PROJECT OBJECTIVES:

The objective of this research activity has been to characterize the residual organic constituents of municipal wastewaters by physical-chemical and biological processes. The refractory organic nitrogen content is of particular concern. Treatment steps are needed for persistent organics that pass through (or are created by) primary treatment, secondary biological treatment, chemical (lime) coagulation/flocculation, filtration and activated carbon processes.

A basic premise of this investigation has been that if the chemical characteristics of residual materials in wastewaters are well-known then public health and aquatic environmental influences can be evaluated and appropriate corrective steps (either by treatment or restrictive use) can effectively manage these refractory materials as required. The strategy of taking corrective action following treatment classification (as contrasted with analytic classification) is known as "process response". From an
engineering point of view, process response allows the immediate determination of the treatment required by classifying the residual organics by appropriate removal techniques.

ACHIEVEMENT OF OBJECTIVES:

Nitrogen-bearing refractory organic residuals of effluents have been identified in this research and treatment steps to remove these potentially harmful organics can now be recommended. In addition, analytic techniques were developed to measure organic and inorganic nitrogen in water in real-time with requisite and specified accuracy, precision and sensitivity. Furthermore, a data base was accumulated not only on treated and untreated wastewaters but also on local rivers, lakes and groundwaters.

RESEARCH PROCEDURES USED:

I. General Statement

Research has shown that there are residual and refractory organics in municipal wastewaters as measured by Total Organic Carbon at approximately 3 mg/l TOC. These organics (1) are aquatically dispersed (either as soluble or micro-colloidal constituents); (2) appear in the effluent of currently used bioreactors (trickling filters and activated sludge units); (3) are non-adsorbable onto activated carbon; (4) are resistant to ozonation; (5) have the unusual characteristic of being cationic at relatively high pH (9-10). Both Kjeldahl and instrumental Total Nitrogen analyses show these residuals to be nitrogen-bearing organics with approximately 2 mg/l as organic nitrogen.

Sensitive and rapid (real-time) measures of the major nitrogen forms were needed for this work; therefore, a major portion of the initial activity
had been the development of instrumental techniques for determining Total Nitrogen (TN) in its numerous and variable forms—either organic nitrogen (Org N), ammonia-ammonium (NH$_3$-NH$_4^+$), nitrates (NO$_3^-$) or others. Real-time capability is now available through the Dohrmann Total Nitrogen analyzer, a major instrument in this activity, for TN (approximately 15 minutes), and coupled with ion selective probes, NH$_3$ (approximately 1 minute) and NO$_3^-$ (approximately 1 minute). These techniques have been correlated to Standard Methods for clean waters and the wastewaters investigated so that interpolation of different analytic techniques can be easily made.

In addition, specific (but slow-time) analysis has identified the residual organic nitrogen as being micro-colloidal polymers of basic amino acids such as lysine.

Pilot plant work with various advanced wastewater treatment steps has shown which unit processes are useful in removing classes of residuals from sewage effluents.

Four theses, 7 technical papers, one workshop/conference are among the accomplishments of these research activities along with the technical conclusions and sundry information dissemination (e.g., other theses, Gordon Conference presentation and data base accumulation). See the List of Publications and Other Information Dissemination Items for the complete citation.

II. Formulations

The formulae listed in Table I summarize many of the conclusions concerning the measurement techniques. These environmental engineering measurements use total or gross (total grouping of a class of organics rather than specific individual methods) since there are so many individual constituents in each category. The nomenclature sheet identifies each parameter, some of which are mentioned in the discussion to follow.
TABLE I

Relationship Between Aquatic Nitrogen Forms in Effluents

\[ TN = \text{Org N} + \text{NH}_4^+ + \text{NH}_3 + \text{NO}_3^- + \text{NO}_2^- \]  \[ 1 \]

\[ \text{NH}_4^+ >> \text{NH}_3 \text{ at neutral pH's} \]  \[ 2 \]

\[ \text{NO}_3^- >> \text{NO}_2^- \text{ in most aerobic sewages} \]  \[ 3 \]

\[ \text{TOxN} = \text{Org N} + \text{NH}_4^+ \]  \[ 4 \]

\[ \text{TOxN} > \text{TKN} = \text{Org N} + \text{NH}_4^+ \]  \[ 5 \]

\[ \text{TOD} > \text{BOD}_{\text{ultimate}} > \text{BOD}_5 \]  \[ 6 \]

\[ \text{TOC (expressed as O}_2 \text{ eq.)} > \text{BOD}_{\text{ultimate}} - \text{TOxN} \]  \[ 7 \]
IDENTIFICATION OF TERMS

TN  mg/l N - Total Nitrogen as measured on the Dohrmann nitrogen analyzer. Includes all aquatic nitrogen forms except N\textsubscript{2}.

Org N  mg/l N - Includes all organic nitrogen and has a higher yield than the standard Kjeldahl techniques.

NH\textsubscript{4}\textsuperscript{+}  mg/l N - Ammonium ions as determined by ion specific probe.

NH\textsubscript{3}  mg/l N - Ammonia - reports as NH\textsubscript{4}\textsuperscript{+} by the technique used.

NO\textsubscript{3}\textsuperscript{-}  mg/l N - Nitrate ion as determined by ion specific probe.

NO\textsubscript{2}\textsuperscript{-}  mg/l N - Nitrite (usually negligible in waters tested).

TOxN  mg/l O\textsubscript{2} - Total Oxidizable nitrogen - includes all aquatic nitrogen except nitrates and N\textsubscript{2} that can be oxidized.

TKN  mg/l N - Kjeldahl technique measurement of organic nitrogen and ammonium.

TOD  mg/l O\textsubscript{2} - Total Oxygen Demand as determined by Ionic Co. Model TOD analyzer. Includes all oxidizable constituents in water.

TOC  mg/l C - Total Organic Carbon as measured by Lira (Model N300) unit. For comparison purposes this is calculated into an O\textsubscript{2} eq. for the purpose of comparison to other parameters used.

BOD\textsubscript{5}  mg/l O\textsubscript{2} - Biochemical Oxygen Demand Classical measurement of (part of the) biodegradable organics in waters. Usually does not include oxidizable nitrogen.

BOD\textsubscript{ultimate} - mg/l O\textsubscript{2} - Long term Biochemical Oxygen Demand. Includes all biological altered aquatic constituents that utilize O\textsubscript{2}.
III. Pilot Studies and Full Scale Plant Survey

Traces of organic nitrogen have been found in the effluents of biological treatment plants (≈ 25 mg/l TN), tertiary (sand filter) plants (≈ 9 mg/l TN) and from columns of activated carbon (≈ 5 mg/l TN). Detailed data on the nitrogenuous components, transition and transformation have been collected through full-scale and model pilot sewage treatment plants; these included several advanced wastewater treatment steps.

A. Progress Response Example

A series of process response tests have been conducted as a method of characterizing the organic residuals in waters. The process response approach can result in the direct selection of an optimum strategy to effect removal of the maximum amount of residuals of concern.

As an example, Figure 1 illustrates one relatively simple process response approach. In the schematic the TOC and TN analyses shown in signal flags, indicate these analyses before and after each unit step - in this example activated carbon column treatment (A/C), ultrafiltration at 500 mu (UF), and electrodialysis (ED). The influent to this set of treatment is unchlorinated secondary sewage effluent (2°).

Each treatment step is operated to be optimally efficient as a removal step. The removal of organic carbon and aquatic nitrogen across the activated carbon is not complete; there is always leakage. Some organic carbon and nitrogen constituents are effectively removed but others pass through, because of physical size, chemical characteristics or other process response characteristics. Organics that are either charged or too large to adsorb in the pores (≈ 50Å) of the carbon can pass through activated carbon treatment.

If the activated carbon effluent is passed through an optimizer electrodialysis (ED) unit and recycled over and over again (for six hours in this
FIG. 1 - SCHEMATIC OF PROCESS RESPONSE TEST ON A SECONDARY EFFLUENT
case) then the charged carbonaceous and nitrogenous organics are removed. ED concentrates charged species either in separate concentration zones or on the electrodialysis membrane surfaces. Note that while the organic carbon content is not much affected, the nitrogen components are largely removed.

If a parallel stream from the A/C unit is sent to an efficient ultrafiltration (UF) unit (molecular sieving at 500 millimicrons) neither the nitrogen nor carbon content is significantly altered.

The conclusions that can be drawn from these tests concerning the nitrogen-bearing organics from secondary treatment are that some (1) are not adsorbable onto activated carbon; (2) are smaller than 500 µm (5000Å) but larger than 50Å (5µm); and (3) are charged since they are influenced in the D.C. electrical field in the ED cell. This information is useful in selecting treatment steps: ion exchange (IEX) and/or electrodialysis (ED) would be appropriate for the removal of the bulk of the organics escaping activated carbon columns. Activated carbon is especially effective in removing non-charged hydrocarbonaceous and benzene-like organics while IEX and ED are effective in removing ions and highly charged colloids.

B. Pilot Plant

As part of this research activity a model pilot plant was built with elements corresponding to conventional and advanced wastewater treatment plants. The operation of this pilot plant was investigated to collect data on nitrogen, carbon and oxygen demand parameters and to follow traces and spikes of group and individual compounds. In the treatment train were 1. flow equalization; 2. primary sedimentation; 3. an activated sludge bio-reactor; 4. lime treatment; 5. recarbonation; 6. sand filtration; 7. activated carbon columns; 8. anion and cation ion exchange columns
and later on, 9. ozonation chamber added. Figure 2 represents, as an example, a trace of the three major forms of aquatic nitrogen through the pilot plant train.
FIG. 2 – EFFECT OF CONVENTIONAL AND ADVANCED WASTE TREATMENT ON SEWAGE

SEWAGE

- NH₃
- ORG. N
- NO₃

POSITION IN ADVANCED WASTEWATER TREATMENT PLANT

TN = 18.6 mg/l
IV. Leakage of Basic Amino Acid Through Activated Carbon Columns

A. Description of Residual

As stated before, previous research (Helfgott, Hunter & Rickert, 1970) (Helfgott & Hart, 1974) (Hart & Helfgott, 1975) has shown that there are persistent refractory organic residuals that are not readily removed either in conventional or some advanced wastewater treatment systems. These materials had been characterized as:

non-settling,
non-biodegradable,
non-absorbant,
resisting coagulation & flocculation,
cationic at high pH (\( \approx 9 - 10 \)),
nitrogen-bearing \((C/N \approx 3/2)\),
non-filterable and dispersed; and
Molecular Weight < 500 \( \mu \text{m/g}-\text{mole}\),
Physical size > 50\( ^{\circ} \) but < 5000\( ^{\circ} \).

It was hypothesized that certain basic amino acids (such as lysine) in small polymeric forms would fit the above description; however, lysine and similar basic amino acids were considered too simple a model since it is generally believed that most naturally occurring materials are biochemically degradable. Research (Helfgott & Hart, 1974) has shown that several refractory organic materials (e.g., humic acids, DDT, chlorinated hydrocarbons and some forms of amino acids) resist degradation. Amino acid polymers are created during biological treatment of sewage; the cell wall structural forms of these materials do not degrade easily although they may be released, re-absorbed and recycled in and out of the micro-organism biomass. Material such as lysine could be the "locking" mechanism by occurring at the ends of
short proteinaceous polymer chains. These ends of the polypeptides are then charged cationically, highly soluble and bio-refractory.

B. Methods and Results

To investigate this working hypothesis, specific analysis on the presence of these materials in treated wastewaters was needed. Secondary sewage effluent that had been settled and filtered (sand filtration at 2 gpm/ft$^2$ in 2 feet of media) was passed through a column of activated carbon and the samples analyzed by direct acid assay.

The activated carbon columns contained 2 feet high granular fresh material with distribution rings at 6 inch intervals to avoid wall effects (leakage of untreated materials along the sides). The columns were run to collect 20 liters at a low rate (1 gpm/ft$^2$) and 20-30 minute contact to assure effective removal of adsorbable organics. A 1 liter aliquot of the composite was taken for amino acid assay.

One set of samples (influent and effluent of activated carbon columns) was run directly and a second set was mildly hydrolized (6 N HCl for 24 hours under vacuum) and lyophilized (freeze-dried to concentrate the amino acids). Hydrolization depolymerizes any protein, polypeptide and such to free amino acids. The results are listed in Tables I and II.

The influent and effluent whose data are shown in Table I had not been specifically prepared for analysis in contrast to the data in Table II. The analysis of NH$_3$ content of secondary sewage effluent at about 17 mg/l from the trickling filter at University of Connecticut is typical of the ammonia content there ($14.5 \pm 7.2 \text{ mg} \text{ NH}_3 \text{ per } 1 \text{L}$) (J. McDermott & T. Helfgott, 1973) and is presented here to confirm the analyses of the Amino Acid Assay System. Note that little, or only trace amounts, of the basic amino acid
TABLE II

Basic Amino Acids and Ammonia in
Activated Carbon Column Influent & Effluent

mg/l as material listed

<table>
<thead>
<tr>
<th></th>
<th>Ammonia</th>
<th>Lysine</th>
<th>Histidine</th>
<th>Arginine</th>
<th>Ornithine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent:</td>
<td>17.36</td>
<td>trace</td>
<td>none</td>
<td>none</td>
<td>0.896</td>
</tr>
<tr>
<td>Effluent:</td>
<td>17.44</td>
<td>trace</td>
<td>none</td>
<td>none</td>
<td>present</td>
</tr>
</tbody>
</table>

---

Feed Material was sand filtered secondary sewage effluent from a Trickling Filter.
are available as free amino acids.

The samples whose analysis is presented in Table II had been hydrolized and lyophilized. The ammonia figure here represents a product of this preparation since the original ammonia (about 17 mg/l \( \text{NH}_4^+ \)) was removed in the preparation. The ammonia data reported are the degradation products of organics (e.g., uric acid, creatinines) and inorganic (e.g., urea) compounds in the samples.

Note the significant presence of three basic amino acids -- lysine, histidine and ornithine -- at concentrations close to those reported in the previous research and in high C/N ratios. The analysis pretreatment undoubtedly depolymerized the amino acids and showed those building block constituents which are not found in a simple form in sewage or effluents but can be released on hydrolysis.

C. Discussion

The refractory residual amino acid polymers which were found can have significant environmental and health impacts. Secondary sewage effluent typically contains about 4-8 mg/l organic nitrogen while subsequent activated carbon effluent contains 2-3 mg/l organic N. In the receiving environment, degradation can take alternate paths. One path, deamination, releases organic acids and ammonia. Ammonia is of course toxic to fish, an oxygen demanding material and a nutrient stimulation of algae (eutrophication). Another path, decarboxylation, results in some physiologically active and harmful amines (Putomains): lysine becomes cadaverine; ornithine becomes putrescine. These materials are associated with decaying and toxic organic debris.

It is suggested that the current biological treatments used for wastewater treatment not only fail to remove these potentially harmful materials but actually produce them by growing high concentrations of bacterial mass.
Table III

Basic Amino Acids and Ammonia in Activated Carbon Influent & Effluent

mg/l as material listed, as C and as N

<table>
<thead>
<tr>
<th>Ammonia</th>
<th>Lysine</th>
<th>Histidine</th>
<th>Arginine</th>
<th>Ornithine</th>
<th>Total Basic Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NH}_4^+$</td>
<td>COOH</td>
<td>COOH</td>
<td>COOH</td>
<td>COOH</td>
<td>COOH</td>
</tr>
<tr>
<td>$\text{H}<em>2\text{N-CH}</em>{(\text{CH}_2)_4}\text{H}_2\text{N}$</td>
<td>$\text{N}$</td>
<td>$\text{C-\text{NH}_2}$</td>
<td>$\text{C-\text{NH}_2}$</td>
<td>$\text{C-\text{NH}_2}$</td>
<td>$\text{C-\text{NH}_2}$</td>
</tr>
</tbody>
</table>

M.W. gm g-mole 18 146.19 155.16 174.21 132.16 ---
pK 9.3 9.7 9.2 12.5 8.7 ---

Influent:

| mg/l as is | 102 | 10.2 | -0- | 6.16 | 2.59 | --- |
| mg/l C | --- | 5.02 | --- | 2.54 | 1.78 | 9.34 |
| mg/l N | 79.3 | 1.95 | --- | 2.05 | 0.55 | 4.55 |

Effluent:

| mg/l as is | 26.8 | 8.18 | -0- | 5.36 | 1.07 | --- |
| mg/l C | --- | 4.02 | --- | 2.21 | 0.49 | 6.72 |
| mg/l N | 20.8 | 1.60 | --- | 1.80 | 0.25 | 3.65 |

--- Samples were Hydrolysed 6 N HCl for 24 hours under vacuum
Feed Material was sand filtered Secondary Sewage effluent from a Trickling Filter.
Cell wall proteinaceous fragments of bacteria decompose only partially to protoses, peptones and polypeptides. The residual amino acids because of their length and structure can form ring structures at the ends of polypeptides and limit further bacterial degradation. Because they are highly soluble, adsorption onto bacteria and activated carbon is limited. The predominant positive charge even up to high pH hinders attachment (by ion exchange mechanisms) onto adsorbed metals (e.g., Fe$^{+++}$ on Ca$^{++}$) at surfaces. These micro-colloids are then a class of treatment refractory materials in effluents.

For all these reasons these residual organics have been found in the effluents of highly treated wastewaters.
CONCLUSION:

Some basic amino acids - lysine, arginine and ornithine - in a polypeptide form, form a class of residual organics found in the effluents of biological treatment and of activated carbon. The mineralization of these materials ought to be completed before these constituents are discharged into the aquatic environment. Ozonation of the effluents of activated carbon or cation exchange columns has been proposed as a physical-chemical method for removing these refractory organic constituents from wastewaters. A series of ozonation experiments were conducted with clean water and lysine and also with effluents and lysine. Ozonation had little effect on lysine. Neither oxidation nor hydrolysis effects were promoted. Ozonation can not be used to remove these residual amino acids.

Alternative methods likely to remove these amino acids from wastewater are those methods that take advantage of the charge on the basic amino acid - either ion exchange or electrodialysis.
References


LIST OF PUBLICATIONS and other Information Dissemination Items


ACKNOWLEDGEMENT:

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ABSTRACT:

The purpose of this research is to characterize by process response and chemical analysis the residual organic constituents in waters especially the nitrogen-bearing components of wastewater treatment effluents following biological treatment and advanced wastewater unit steps such as activated carbon.

Special real-time (quick instrumentation measurement) techniques have been developed to make necessary precise, accurate and sensitive determinations necessary to measure nitrogen in its transient forms (from organic nitrogen to ammonia to nitrate) as the nitrogen passes through the several unit operations and processes necessary in wastewater treatment to remove nitrogenous organics.

Research has shown that there are residual and refractory organics in municipal wastewaters as measured by total organic carbon (≈ 3 mg/l TOC), that are dispersed non-adsorbable onto activated carbon, are cationic at high pH (≈9-16) and resist ozonation degradation.

The residual organics have been specifically identified as some basic amino acids - lysine, arginine and ornithine - in polypeptide form.

These small, charged microcolloids can be removed from treatment water by processes that take advantage of the charge on these nitrogenous organics. The processes that can remove the polymeric forms of basic amino acids are ion exchange and electrodialysis.
KEYWORDS:

residual nitrogenous organics
activated carbon
ozonation
ion exchange
electrodialysis
effluents of biological treatment
basic amino acids