction has been recognized for over a half century in food processing as the browning reaction.¹⁰ In wastewater analyses this reaction may occur during the unit operations of freeze drying, vacuum evaporation, and acid hydrolysis because a characteristic brown color is observed in all these operations. The brown supernate from the

anaerobic digestion of sludge suggests that the browning reaction also takes place in sludge digesters. A recent publication¹¹ indicates the possibility that browning takes place in anaerobic wastewater treatment.

Conclusions

1. By the procedures selected for this investigation, approximately 80 percent of total-N could be accounted for. In Madison raw wastewater, free $\text{NH}_3\text{-N}$ averaged 60 percent of total-N. Urea-N averaged 7.6 percent of total-N. Total amino acid-N averaged 12.5 percent of total-N. In Cross Plains raw wastewater, free $\text{NH}_3\text{-N}$ averaged 60 percent of total-N. Urea-N averaged 14.5 percent of total-N. Total amino acid-N averaged 7.1 percent of total-N. Because only four samples of wastewater were analyzed for particulate-N, the following conclusions must be considered tentative. An average of 27 percent of particulate-N was in the form of total amino acid-N, and particulate-N represented 67 percent of organic-N.

2. Two micro-procedures adapted from soils methods (urea and amino acid-N) have been scaled up to fit into the macro-procedures that are compatible with the equipment normally available in the laboratory of a waste treatment plant.

3. Free amino acid-N was detected only four times in a total of 20 analyses of Madison area wastewater.

4. If the browning reaction occurs in vacuum evaporation, freeze drying, and acid hydrolysis of wastewater, then these three operations are potential sources of error in subsequent determinations for specific forms of nitrogen. Separation and removal of carbohydrates and hydroxybenzene compounds before any of the three operations listed should improve the accuracy of analyses for specific forms of nitrogen.

The browning reaction may prove to be a significant source of color in

wastewater sludge digesters, pulp digesters, wool-scouring liquors, and brewery, distillery, and pharmaceutical waste streams.

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