Measurement of dissolved free and combined amino acids in unconcentrated wastewaters using high performance liquid chromatography

David R. Confer, Bruce E. Logan, Brian S. Aiken, David L. Kirchman

ABSTRACT: Dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA, e.g., discrete dissolved polyptides and proteins as well as amino acids, peptides, and proteins adsorbed to humic material) were measured in unconcentrated domestic wastewater at a detection limit of 10 to 100 nM (coefficient of variation <0.10 at 100 nM) using precolumn ortho-phthalaldehyde derivatization, reverse-phase high performance liquid chromatography separation, and fluorometric detection. Wastewaters were sampled at three types of wastewater treatment plants: trickling filter, conventional activated sludge, and pure-oxygen activated sludge. Raw wastewaters contained the highest concentrations (58 μM) of dissolved total amino acids (DTAA) with 7% as DFAA. The DTAA were between 28 and 40 μM in primary clarifier effluents with 7% to 29% as DFAA. Removal percentages during biological treatment were >70% for DFAA and 23% to 79% for DCAA. Molecular weight separations indicated that most dissolved organic carbon (DOC) (>68%) in the trickling filter influent had small molecular weights (<1 000 atomic mass unit [AMU]), whereas the majority of DTAA had large molecular weights (>10 000 AMU). The DTAA were a small fraction (8% to 13%) of DOC in both influent and effluent samples. Water Environ. Res., 67, 000 (1995).

KEYWORDS: activated sludge, amino acid, proteins, trickling filter.

The ability to accurately measure the various forms of organic carbon present in wastewater is an important factor in understanding the biochemical processes that remove contaminants during biological wastewater treatment. Dissolved organic nitrogen is an essential component of all aquatic systems, and concentrations can range from few micrograms per liter in natural waters such as lakes and oceans to 20 mg L⁻¹ or more in domestic wastewaters (Standard Methods, 1992). Examples of organic nitrogen include amino acids, peptides, proteins, nucleic acids, and urea. The amount of organic nitrogen that consists of dissolved amino acids can be determined independently from other forms of organic nitrogen. Dissolved amino acids are usually separated into dissolved free amino acids (DFAA), total amino acids (DTAA), or combined amino acids (DCAA); DCAA is the difference between DTAA and DFAA.

Measurement of DTAA and DFAA in wastewater samples has been difficult because of the analytical procedures used and the low concentrations of amino acids in wastewaters. The earliest studies (Sastry et al. 1958) used paper chromatography, whereas more recent methods have been based on ion exchange chromatography using ninhydrin derivatization with ultraviolet detection (Stein and Moore 1948). However, because the detection limit (approximately 2.5 μM) for each amino acid using the ninhydrin method is much higher than amino acid concentrations in typical domestic wastewater, sample concentration is usually necessary. In addition to the time and effort required to concentrate samples (for example, by rotoevaporation), concentration procedures have the potential for loss of organic nitrogen components and sample alteration.

To measure total, combined, and free amino acids in wastewaters without sample concentration, we adapted a fluorescent marker technique developed by Lindroth and Mopper (1979) to study amino acids in natural waters. In this method, amino acids are labeled using ortho-phthalaldehyde (OPA), separated using reverse-phase high performance liquid chromatography (HPLC), and analyzed using fluorometric detection. The accuracy of the original test for DTAA was increased by using a vapor-phase acid hydrolysis procedure that gives higher DTAA than liquid-phase acid hydrolysis (Tsugeta et al. 1987; Keil and Kirchman 1991). Because previous studies did not use vapor-phase hydrolysis, earlier estimates of wastewater DTAA may be low. For natural water samples, individual amino acids can be measured as low as 1 nM. Modification of this method in our laboratory for wastewater samples resulted in a higher detection limit for each amino acid of 10 to 100 nM, and this level of sensitivity was sufficient to measure both DFAA and DTAA in raw and treated wastewaters.

The main objective of this study is to determine concentrations of amino acids in wastewaters without sample preconcentration and to use vapor-phase hydrolysis for DTAA measurement. We measured the concentration and percentage removal of both forms of amino acids (DFAA and DCAA) at three different types of wastewater treatment plants (trickling filter, conventional activated sludge, and pure-oxygen activated sludge). Previous wastewater studies did not report the portion of dissolved organic carbon (DOC) attributable to DTAA, so at two of the treatment plants we compared DTAA and DOC concentrations. In addition to proteins, peptides, and amino acids, the total DOC pool in wastewater also contains lipids, carbohydrates, and humic matter. At the trickling filter plant, we used ultrafiltration to separate both DOC and DTAA into a small (<1 000 atomic mass unit [AMU]), intermediate (1 000 to 10 000 AMU), and large (>10 000 AMU) molecular weight fractions to compare the mass distributions of DOC and amino acids in domestic wastewaters.
Methods

Sample collection and preparation. Wastewater samples were collected from three wastewater treatment plants: the Roger Road biotower and the Ina Road pure-oxygen activated sludge plant, both in Tucson, Ariz., and a conventional activated sludge plant in Lewes, Del. Samples were taken at the Roger Road plant at three process train locations: after primary sedimentation (primary clarifier), before biotower application at a location that included both primary clarifier effluent and recycled effluent (biotower influent), and after treatment (biotower effluent). Samples from the Ina Road plants were taken after primary sedimentation (primary clarifier) and after activated sludge treatment (aerator effluent). The Lewes plant has no primary clarifier, so samples were either raw wastewater or aeration tank effluent (aerator effluent).

All samples (0.5 L) were collected in 1-L Nalgene bottles pre-rinsed several times with the wastewater sample to be collected, placed on ice; and returned to the laboratory within 30 minutes. Dissolved fractions were prepared by serial vacuum filtration through 5-μm (once) and 0.2-μm (twice) polycarbonate filters (25 mm, Poretics) on a 10-place manifold (Hoefer Scientific) at less than 250 mm Hg to minimize cell rupture (Fuhman and Bell 1985). Samples were either air-dried immediately or frozen (−20°C) for later analysis. Control experiments indicated that samples could be frozen for several weeks with no noticeable sample degradation. Filtered, frozen samples from the Lewes plant were packed in dry ice and sent by overnight mail to the University of Arizona for analysis. Sampling dates, locations, and the measurements performed on each sample are summarized in Table 1.

HPLC analysis of amino acids. Precolumn OPA derivatization and HPLC were used to measure dissolved free amino acids in unhydrolyzed samples and DTAA after hydrolysis. The DCAA are calculated as the difference between these concentrations (DTAA – DFAA). The derivatization reagent was prepared by dissolving of 10 mg OPA (Sigma, purest grade) in 200 μL methanol and adding 200 μL borate buffer (pH 12.5, 0.8 M) and 10 μL mercaptoethanol (Sigma). This reagent fluorescently labels primary amines (excitation: 320 nm; emission: 450 nm). A Gibson model 231 autosampler with a model 420 diluter, a Beckman model 420 HPLC, an Alltech OPA-HS reverse-phase column (100 × 4.6 mm), a St. John's fluorometric detector, and a Jones Chromatography JCL-6000 PC-based data acquisition system were used in all analyses.

The analytical procedure consisted of addition of α-amino-butyric acid (1 μM) to samples as an internal standard to monitor system response, followed by the OPA derivatization solution (10 μL/mL sample). The sample was allowed to react for 2 minutes to label amino acids and stopped by lowering the pH with acetic acid (5 μL/mL sample). Between 100 to 200 μL was injected into the column. The elution gradient recommended by Alltech for the OPA-HS column was used, but the flow rate was reduced from the recommended 2 mL/min−1 to 1 mL/min−1 to increase peak resolution. Sodium acetate (0.05 M, pH 5.8 with 2% tetrahydrofuran) and pure methanol were used as the secondary and mobile phases. Standards were made by addition of asparagine (Sigma) and glutamine (Sigma) to an amino acid mixture (Fisher Chemical Co.). All analyses were run in triplicate. Individual amino acid concentrations were determined by comparing sample chromatogram peak areas with peak areas of the standard amino acid mixture. This method allowed the detection and resolution of aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, threonine, alanine, arginine, tyrosine, methionine, phenylalanine, isoleucine, and leucine. Valine and tryptophan coeluted.

Free amino acid analysis. The original protocol developed by Lindroth and Mopper (1979) could not be used for wastewater analysis because of the extremely high concentration of ammonia and volatile amines in wastewater samples. These compounds form fluorescent products during OPA derivitization and coelute with tyrosine, methionine, valine, tryptophan, phenylalanine, isoleucine, and leucine, making detection and quantification of these amino acids impossible. Ammonia and volatile amines were found to be as high as 10−2 M in wastewater samples with an Orion model 9512 ammonia electrode. Ammonia and volatile amines needed to be reduced to ≤10−5 M before derivatization (Figure 1) to resolve the obscured amino acids. Amino acid concentrations of approximately 10−3 M can be detected in the presence of higher (approximately 10−3 M) concentrations of ammonia and volatile amines because the relative fluorescence of OPA-derivatized amino acids (fluorescence per mole) is much higher than that of OPA-derivatized ammonia and volatile amines.

Ammonia and volatile amines were removed by a gas-phase stripping procedure. A filtered wastewater sample (2 to 5 mL) was transferred to a muffled, 20-mL borosilicate glass scintillation vial, weighed, and the pH adjusted to 11 to 11.5 using a borate buffer (pH 12.5, 0.08 M NaBO3, adjusted to pH 12.5 with 10 M

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Date</th>
<th>Process</th>
<th>Location</th>
<th>DTAA</th>
<th>DFAA</th>
<th>DOC</th>
<th>Molecular Weight separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/23/92</td>
<td>TF</td>
<td>Roger Road</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5/27/92</td>
<td>PAS</td>
<td>Ina Road</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6/18/92</td>
<td>TF</td>
<td>Roger Road</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7/9/92</td>
<td>TF</td>
<td>Roger Road</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7/16/92</td>
<td>TF</td>
<td>Roger Road</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7/16/92</td>
<td>TF</td>
<td>Roger Road</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7/28/92</td>
<td>CAS</td>
<td>Lewes</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
To avoid sample contamination, pH was monitored on a parallel sample aliquot using pH paper (Hydrion) during buffer addition. After the approximate amount of buffer required was determined on the parallel sample, this amount was added to the analytical sample and pH confirmed by removing 20 µL and testing it with pH paper. Increasing the sample pH shifted the ammonia-volatile amine equilibrium from the nonvolatile ionized forms (R-NH₃⁺, R = H or organic) to the volatile R-NH₂ forms. The pH-adjusted sample was then purged with N₂ gas for at least 30 minutes. Gas flow was adjusted to provide vigorous bubbling, but no spattering, to prevent sample loss. Because water evaporation reduced the weight of the buffered sample to less than that of the original sample, ultrapure water (Milli-Q, Millipore Corp.) was added to restore the purged sample to the original sample weight. The sample was then analyzed using OPA derivatization and HPLC separation protocol described previously.

**Total amino acid analysis.** The method used for total amino acid analysis was essentially the rapid vapor-phase hydrolysis method of Tsuchita et al. (1987) as modified by Keil and Kirchman (1991). Vapor-phase hydrolysis was used because it has been shown to give higher DTA concentrations than the liquid-phase hydrolysis method because there is less loss of amino acids during vapor-phase hydrolysis (Keil and Kirchman, 1991). Ascorbic acid (10 µL, 20 mg mL⁻¹ solution) was added to filtered wastewater samples (150 µL) in 6 mm × 100 mm-muffled test tubes to prevent amino acid oxidation in the presence of NO₃⁻ (Robertson et al., 1987). The samples were evaporated to dryness under a stream of nitrogen and inserted into wide-mouth ampules (10 mL) containing an acid hydrolysis mixture of 70 µL of high-purity constant-boiling HCl (Pierce Chemical Co.) with 10% trifluoroacetic acid (Sigma) and 0.1% phenol (Fisher). This solution was purged with argon gas for 5 minutes, and the ampules sealed under vacuum (>600 mm Hg). Samples were hydrolyzed for 30 minutes at 156°C. After cooling, the ampules were opened, and the sample tubes removed and dried under vacuum (>600 mm Hg) for 10 minutes to remove any acid. Samples were redissolved in 450 µL of borate buffer (pH 10.5, 0.08 M) and analyzed for amino acids by HPLC.

**DOC analysis.** Nonpurgable DOC was measured using a Shimadzu 5000 DOC analyzer, autosampler, and a two-point calibration curve with blank correction. Standards and samples were acidified to pH 2.5 and purged of inorganic carbon (CO₂) before analysis.

**Molecular weight distribution of amino acids and DOC.** Molecular weight separations were performed using two ultrafiltration membranes with different nominal molecular weight (NMW) cutoffs. Bacteria were removed from samples by centrifugation (Beckman, Model J2-21) at 5 000 × g for 1 hour, and vacuum-filtered twice through 0.2-µm nylon filters (MSI) at <250 mm Hg. Part of the sample was analyzed (in triplicate) for DTAA and DOC. Molecular weight separations were conducted in 200-mL stirred ultrafiltration cells (Amicon, Model 8200) under nitrogen at 55 to 65 psig using Amicon 1 000 NMW (YM1) and 10 000 NMW (YM10) cutoff cellulose membranes. All membranes were initially conditioned according to the manufacturer's instructions, stored face down in distilled water or a 5% ethanol solution at 4°C, and discarded after 10 ultrafiltration cycles. Before each use, at least 50 mL of distilled water was passed through the membrane. Bulk samples were obtained by loading 160 mL of 0.45-µm filtrate into the ultrafiltration cell, discarding 10 mL, and collecting 120 mL. Bulk samples were analyzed for DOC and DTAA.

As a result of membrane rejection, the permeate concentration from a batch ultrafiltration cell will always have a concentration less than or equal to the concentration of organics in the original sample. To account for membrane rejection, DOC size distributions were calculated using the permeation coefficient (Logan and Jiaron 1990) model

$$\ln C_p = \ln (pC_{0}) + (p - 1) \ln F$$

Where

$$p = \text{the permeation coefficient}$$

$$F = 1 - (V_f/V_o) = \text{the fractional reduction of retentate volume at time } t.$$  

Estimates of $p$ and $C_{0}$ were made from a linear plot of $\ln (C_p)$ versus $\ln (F)$ where $(p - 1)$ and $\ln (pC_{0})$ are the slope and y-intercept, respectively.

A minimum of six permeate fractions were manually collected per membrane during each molecular weight separation. Samples (approximately 5 mL) were collected in 20-mL glass scintillation vials. Permeate flow was monitored between fraction collection by measuring the permeate volume versus time. After collection, the samples were weighed, and the total flow from the membrane was calculated by converting the change in weight to a change.
in volume, assuming a sample density of 1 g cm\(^{-3}\) and dividing by the collection time period. Collection times were distributed throughout the ultrafiltration cycle with a majority of the samples collected toward the end of the cycle. Samples were not collected before 10 mL of permeate had passed through the membrane or if less than 20 mL of retentate remained in the ultrafiltration cell.

Samples analyzed for DOC were divided into three molecular weight fractions: a small fraction (\(C_m < 1000\) AMU), an intermediate fraction (1000 to 10,000 AMU, determined by difference of \(C_m\) calculated for the 1000 and 10,000 AMU membranes), and a large fraction (>10,000 AMU, calculated as the difference between the total DOC concentration and the \(C_m < 10,000\) AMU).

**Results**

**Individual amino acid concentrations in wastewaters.** A typical amino acid profile of Roger Road biotower effluent is shown in Table 2. Fifteen amino acids were resolved, with only valine and tryptophan co-eluting. In hydrolyzed samples (DTAA), individual amino acid concentrations ranged from 1.0 to 2.1 \(\mu\)M, whereas individual amino acid concentrations in unhydrolyzed samples (DFAA) were all less than 0.64 \(\mu\)M. The DFAA were measured with a coefficient of variation of 0.11, and DTAA were measured with a coefficient of variation of 0.01.

**Distribution of amino acids in raw sewage and primary effluents.** The highest DTAA concentrations were measured on raw wastewaters at the Lewes activated sludge plant. Raw wastewater entering the aeration tank contained 58 \(\mu\)M DTAA with only 7% as DFAA (Figure 2a). However, because this plant did not have a primary clarifier, a direct comparison of primary clarifier effluents at all three plants could not be made.

The DTAA concentrations in primary clarifier effluent at the

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>DFAA, (\mu)M</th>
<th>DTAA, (\mu)M</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>0.04 ± 0.02</td>
<td>1.67 ± 0.03</td>
</tr>
<tr>
<td>GLU</td>
<td>0.14 ± 0.01</td>
<td>1.52 ± 0.01</td>
</tr>
<tr>
<td>SER</td>
<td>0.64 ± 0.04</td>
<td>1.29 ± 0.01</td>
</tr>
<tr>
<td>GLN</td>
<td>0.31 ± 0.04</td>
<td>nd</td>
</tr>
<tr>
<td>HIS</td>
<td>nd</td>
<td>1.11 ± 0.01</td>
</tr>
<tr>
<td>GLY</td>
<td>0.19 ± 0.02</td>
<td>2.14 ± 0.01</td>
</tr>
<tr>
<td>THR</td>
<td>0.20 ± 0.01</td>
<td>1.38 ± 0.01</td>
</tr>
<tr>
<td>ALA</td>
<td>0.10 ± 0.03</td>
<td>1.35 ± 0.01</td>
</tr>
<tr>
<td>ARG</td>
<td>0.23 ± 0.02</td>
<td>1.64 ± 0.01</td>
</tr>
<tr>
<td>TYR</td>
<td>0.02 ± 0.01</td>
<td>1.05 ± 0.02</td>
</tr>
<tr>
<td>MET</td>
<td>nd</td>
<td>1.01 ± 0.01</td>
</tr>
<tr>
<td>VAL/Leu/Ile</td>
<td>nd</td>
<td>1.23 ± 0.01</td>
</tr>
<tr>
<td>PHE</td>
<td>nd</td>
<td>1.04 ± 0.01</td>
</tr>
<tr>
<td>ILE</td>
<td>0.10 ± 0.01</td>
<td>1.11 ± 0.02</td>
</tr>
<tr>
<td>LEU</td>
<td>nd</td>
<td>1.22 ± 0.02</td>
</tr>
<tr>
<td>Total</td>
<td>1.97 ± 0.21</td>
<td>18.76 ± 0.19</td>
</tr>
</tbody>
</table>

\(0.09\ \text{mg DOC L}^{-1}\) \(1.08\ \text{mg DOC L}^{-1}\)

\(\text{nd} = \text{below detection limit.}\)

Figure 2—Influent and effluent free and combined amino acids at the Ina Road pure-oxygen activated sludge (upper chart) wastewater treatment plant (Experiment 2) and at the Lewes, Delaware conventional activated sludge wastewater treatment plant (lower chart) (Experiment 7).

two Tucson treatment plants were between 28 and 40 \(\mu\)M with DFAA accounting for 7% to 29% of the total. Primary clarifier effluent from the Ina Road plant contained 40 \(\mu\)M DTAA with 16% DFAA (Figure 2b). Primary effluent at the trickling filter plant consistently contained approximately 30 \(\mu\)M DTAA with no apparent differences between summer and winter or time of day. The DFAA accounted for 21% to 29% of DTAA (Figure 3). The DTAA concentrations in the trickling filter influent were more variable than primary clarifier concentrations because tower influent contained both recycled effluent (treated wastewater) and primary clarifier effluent. Therefore, during low-hydraulic flow rates into the treatment plant, more treated effluent
The two activated sludge plants removed a larger percent of all forms of amino acids than the trickling filter. Removal percentages in the aeration basin at the pure-oxygen plant were 89% for DFAA and 72% for DCAA (Figure 2a). The conventional activated sludge plant had aeration basin removal percentages of 90% for DCAA, and >98% removal of DFAA (≤0.06 μM DFAA in the effluent, Figure 2b).

The DTAA were consistently a small fraction of the total DOC measured at two of the wastewater treatment plants. The conventional activated sludge plant influent DTAA were 13% of total DOC, whereas the effluent amino acids were only 4% of total DOC (Figure 4). The DOC, 8% to 12% in the biotower influent and 6% to 16% in the biotower effluent, consisted of DTAA (Figure 5).

The DTAA removal was less than DOC removal in the trickling filter, but the reverse was observed at the activated sludge plant (Table 3). The DOC removal percentages in the trickling filter were 40% to 66% across the biotower (Table 2, Figure 5), whereas DTAA removal was only 20% to 55%. At the activated sludge plant, 77% of the DOC was removed, whereas 92% of DTAA were removed. The higher removals for both amino acids and DOC at the conventional activated sludge plant is at least partly because of the fact that removals are based on the higher influent concentrations of raw sewage because primary clarification is not used at the Lewes plant. The DOC was not measured at the pure-oxygen activated sludge plant. The absence of a correlation between the percent of DOC and DTAA removed by the trickling filter may be due to differences in biodegradability between DTAA and DOC. For example, the lowest DOC removal rate (40%, Experiment 3) occurred during a low-flow period that required high-recycle ratios. The low DOC removal rate probably reflects a high percentage of relatively nonbiodegradable DOC in recycled effluent.

Molecular weight distributions. The DOC in the trickling filter influent was predominantly small molecular weight (<1 000 AMU) material, although the majority of DTAA were large molecular weight (>10 000 AMU) material (Figures 6 and 7). Effluent DOC concentrations (uncorrected for membrane rejection) decreased 75% in the low molecular weight fraction, 42% in the intermediate molecular weight fraction (1 000 to 10 000 AMU) but increased (20%) in the large molecular weight fraction compared to influent values.

Effluent DTAA concentrations (uncorrected) decreased by 80% in the high molecular weight fraction, by 17% in the intermediate weight fraction, but increased substantially (69%) in the low molecular weight fraction. The large concentrations of amino acids in the <1 000 AMU fraction do not contradict the relatively low DFMA concentrations because the <1 000 AMU fraction includes both DFMA and small polypeptides.

Using a permeation coefficient only slightly modified the DOC size distribution and therefore did not alter the apparent increase in the larger molecular weight size fraction (Figure 6b). The small increase in the large size fraction from 2.0 to 2.4 mg L⁻¹ is within the experimental error for DOC measurements and the permeation coefficients. Permeation coefficients were not separately determined for amino acids. It was concluded from other experiments that estimates of permeation coefficients were not statistically significant at the low amino acid concentrations present in wastewater. Unfortunately, permeation coefficients determined for DOC separations are not applicable to amino acids.

Figure 3—Primary clarifier influent and effluent free and combined amino acids at Roger Road trickling filter wastewater treatment plant in Experiment 1 (upper chart) and Experiment 3 (lower chart).

was recycled, lowering amino acid concentrations applied to the trickling filter. Because of these variations in the amount of recycled effluent, biotower influent DTAA concentrations varied from 12 to 24 μM DTAA with 25% to 31% present as DFMA.
Table 3—Comparison of amino acid and DOC process removals at Roger Road trickling filter (TF) and Lewes, Delaware conventional activated sludge (CAS) wastewater treatment plants.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Process</th>
<th>Influent concentrations, mg DOC L⁻¹</th>
<th>Removal, % of influent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DTAA</td>
<td>DCAA</td>
</tr>
<tr>
<td>1</td>
<td>TFₕ</td>
<td>0.60</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>TP</td>
<td>1.35</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>TP</td>
<td>1.35</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>PP</td>
<td>1.50</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>CASₜ</td>
<td>3.69</td>
<td>3.50</td>
</tr>
</tbody>
</table>

*Influent is biotower influent.
Not measured.
*Influent is raw wastewater.

Acid molecular weight separations. The permeation coefficient for effluent DOC (P = 0.43) in the small molecular weight fraction, when applied to DTAA, produces 0.90 mg DTAA L⁻¹ <1000 AMU. This is unacceptably high because it is larger than the measured value of 0.84 mg-DTAA L⁻¹ for the small and intermediate molecular fractions combined.

Discussion
This study shows that amino acids in wastewaters can accurately be determined at nanomolar concentrations using the OPA HPLC method as long as interfering ammonia and volatile amines are removed before analysis. The DTAA concentrations in raw and treated wastewater are within ranges reported using other methods, although the percentage of DFAA in primary effluent (7% to 29%) falls within a narrower range than previously reported values. Kahn and Wayman (1964) determined that raw wastewater in Denver Colorado contained 280 mg DTAA L⁻¹ (approximately 2.3 mM) with a substantial fraction (41%) present as DFAA. Primary effluent contained 65 mg L⁻¹ total amino acids with 46% free amino acids. The percent of the sample that was DFAA reported by Kahn and Wayman was much larger than that reported in an earlier study in India (Sastry et al. 1958) where DFAA were only a small proportion (≤1.5%) of DTAA in wastewaters. Other investigators have also determined that DFAA are a small percent of DTAA. Hanson and Lee (1971) found an average of approximately 20 mg DTAA L⁻¹ in raw wastewaters at two Wisconsin wastewater treatment plants with only 20% of samples containing more than 2 mg L⁻¹ (the detection limit) of DFAA. The low concentrations of DFAA reported in earlier studies may be due to longer preparative procedures used in other methods. The DFAA are rapidly assimilated by bacteria, and improper sampling handling or long

Figure 4—Influent and effluent DOC and DTAA at Lewes, Delaware conventional activated sludge wastewater treatment plant Experiment 7.

Figure 5—Influent and effluent DOC and DTAA at Roger Road trickling filter wastewater treatment plant Experiments 3, 4, and 5.
process times can decrease measured values. Conversely, high concentrations of ammonia or nonamino acid primary amines can result in artificially high DFAA values.

A commercially available colorimetric protein assay (Pierce Comassie Plus, Pierce Chemical Co.) based on the Bradford protein method (Bradford 1976) indicated protein concentrations of 0.5 mg DOC L\(^{-1}\) in Roger Road biotower influent when measured against a bovine serum albumin standard (data not presented). The DTAA concentrations determined using HPLC are at least three times higher (Table 3, Figure 7). Raunkjaer et al. (1994) notes that the Lowry protein method (Lowry et al., 1951) gives wastewater protein concentrations four to seven times higher than the Bradford method. The Bradford method reacts with polypeptides and proteins larger than 2 000 AMU, but the molecular weight distribution of Roger Road biotower influent and effluent indicates that a substantial portion of DTAA in wastewater can be less than 2 000 AMU (Figure 7) and therefore nonreactive. The HPLC that quantifies individual amino acids or the Lowry method that reacts with individual peptide bonds therefore give more accurate estimations of wastewater protein concentrations than the Bradford method.

There are several similarities between the amino acid distributions observed in natural systems and those determined in this study for wastewaters. First, the ratio of DFAM:DTAA in wastewaters is similar to values found in natural waters. For example, Coffin (1989) found DFAM in the Delaware estuary accounted for 1.5% to 35% of the DTAA with typical values approximately 10%. Second, the percentage of DOC that is DTAA in primary effluent, biotower influent, effluent, and activated sludge effluent (6% to 13%) is also typical of values found in natural systems. In a study of organic matter in a eutrophic polder lake in The Netherlands, de Haan and de Boer (1979) found DTAA accounted for approximately 6.5% of the total DOC. Third, molecular weight distributions of DTAA in natural waters are similar to those obtained in this study for wastewater. De Haan and de Boer (using Sephadex gel permeation chromatography) found that 50% to 70% of DTAA were contained in a large (> 5 000 AMU), 20% to 35% in an intermediate (500 to 5 000 AMU), and 5% to 15% in a small (< 500 AMU) molecular weight fraction. Even though different size classes and separation techniques were used in the two studies, de Haan and de Boer's values agree with the wastewater distributions of 57% in the large (> 10 000 AMU), 20% intermediate (1 000 to 10 000 AMU), and 23% in the small (< 1 000 AMU) molecular weight fractions measured on biotower influent.

The predominance of low molecular weight DOC in wastewater at the trickling filter plant agrees with data previously obtained at the same plant (Amy et al. 1987). The DOC at this plant has been found to be primarily concentrated in the small and large molecular weight fractions with very little material in the intermediate (1 000 to 10 000 AMU) size fraction. This type of bimodal size distribution has been found at other wastewater treatment plants. Levine et al. (1985) determined molecular weight distributions of DOC at three California wastewater treatment plants using gel permeation chromatography. At the Univ. of Calif. Davis plant, the molecular weight distribution of DOC in primary clarifier effluent was bimodal, with average molecular weights of 10 000 and 20 000 AMU. At the San Diego and South Lake Tahoe plants, however, primary clarifier effluent was predominantly centered around 10 000 AMU. In the Levine study, however, the lowest molecular weight size marker was 12 400 AMU, so that the ability of the method to differentiate material with lower molecular weights was limited. Much of the material reported to be 10 000 AMU, therefore, could have had lower molecular weights.

In studies at two wastewater treatment plants we found no correlation between DTAA and DOC removals. Removals of DOC were larger than DTAA removals at the activated sludge
plant but lower at the trickling filter plant. The generality of this observation, however, is not known because of substantial differences in process trains at the two plants. The activated sludge plant, for example, did not have a primary clarifier. Unless DTAA removal is the same in primary clarifiers as in biological reactors, a direct comparison between the two plants cannot be made. In addition, wastewater applied to the trickling filter was a combination of recycled effluent and primary clarifier effluent that may have concentrated nonbiodegradable material, especially DTAA. The lower removal rate of DTAA compared to DOC in the biotower may be due to the relatively lower biodegradability of large as compared to small molecular weight compounds. Molecular weight distributions of biotower influent showed that 57% of DTAA was associated with the large molecular weight fraction, whereas 68% of total DOC was less than 1,000 AMU. Relationships between DTAA and DOC removals require further study.

In conclusion, the use of HPLC for the measurement of amino acids in unconcentrated wastewater samples has been shown to be accurate and precise at concentrations two to three orders of magnitude lower than other available techniques. It is a powerful technique that will allow investigators greater access to questions of protein, peptide, and amino acid concentrations in wastewaters during treatment processes than has previously been possible.

Acknowledgments

Credits. This research was supported by National Science Foundation grant BCS-8912893. Lucy Feingold assisted with Delaware wastewater samples.

Authors. David Confer and Brian Aiken are graduate students, and Bruce Logan an associate professor in the Environmental Engineering Program, Department of Chemical and Environmental Engineering, University of Arizona. David Kirchman is a professor in the College of Marine Studies, University of Delaware. All correspondence should be sent to Bruce Logan, Department of Chemical and Environmental Engineering, 120 Harshbarger Bldg., University of Arizona, Tucson, AZ 85721.

Submitted for publication August 8, 1993; revised manuscript submitted January 20, 1994; accepted for publication February 7, 1994. Deadline for discussions of this paper is May 15, 1995. Discussions should be submitted to the Executive Editor. The authors will be invited to prepare a single Closure for all discussions received before that date.

References


