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Title: Investigation of Humic Substances in Secondary Effluents

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INVESTIGATION OF HUMIC SUBSTANCES IN SECONDARY EFFLUENT

FINAL REPORT

July 1983

by

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Supported by

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Grant No. CME8008683

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1. INTRODUCTION

1. INTRODUCTION

The objective of the present work was to develop an isolation scheme to separate humic substances from the various streams of a sewage treatment plant, characterize them by non-destructive and destructive methods, identify the above degradation products using advanced instrumental analyses such as GC-MS, and relate these observations to the structure of the humic substances.

The immediate value of the work is to characterize the quality of advanced wastewater treated effluent for potential reuse in the domestic water supply. Inherent in such knowledge will be the ability of assessing the effectiveness of the individual advanced wastewater treatment processes and processes in combination for removing these materials of public health concern. This is specially true when the treated effluent is subjected to chlorination for reuse. The formation of trihalomethanes (THM) resulting from chlorination of precursor materials, such as the humic substances, is of public health concern.

Humic substances were isolated from secondary effluents of Atlanta trickling filter plant and Savannah activated sludge plant. River water humus was isolated from the Satilla river in large quantities. Conditions of experiments were first tried out with the river water humus before ap;lying them to the secondary effluent humus. Such exploratory experiments with river water humus were performed in the study of oxidative degradation with potassium permanganate under different conditions of severity. The condition of the oxidation was found to play an important role in determining the aromat-c and aliphatic constituents of the product mixture

(Reuter, Ghosal, Chian and Giabbai, 1983). In addition, findings of previous workers on oxidation studies have been analyzed, the aromatic fractions in these samples have been recalculated and these corrected values have been compared with those obtained by ^{13}C-NMR studies (Ghosal and Chian, communicated to J. Soil Sc. Soc. Amer.).

Details of the experiments conducted and data collected as well as their significance are presented in this report.

2. METHODS AND MATERIALS

2. METHODS AND MATERIALS

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2.1 Description of the Wastewater Treatment Plants

Flow diagram of Savannah treatment plant is presented in Fig. 1 and that of Atlanta is presented in Fig. 2. Other information on the exact locations, treatment processes, design capacities and average flow rates are presented in Table 1.

Table 1

Characteristics of the Treatment Plants

City	Location	Process	Design Capacity	Average Flow Rate
1. Atlanta	Intrenchment Creek	Trickling Filter	20 mgd	12-14 mgd
2. Savannah	President Street	Activated Sludge	20 mgd	16.8 mgd

The BOD_5 and flow rates of the raw sewage influent and secondary effluent of Savannah plant covering a period from January through August, 1982, are presented in Fig. 3. It is seen from Fig. 3 that an average of 80% removal of BOD_5 was obtained with the treatment plant. Although the average influent BOD_5 was maintained consistently at 200mg/ℓ, the effluent BOD_5, which exceeded the required 30-day average of 30 mg/ℓ was somewhat less desirable. This indicates that the Savannah plant was operated at a marginal level.

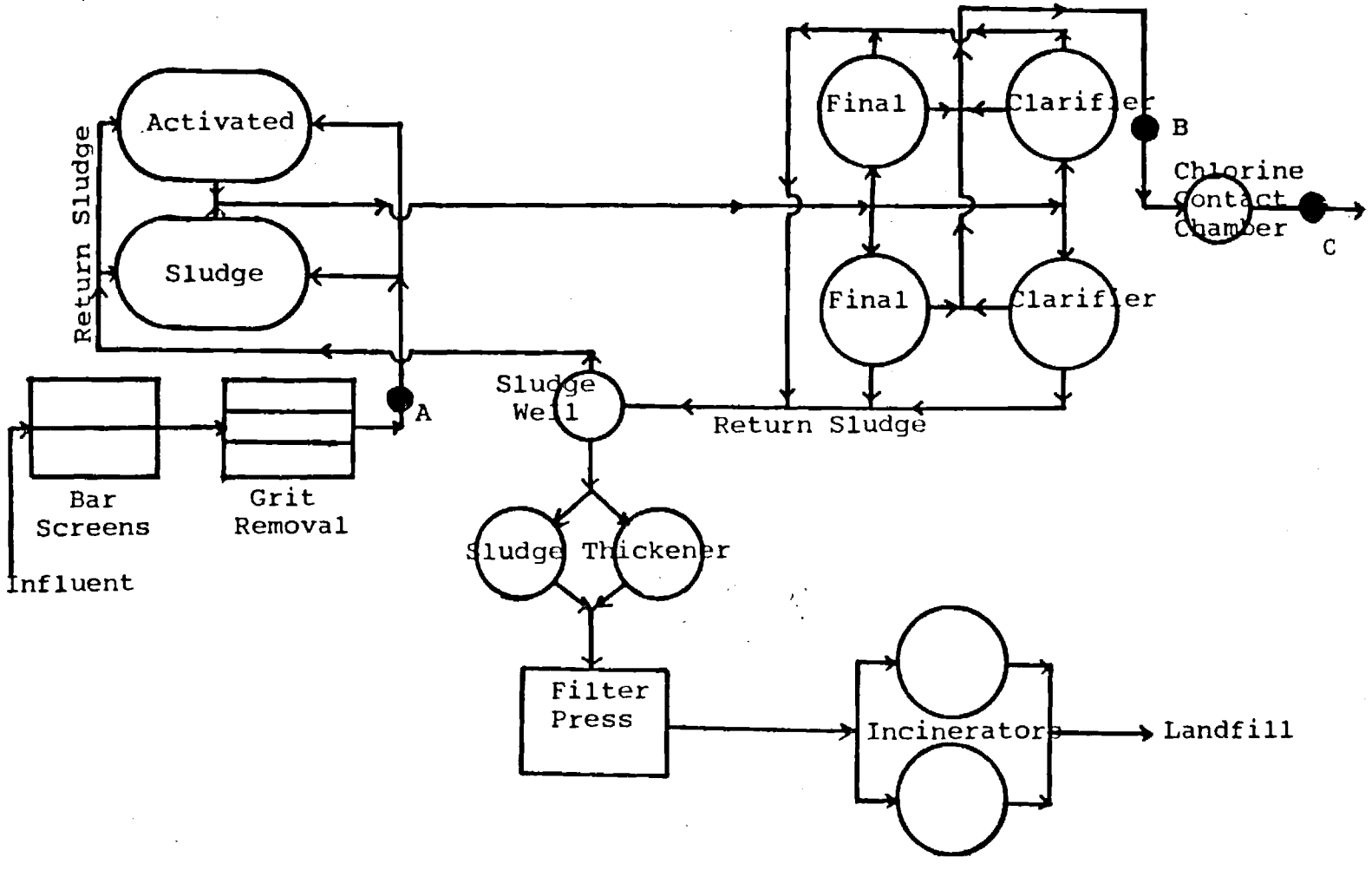

Figure 1. Flow diagram of Savannah activated sludge treatment plant.

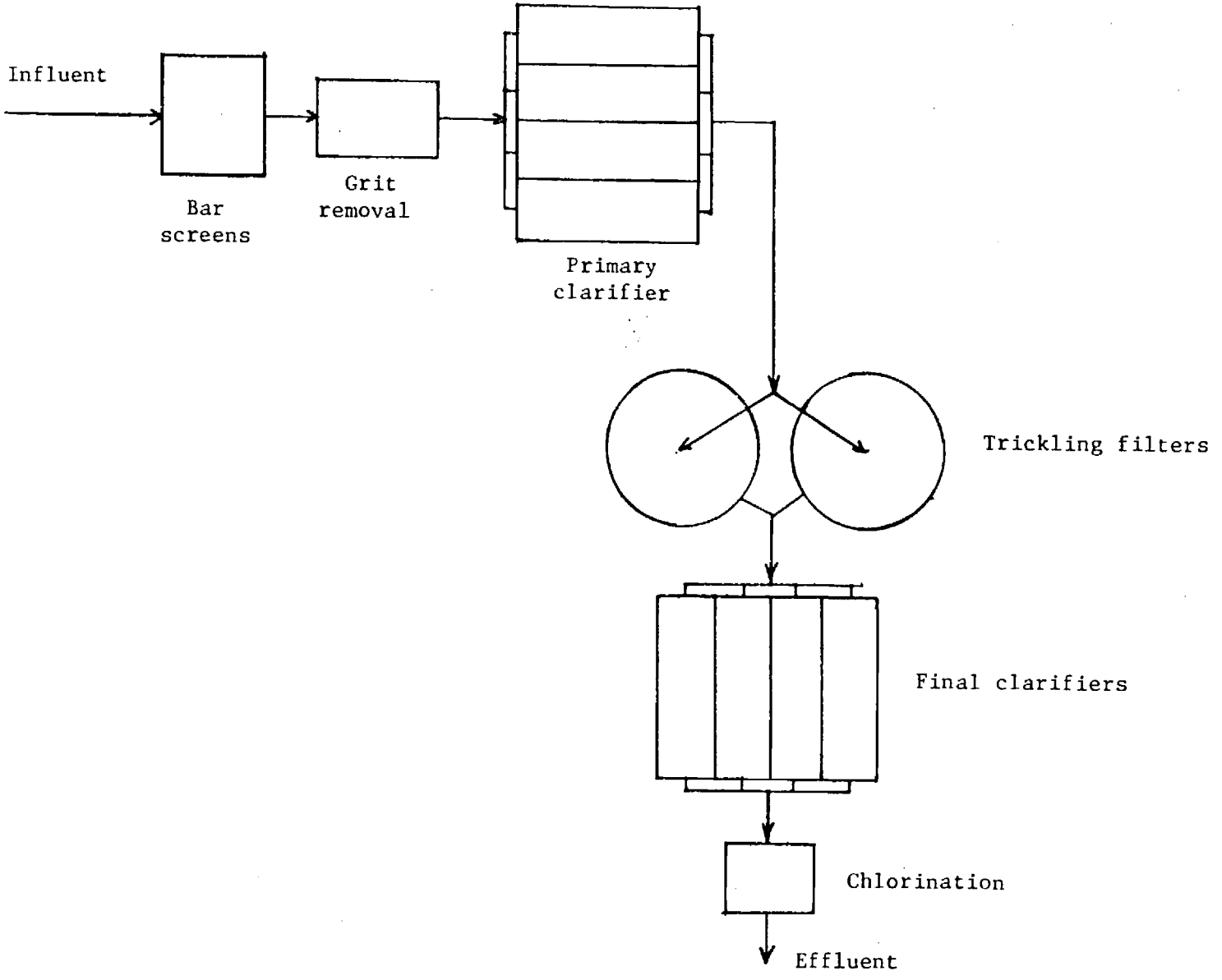

Figure 2. Flow diagram of Atlanta trickling filter treatment plant.

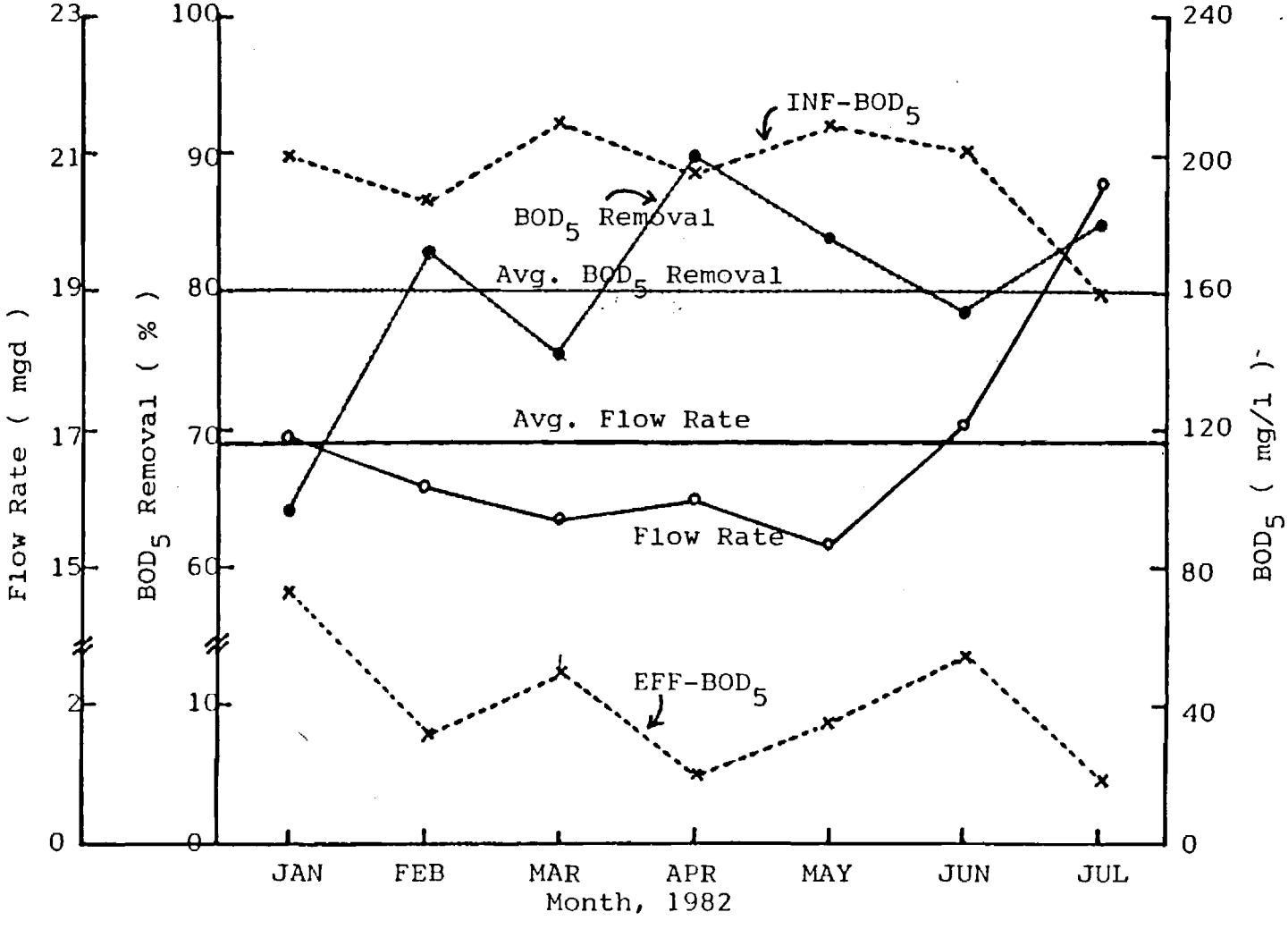

Figure 3 . Monthly average flow rate and BOD_5 removal of Savannah activated sludge treatment plant.

2.2 Sampling

Grab samples of the influent and secondary effluent before and after chlorination were collected at the Savannah plant between June 16 to 23, 1982. Four hundred (400) liters of the influent were taken from the discharge of the grit chamber. Twelve hundred (1200) liters of the effluent and 2200 liters of the chlorinated effluent were taken from the discharge weir of final clarifiers and the discharge of the chlorine content tank respectively. Two hundred (200) liters of sample were collected at each sampling point at about 10AM and humic substances were collected the same day according to the procedure described in Section 2.3.

The samples used for the determination of the gross properties, such as BOD_5, COD and TOC were frozen after filtration through a 0.45μ glass fiber filter. Analyses were completed within seven days according to procedures outlined in Standard Methods. TOC was determined on a Beckman Model 915 TOC Analyzer.

Grab samples of secondary effluent were collected prior to chlorination at the Atlanta plant in July, 1981. The sample was allowed to settle in a drum for several hours before sample was drawn out from the top for processing. Sodium azide (5g/200 liters of sample) was added to prevent growth of bacteria.

2.3 Isolation of organic fractions

Secondary effluents were filtered through a coarse glass filter, acidified to pH < 2 by addition of hydrochloric acid and passed over XAD-8 resin. The adsorbed hydrophobic acids were desorbed with aqueous triethylamine. The excess triethylamine was removed under reduced pressure and the residual aqueous solution was desalted by batch treatment with a cation exchange resin (AG-50W-X8, H^+ form). This desalting step caused the precipitation of humic acid. The suspension was filtered and the filtrate was processed to isolate fulvic acid, while the residue was processed to isolate humic acid. A schematic diagram of the isolation procedure is presented in Figure 4.

Humic Acid (I). The residue (see Figure 4) was treated with 0.1N sodium hydroxide to dissolve the humic acid, and filtered on a sintered glass filter to separate the exchange resin. The resin was regenerated by treatment with 2N NaOH, followed by distilled water and 2N HCl. The filtrate was acidified with HCl and the floccular precipitate separated by centrifugation. The collected humic acid was redissolved in 0.1N NaOH, reprecipitated with HCl, washed several times with distilled and deionized water and separated once more for purification.

Purification of humic acids was found necessary whenever the ash content was high. The samples redissolved in NaOH solution (0.1N), centrifuged to eliminate any precipitate, acidified with HCl, centrifuged and decanted. The residual solid was treated with distilled water, centrifuged and the liquid was decanted. The process was repeated thrice and finally, the brown solid suspension in water was lyophilized to obtain humic acids.

Fulvic Acid (II). The filtrate was extracted with ethylacetate to remove extractable organic acids (III). The residual solution was lyophilized and dried in a vacuum dessicator over sodium hydroxide for several days to yield fulvic acid, (see Figure 4).

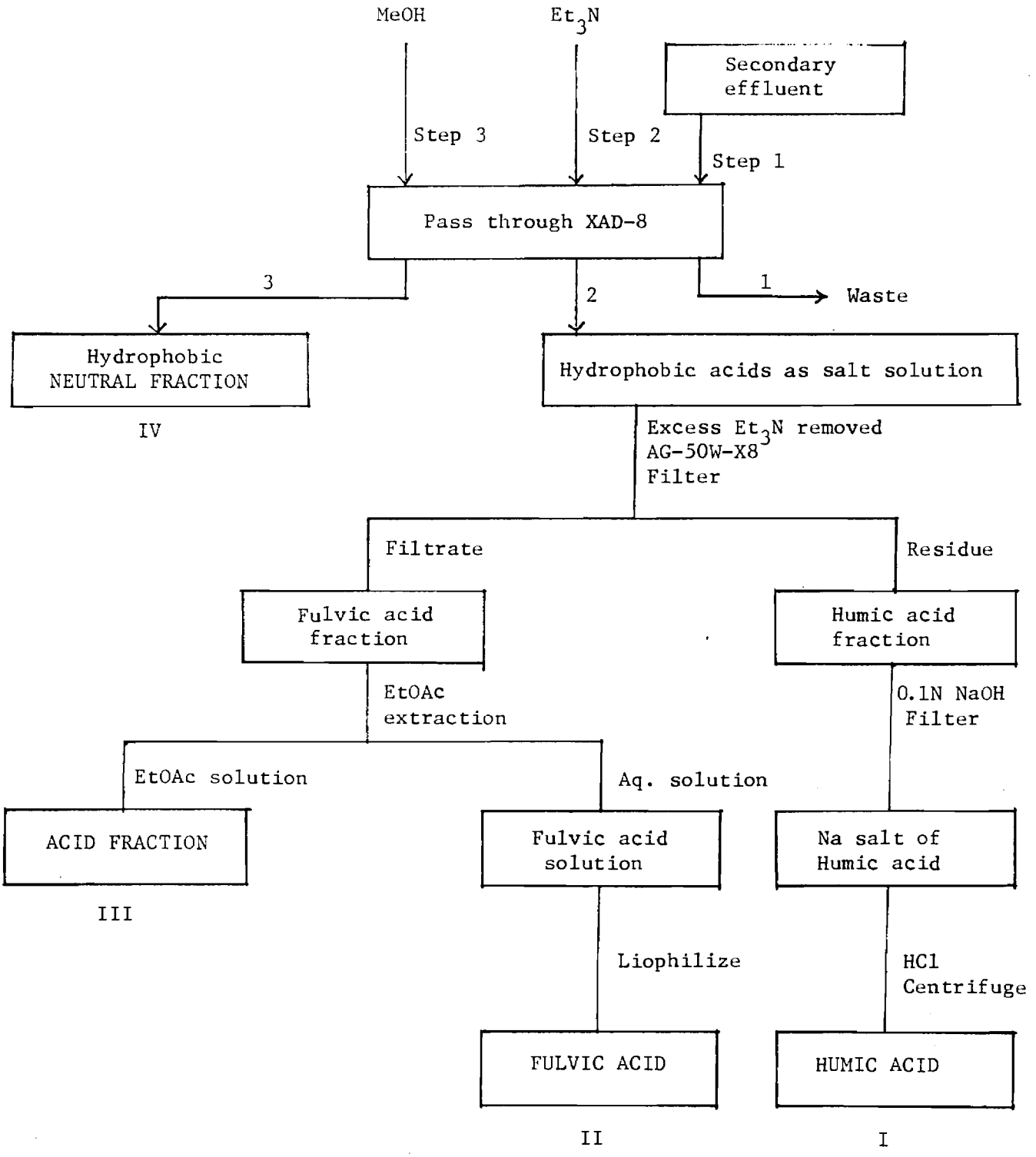

Figure 4. Schematic diagram of isolation procedure.

Acid Fraction (III). This fraction was obtained by extracting the filtrate separated from the precipitated humic acids with ethyl acetate.

Hydrophobic neutral fraction (IV). After eluting the hydrophobic acid fraction, the XAD-8 column was washed with water and then eluted with methanol to obtain hydrophobic neutral compounds. Methanol was evaporated, the residue redissolved in CH_2Cl_2 and the solution analyzed by GC-MS.

2.4 Acid Hydrolysis

Humic substances (0.1 g) and hydrochloric acid (6N, 5 ml) were sealed in a glass ampule under nitrogen and heated at 100° for 22 hrs. The acidic solution was filtered and the filtrate was analyzed for amino-acids. The residue was suspended in water, neutralized to about pH 7 and filtered over an ultrafiltration membrane (UM-02, Amicon, Bedford, MA, having a molecular weight cutoff of 1000) to separate substances of smaller molecular weight. The residue was dried and its infrared spectrum was recorded.

2.5 Amino Acid Analysis

Analysis of amino acid was done according to Spackman et al., (1958) at the Center for Disease Control, Atlanta, GA on a Beckman 121 automatic amino acid analyzer.

2.6.1 Elemental Analysis

Carbon, hydrogen, nitrogen, halogen, sulfur, and inorganic residue analyses of humic and fulvic acids were performed by a commercial analytical laboratoy (Atlantic Microlab, Inc., Atlanta, GA). For the determination of each element, a modified Pregel technique was used which employed the following standard compounds for each element:

Acetanilide for C, H, and N
Sulfanilamide for S

The samples were dried to constant weight in vacuo over P_2O_5 for several days before analysis.

2.6.2 Analysis of Acid Functions

Carboxyl groups and total acid groups in the samples have been determined in two separate analyses. The following procedures have been followed:

2.6.2.1 -COOH Group:

To an accurately weighed sample (about 10 mg) in a 5 ml vial was added 3 ml of CO_2 free water and precisely 0.2 ml of 0.1N sodium hydroxide solution. The mixture was stirred with a micro magnetic stirbar till solution was complete. The solution was added to a freshly prepared solution (0.2N;100ml) of sodium acetate whose pH was recorded (pH_o) before the admixture. The vial was washed with three aliquots of CO_2 free water (1 ml) and the washings were added to the bulk solution. Precisely 0.2 ml of acid (0.1N) was added to neutralize the alkali added initially. The pH of this mixture was noted, and it was lower than pH_o due to addition of the acid in the sample. The sample was titrated with sodium hydroxide solution (0.1N) from a micro-burette to pH_o. The volume of the sodium hydroxide consumed was noted. A blank was run without adding the sample.

Calculation:

Strength of NaOH = X

Volume of NaOH consumed = $V - V_o$

with sample = V

blank = V_o

Weight of sample = w mg

$$\text{-COOH group} = \frac{X(V - V_o)}{w/1000} \text{ meq/gm.}$$

The procedure described here for determination of -COOH group was developed in our laboratory. A sample of humic substances extracted from aquatic sediments was used for method validation. Eleven determinations of the -COOH group on the same sample gave the following results:

-COOH Group Content of Sample A-1

No.	-COOH group	Mean	SD	CV
1	4.64 meq/gm			
2	4.44			
3	4.79			
4	4.81			
5	4.78			
6	4.82	4.76 meq/gm	0.14	2.9%
7	4.87			
8	4.85			
9	4.82			
10	4.83			
11	4.78			

Estimation of -COOH of the same sample by the method of Perdue, et al., 1980 yielded:

Mean = 4.27 meq/gm

SD = 0.08

CV = 1.9%

from 4 determinations.

2.6.2.2 Total Acidity

The procedure developed by Schnitzer and Gupta (1965), and Schnitzer and Khan (1972) were adopted with some modification for the determination of total acidity of the samples. The procedure is based on the assumption that all acidic hydrogens of the macromolecule react with an excess of barium hydroxide solution. The excess base is back-titrated with standard acid to measure base consumption by the organic functional groups.

All operations were carried out under N_2 atmosphere. Standard barium hydroxide solution (5 ml, 0.0764 N) was added to an accurately weighed sample (about 10 mg) in a 125 ml Erlenmeyer flask followed by CO_2 free water (25 ml). The solution was stirred for 0.5 to 1 hr. The mixture was filtered on Whatman 40 filter paper, the residue washed with water, and the filtrate was titrated with standard hydrochloric acid (.0214 N). A blank was run under

Calculation:

Strength of HCl = X

Volume of HCl consumed = $V_o - V$

with sample = V

blank = V_o

$$\text{Total acidity} = \frac{X(V_o - V)}{w/1000} \text{ meq/gm}$$

A sample of humic substances (A-1) derived from aquatic sediments was used to determine the reproducibility of the results of this prodcedure. The results are shown below.

Total Acidity of Sample A-1

No.	Total Acidity	Mean	SD	CV
1	11.24 meq/gm			
2	10.86			
3	10.89	11.1 meq/gm	0.35	3.2%
4	11.59			
5	11.20			
6	10.59			

2.6.2.3 Direct Titration:

Direct titration curves were obtained by potentiometric titration of the humic substances in 0.1M NaCl with NaOH as a titrant using a Fisher Accumet Model 144 pH meter (Pittsburgh, PA) with a Sargent-Welch glass electrode and calomel reference (Skokie, IL).

Net titration curves were derived by subtracting reagent blank titration from the sample titration.

2.6.3 Infrared Spectroscopy

Infrared spectra were recorded on a Perkin-Elmer 621 or 237B spectrophotometer (Norwalk, CT). Samples were dried over P_2O_5 *in vacuo* for several days to constant weight. Spectroanalytical KBr was dried overnight at 105-110°C. Approximately 1-2 mg samples were mixed with 100-150 mg KBr. The mixture was ground to a fine powder and pressed by Torque Kit (provided by the manufacturer). The resulting pellets were transluscent brown and showed good dispersion of the samples.

Considerable attention was paid in preparation of the pellets for IR analysis to prevent moisture interference.

2.6.4 Apparent Molecular-Weight Distribution

A sample of humic/fulvic acid (20-30 mg) was dissolved in 200 ml of borax buffer (.025 M borax and 0.1M sodium hydroxide in the ratio of 50:18.3 by volume, pH = 10). The apparent molecular-weight distribution of the dissolved organics was determined by a modified procedure according to DeWalle and Chian (1977).

(a) Ultrafiltration

The solution (200 ml) was concentrated to give 50 ml of retentate on UM05 ultrafiltration membrane (Amicon Corp., Lexington, MA), having a molecular weight cut off at 500 Dalton in a static test cell at a nitrogen pressure of 35 psig.

(b) Gel Permeation Chromatography

A 10-mm i.d. glass column filled with pre-swollen Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway,NJ) to a bed height of 410 mm was used for gel permeation chromatography. The flow rate was maintained at 0.3 ml/min. TOC measurement and/or absorbance at λ = 230 nm in the uv region was utilized for monitoring the eluent.

A standard curve was plotted with Blue dextran (MW 2,000,000 Dalton) and glucose (MW 180 Dalton) to determine the exclusion limits of the column used. Blue dextran had a mean exclusion volume of 13.8 ml (V_o) and glucose had a mean exclusion volume of 36.0 ml (V_t). Thus the exclusion range was determined as 13.8 - 36.0 ml.

A sample (0.5 ml) of the retentate from (a) was injected into the column, and was eluted with the borax-buffer. Four fractions were collected as described below:

No.	Volume
0	11.1 ml
1	6.9
2	7.5
3	10.5

A schematic diagram of the steps described is presented in Figure 5.

2.6.5 Thermogravimetric Analysis

Thermogravimetric analysis was obtained on a Perkin-Elmer, Model TGS-2 (Norwalk, CT) An accurately weighed sample of humic or fulvic acid (about 10 mg) was thermally decomposed in a temperature controlled reactor with a temperature gradient of 10°C/min up to 800°C in a nitrogen atmosphere. Weight loss of the sample by combustion with increasing temperature was monitored along with the derivative curve (DTG: differential thermogravimetric analysis curve).

2.6.6 ^{13}C-NMR Spectroscopy

Solid state ^{13}C NMR spectra were recorded on a Bruker WH-400 instrument (Billerica, MA) at a spinning rate of 4.0 KHz at the Department of Chemistry, University of South Carolina, Columbus.

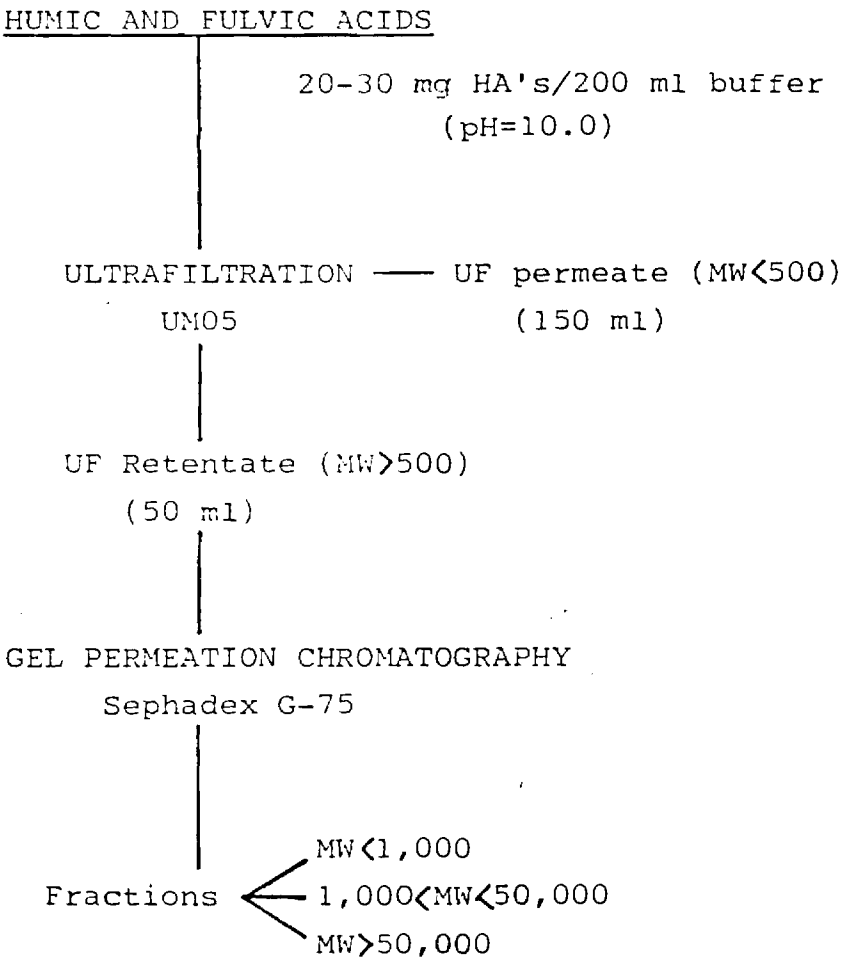

Figure 5. Determination of molecular-weight distribution of humic and fulvic acids

2.7 Permanganate Oxidation

Oxidative degradation studies of river water humus with $KMnO_4$ have been published. A reprint is enclosed. This study shows that the yield of oxidation products is very low and that most of the starting material is lost as CO_2, CO etc. The best yield is obtained by oxidation with $KMnO_4$-crown ether. This experiment is done by first methylating the humic substances with diazomethane and then oxidizing the modified humus with $KMnO_4$-crown ether in CH_2Cl_2. The success of the experiment depends much on the solubility of the methylated humus in CH_2Cl_2.

Humuc substances derived from secondary effluents do not give a methylated product soluble in CH_2Cl_2. Using NaH and CH_3I as methylating agent (Wershaw et al., 1981) may cause C methylation over and above methylation of acidic -OH groups and possibly include alcohols. Even though the product may be soluble in CH_2Cl_2, the chemical changes introduced into the humus are complex and nonreproducible.

2.8 Neutral Fraction Analysis

Fraction IV (see figure 4) was evaporated to dryness and the residue was dissolved in CH_2Cl_2 (1 ml). The solution was analyzed by GC-MS under conditions described in Section J. An SE-54 capillary column (30m x 0.3mm i.d.) was used for gas chromatography and oven temperature was programmed from 40°C (2 min) to 280°C at 10°C/min.

2.9 Acidic Fraction Analysis

Fraction III (see figure 4) was concentrated and the residue treated with excess ethereal diazomethane. The solvent was evaporated, the residue dissolved in CH_2Cl_2 (1 ml) and the solution analyzed by GC-MS under conditions described in Section J. An SE-52 capillary column (30 m x 0.3 mm i.d.) was used for gas chromatography and oven temperature was programmed from 40°C (4 min) to 280°C at 15°C/min except in the analysis for the influent (AS) sample which it was programmed from 40°C (2 min) to 280 C at 10°C/min.

2.10 Gas Chromatography-Mass Spectrometry-Data System

The GC/MS analysis was performed on a Hewlett-Packard 5830A gas chromatograph (Avondale, PA) equipped with capillary column injector port, interfaced to a FINNIGAN 4000 mass spectrometer (Sunnyvale, CA) equipped with Incos Data System (Southboro, MA). The separation of the organic components was carried out on a 30-m x 0.3 mm i.d. glass capillary column, deactivated according to Grob and Grob (1980) and coated by the static method (1978) with approximately 0.25 µm film thickness of SE-54 or SE-52 silicone gum phase (Applied Science; State College, PA).

Ultra-high purity helium was employed as carrier gas. Aliquots of 0.1 to 0.2µl of the solution were injected in splitless mode and the chromatography was operated under the oven temperature program as stated in sections 2.8 and 2.9. The glass capillary column was directly connected to the ionization source by means of a 40 cm x 0.1 mm i.d. fused silica tubing that operated as sample transfer line. The mass spectrometer was operated under the following conditions.

Ionization mode	EI
Emmission current	0.5 mA
Electron energy	70 eV
Preamp sensitivity	10^{-7} A/V
Electron multiplier	1500 V
Mass range	41-500 amu
Scan rate	1 scan/sec

Perfluorotributylamine (FC43) was used initially to tune the ion source; 20 to 30 ng/µl of decafluorotriphenylphosphine (DFTPP) was subsequently injected through the GC in order to check the tune obtained, the column and the transfer line performances.

The INCOS data system was used to match the mass spectra of the eluting GC peaks with those authentic samples stored in the library. Two indices, FIT and PURITY, are provided by this system as measures of agreement between sample and reference mass spectra. A FIT index of $\geq$850 (out of 1000), and a PURITY index of $\geq$500 (out of 1000) were accepted as a good match. In addition, a visual

check, subtraction of the library spectrum from the sample spectrum and logical fragmentation patterns were the criteria examined for the tentative identification of the mass spectra.

2.11 Chlorination of River Water Humus

River water humus (0.6g) was treated with chlorine in distilled water (225 ml of Cl_2-water with a concentration of 4.272 g Cl_2/litre, i.e., 0.96 g Cl_2, Cl/C atomic ratio 1:1). The pH was adjusted to 7 by adding $NaHCO_3$. The mixture was stirred for 1 hr., acidified with HCl to pH 2 and the solution passed through a column of XAD-8 resin. The adsorbed acids were eluted with aqueous triethylamine solution. Excess triethylamine was removed in a rotary evaporator, the residual solution desalted with ion exchange resin (AG 50W-X8, H^+ form) and filtered. The filtrate was extracted with ether to remove smaller organic molecules, and the residue was lyophilized to give a solid product which was extracted with acetone and filtered. The filtrate was stripped of solvent in a rotary evaporator to yield a dark product (0.112 g, 18%).

2.12 Metal Analysis

Two digestion procedures were utilized in the preparation of samples for metal analysis.

Method I: The dried sample was ashed in an electric furnace at 525 ± 25°C overnight to burn away carbon. After cooling, 5 ml of 6N HCl (Ultrix, Phillipsburg, NJ) was added and evaporated to dryness on a water bath. This process was repeated a second time, finally 5 ml of 6N HCl were added and the solution was heated for five minutes, and quantitatively transferred to a 50 ml volumetric flask. The volume was adjusted with deionized and distilled (d+d) water.

Method II: 5 ml of HNO_3 and 2 ml of $HClO_4$ were added to the dried sample.

The sample was then covered and heated on a water bath and allowed to evaporate to a final volume of 3-5 ml. 10-15 ml of d+d water was added to the sample and the digested solution filtered through an acid-washed filter paper into a 50 ml volumetric flask. The filter paper was washed and the filtrate diluted to volume with d+d water.

In both digestion methods reagent blanks were carried through equivalent procedures.

Metal analyses were performed on a Perkin Elmer (Norwalk, CT) model 703 atomic absorption spectrophotometer and HCA 2200 graphite furnace. Background correction and gas interrupt were routinely utilized.

Standards were diluted from fresh 1000 ppm stock solutions using the matrix appropriate to the digestion procedures.

3. RESULTS AND DISCUSSIONS

3. RESULTS AND DISCUSSIONS

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3.1 Plant Selection

The city of Savannah Water Pollution Control Plant (1400 East President Street, Savannah, Georgia) was selected for the present study for the following reasons:

1. The plant is an activated sludge facility.
2. The plant is new and well monitored.
3. It has good laboratory facilities.
4. The raw wastewater is almost entirely of domestic origin with little industrial impact.

Some humic substances were also isolated from the effluent of the City of Atlanta Water Pollution Control Plant (Intrenchment Creek, Atlanta, Georgia). This plant is a trickling filter facility.

3.2 Samples

One sample (secondary effluent before chlorination) was collected at the Atlanta plant. Three samples were collected at the Savannah plant. A list of all the samples collected is provided in Table 2.

Table 2
List of samples collected

No.	Plant location	Sampling point	Abbreviation
1.	Atlanta (Trickling filter)	(a) Effluent	TFE
2.	Savannah (Activated sludge)	(a) Influent	ASI
		(b) Effluent	ASE
		(c) Chlorinated effluent	ASCE

3.3 Isolation

Organic matter in river water has been fractionated by adsorption onto macroreticular resins to give six operationally defined fractions; namely, hydrophobic acids, bases and neutrals, and hydrophilic acids, bases and neutrals (Leenheer and Huffmann, 1976; Leenheer, 1981). The hydrophobic acid fraction is mostly composed of aquatic humus. The adsorption of hydrophobic acids on Amberlite XAD-8 requires protonation of the carboxylate anions. This is accomplished by acidifying the sample with concentrated HCl to pH < 2. The adsorbed material can be desorbed from the XAD resin by one of three alternative aqueous bases: 2M ammonium hydroxide (Mantoura and Riley, 1975); 0.1M NaOH (Leenheer, 1981); or 0.15M triethylamine (Perdue, 1979). The last was chosen because excess triethylamine can be easily removed due to its volatility. Unlike ammonia, the tertiary amine does not react with phenols and it is not as strong a base as NaOH which may cause or catalyze oxidative changes in humic substances.

The yields of fulvic(FA) and humic acids (HA) isolated from the three different streams of the Savannah treatment plant and the effluent of the Atlanta plant are shown in Table 3. Table 4 gives the concentrations of humic substances (gm/L) and their percentage of total organic carbon (collected at Savannah treatment plant). The concentration of fulvic acids is very low in all the streams. The fulvic acid fractions isolated were all initially liquid with strong pungent odor. The liquid persisted even after long periods of lyophilization. The samples were redissolved in water, treated with cation exchange resin (AG 50W-X8, H^+ form), filtered and the filtrate lyophilized. The process was repeated twice before any solid fulvic acid could be obtained. A significant position of the fulvic acid fractions was lost during the course of this treatment.

Table 3
Yields of humic acids and fulvic acids isolated from different streams of treatment plants

No.	Source	Volume Processed	Weight of Humic Acid HA	Fulvic Acid FA
1.	Atlanta plant Effluent	972 L	1.91 g	2.64 g
2.	Savannah plant Influent	360 L	7.27 g	0.90 g
	Effluent	900 L	1.54 g	0.31 g
	Chlorinated Effluent	1980 L	1.36 g	0.14 g

3.4 Characterization

3.4.1. Elemental Analysis

Analysis of elements present in the humic substances are exhibited in Table 5. The fulvic acids isolated from the secondary effluent and chlorinated secondary effluent show unusually low values for carbon and high values for sulfur, ash and chlorine in comparison to the other samples. Such low carbon has not been encountered in either soil or aquatic fulvic acid samples (Stevenson, 1982).

Table 5
Elemental composition of humic substances on ash-free basis.

Sample **	C	H	O	N	S	Cl	Ash
ASI-HA	63.0	9.2	20.3	6.4	1.1	0	10.7
ASI-FA	48.8	6.7	37.9	3.2	3.3	0	5.5
ASE-HA	53.1	7.6	28.4	9.6	1.3	0	3.6
ASE-FA	39.0	5.3	36.0	3.4	9.6	6.7	27.0
ASCE-HA	51.2	7.3	31.0	9.3	1.2	0	4.0
ASCE-FA	36.5	5.4	39.9	4.2	9.2	4.8	13.7
TFE-HA	53.9	8.2	29.6	6.9	1.4	*	2.6
TFE-FA	54.1	6.8	30.2	6.5	2.4	*	3.6

*Not analyzed
**See Tables 2 and 3 for abbreviations

Table 4

Concentration of humic substances in the different streams of Savannah treatment plant.

Source		TOC (mg/L)	Weight of HS(g)	Volume sample processed(L)	Concentration (mg/L)	% C in HS	Concn. of HS mg C/L	C in HS as % of TOC	
Influent	HA	88.5	12.12	360	33.7	56.2	18.9	21.4	23.6
	FA		1.50	360	4.2	46.2	1.9	2.2	
Secondary effluent	HA	19.2	2.74	900	3.0	48.2	1.4	7.3	12.0
	FA		2.33	900	2.6	36.1	0.9	4.7	
Chlorinated secondary effluent	HA	10.0	3.88	1980	2.0	47.2	0.9	9.0	11.0
	FA		1.13	1980	0.6	31.5	0.2	2.0	

C values have been used without correcting for ash because the weight of C is here estimated from the gross weight of the isolated humic substances.
HS = Humic substance
HA = Humic acid
FA = Fulvic acid

3.4.2 Infrared Spectra

Infrared spectra of the humic and fulvic acids and their acid hydrolysis products (H) from the sources indicated are presented in Figures 6 to 12.

The broad adsorption bands are indicative of the presence of -COOH, -OH, -CONH and -C-O-C- groups (as explained in Table 6) in the humic substances. The hydrolyzed products show a comparative reduction in the C=O peak as a whole, especially at the lower range of wave numbers,concornitant with a reduction in the amide II band. These results confirm the view that the high nitrogen content of the humic substances is due to incorporation of proteinaceous material giving ruse to hydrolyzable -CONH- linkages. Hydrolysis also effects reduction of the adsorption band in the 1100 cm^{-1} region indicating hydrolysis of polysaccharide-like material.

Table 6
Interpretation of infrared spectra of humic substances from secondary effluents.

Absorption due to	Region in Wave-number	Interpretation
OH stretching	3300	Alcoholic, phenolic, enolic and carboxylic -OH groups.
CH stretching	2920	Aliphatic C-H
C=O stretching	1800-1600	-COOH, ketone, ester, amide (I), quinone and conjugated ketone.
C=N stretching	1540	COO^- and amide (II).
C-O stretching	1050	polysaccharide-like substances.

3.4.3 ^{13}C-NMR

^{13}C solid state NMR spectra of the effluent and chlorinated effluent are presented in Figures 13 and 14. The spectra are divided into 4 regions:

I. 60-50 - alkane carbons.

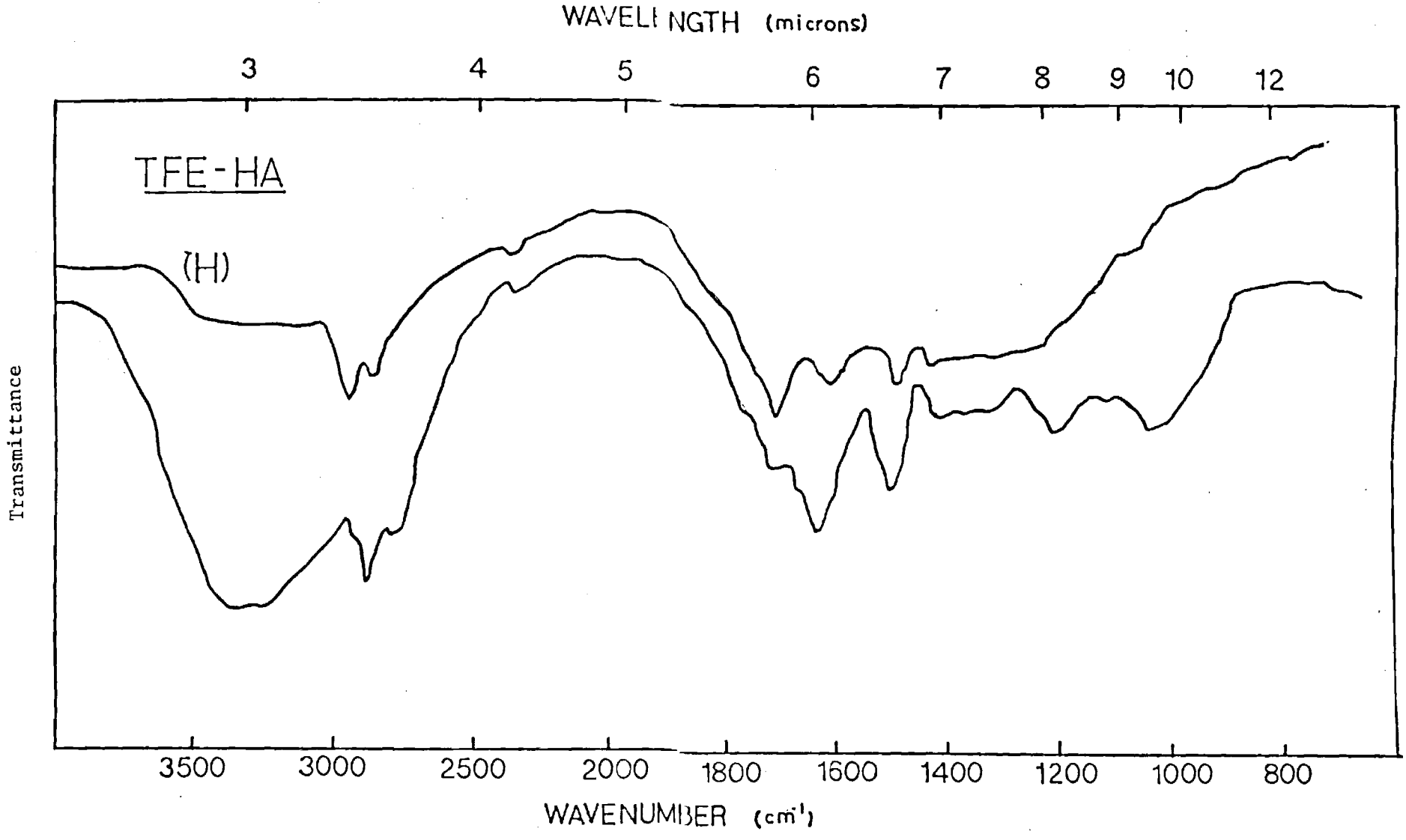


Figure 6. Infrared spectrum of TFE-HA and its hydrolysis product (H).

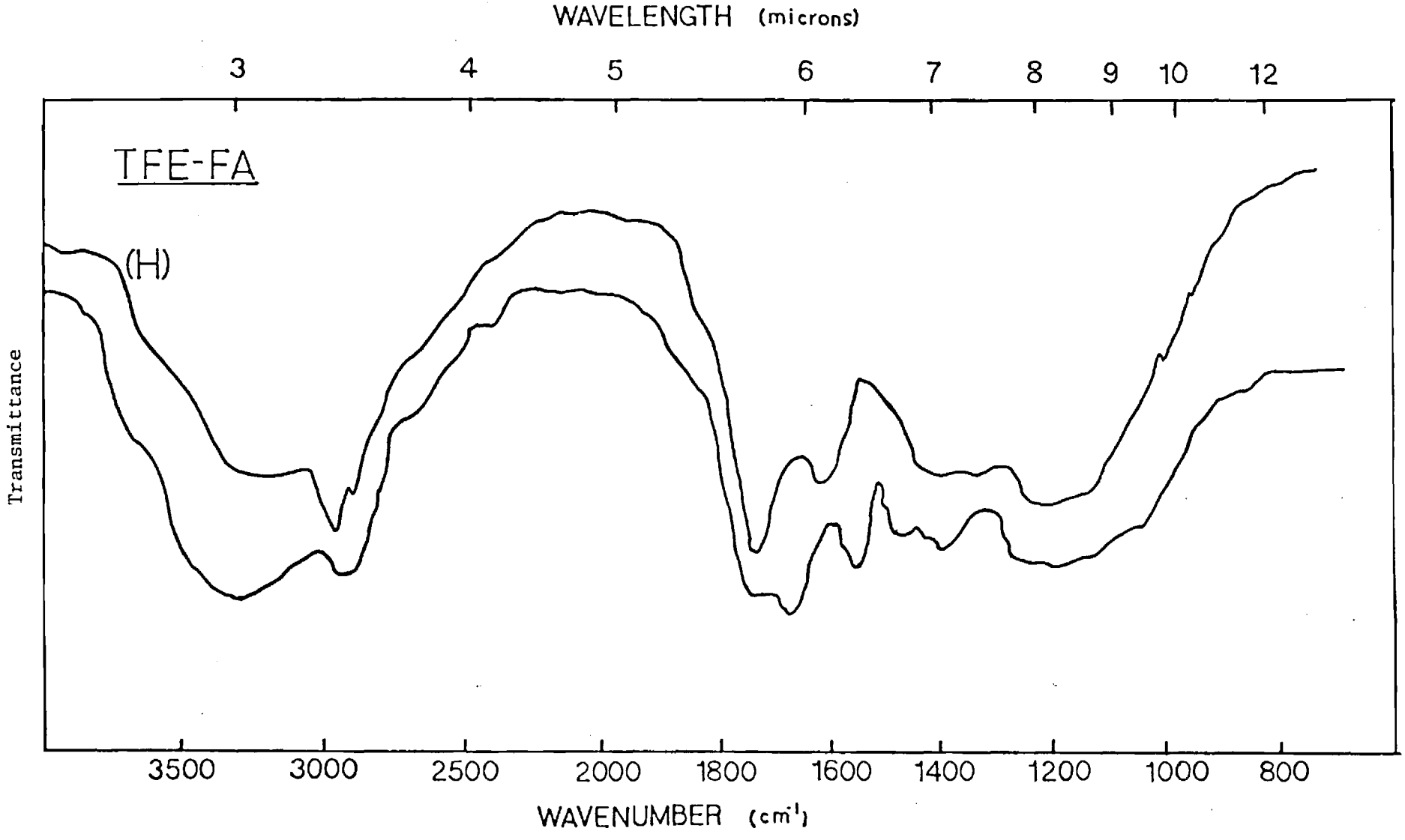


Figure 7. Infrared spectrum of TFE-FA and its hydrolysis product (H).

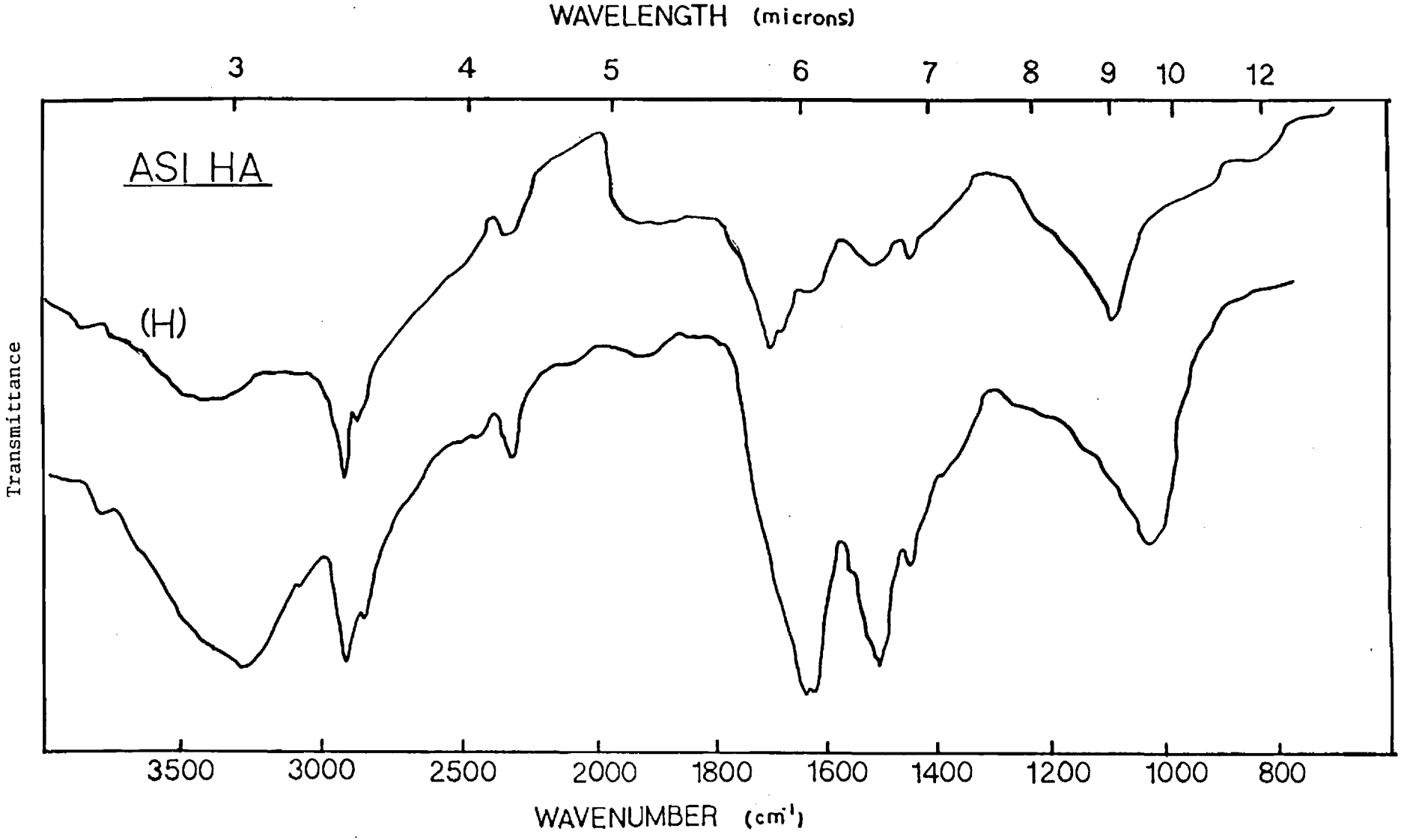


Figure 8. Infrared spectrum of ASI-HA and its hydrolysis product (H).

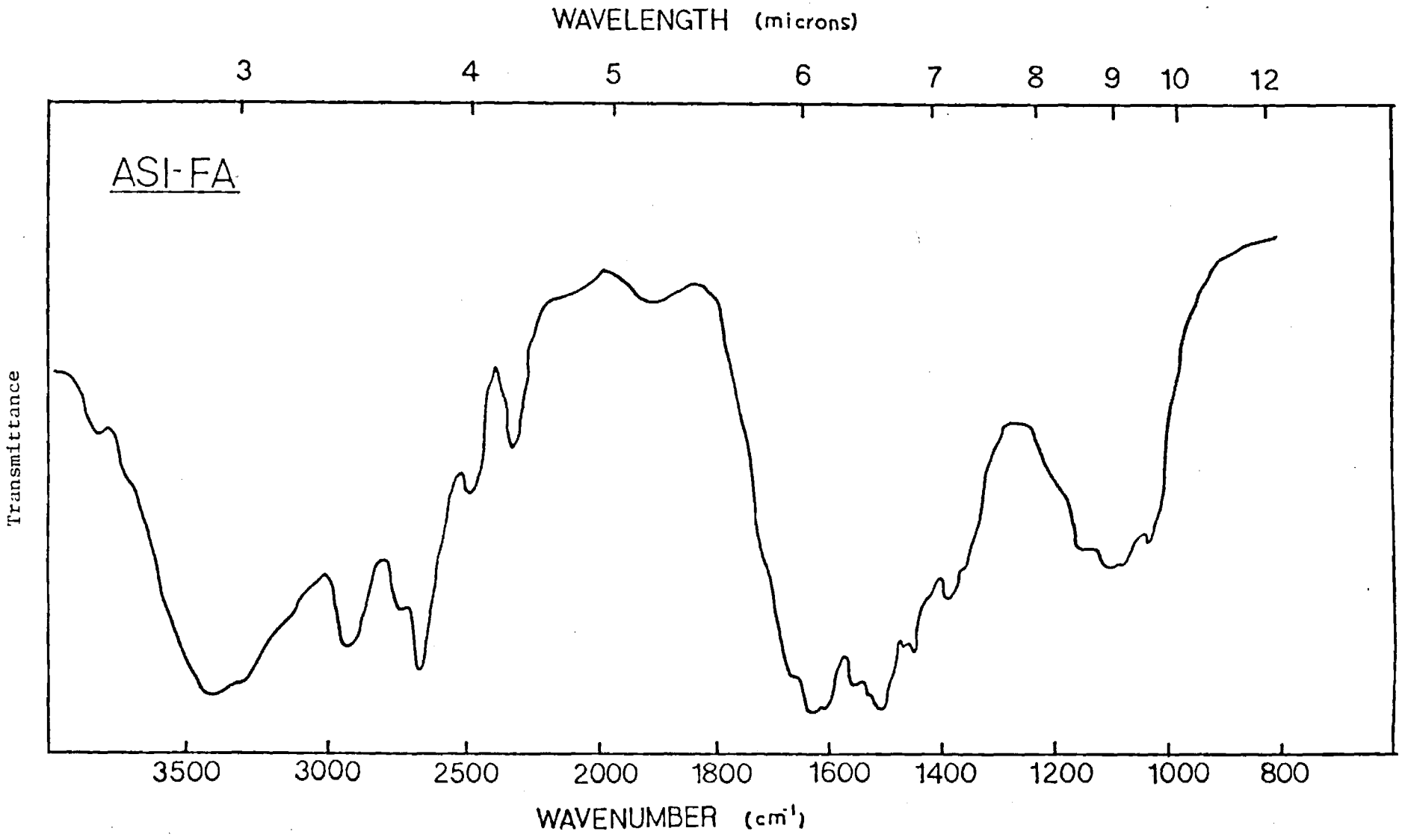


Figure 9. Infrared spectrum of ASI-FA

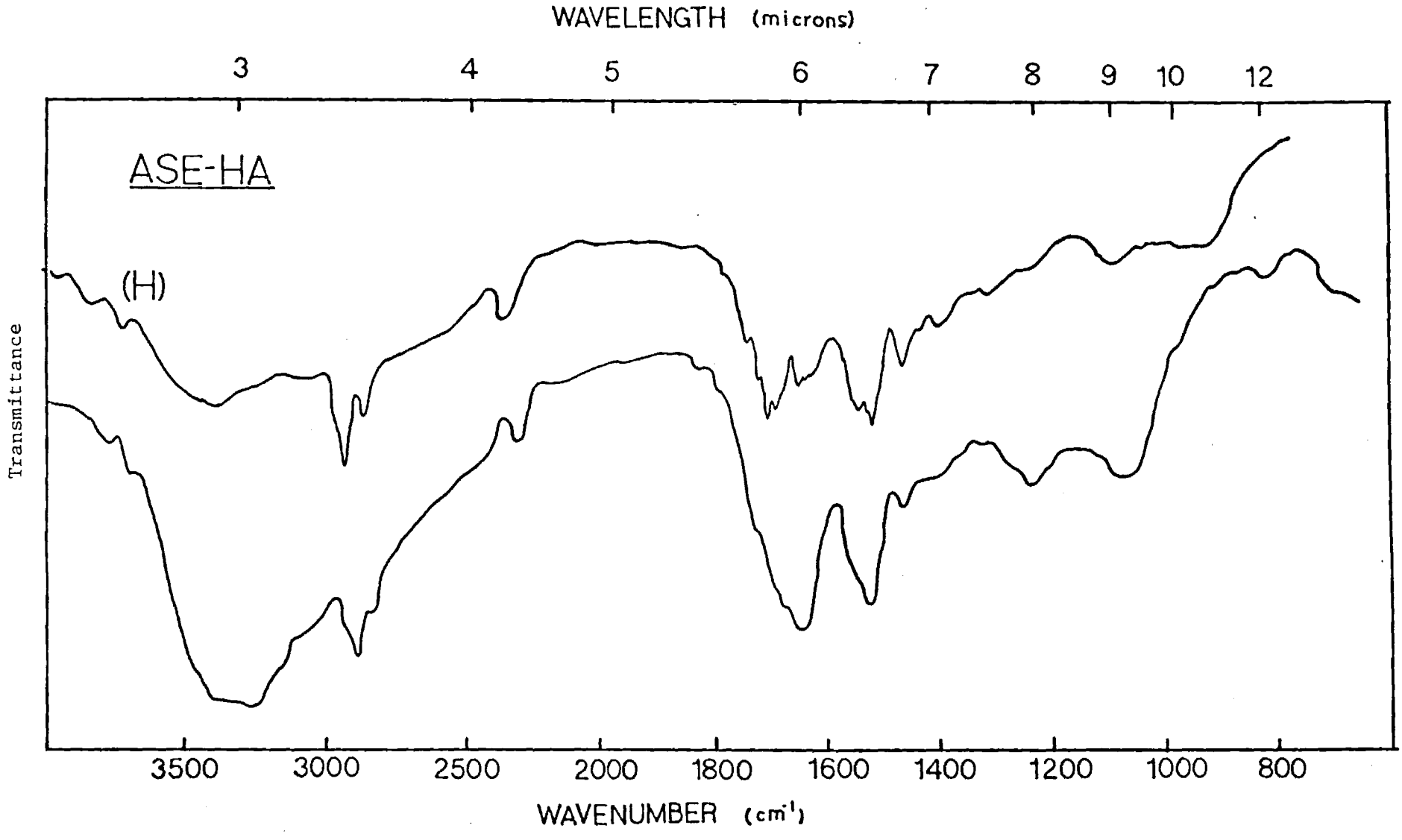


Figure 10. Infrared spectrum of ASE-HA and its hydrolysis product (H).

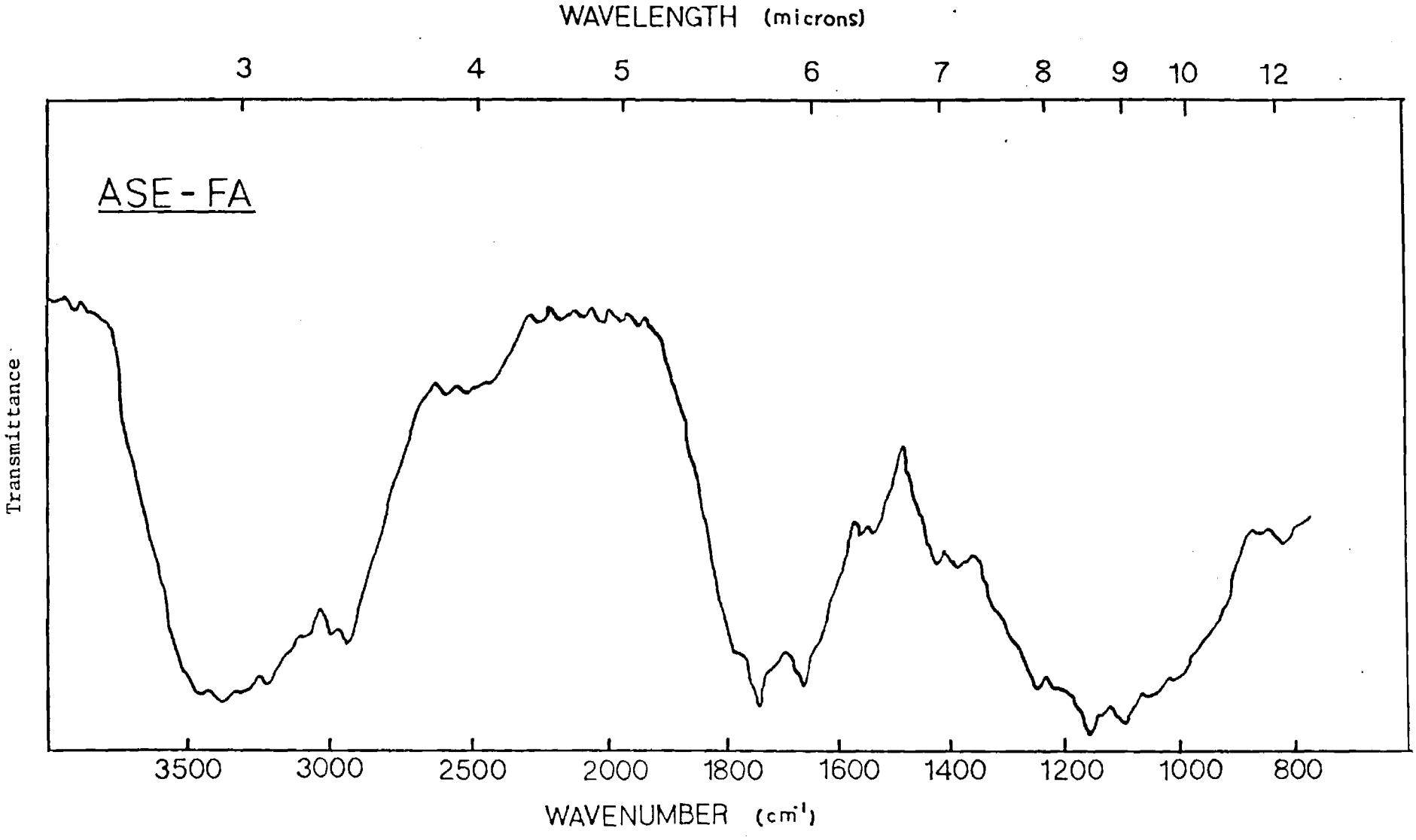


Figure 11. Infrared spectrum of ASE-FA and its hydrolysis product (H).

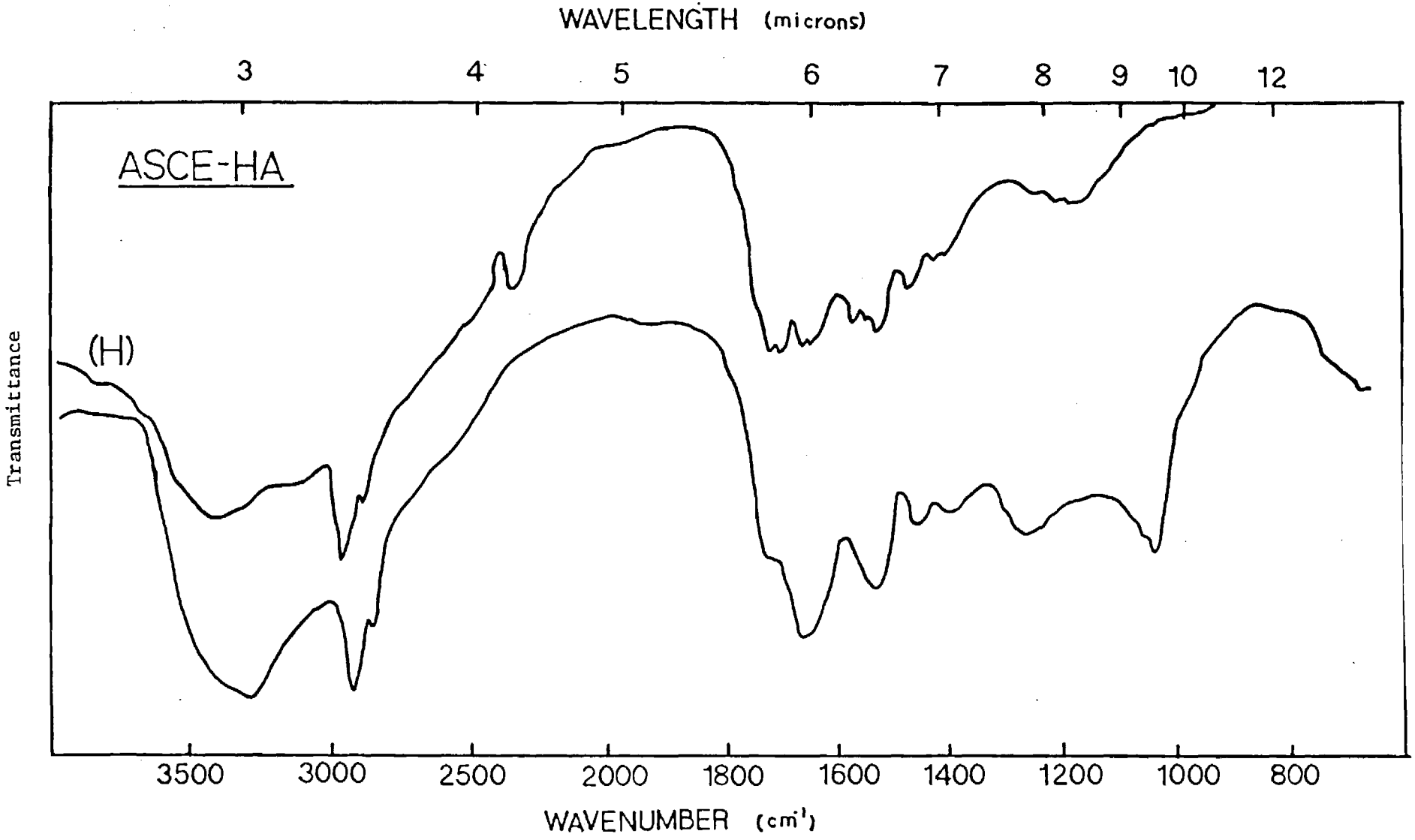


Figure 12. Infrared spectrum of ASCE-HA and its hydrolysis product (H).

II. δ50-110 - carbons bonded with oxygen by single bond

III. δ110-160 - aromatic and aliphatic sp^2 carbons

IV. δ160-190 - carbonyl and carboxylic carbons (C=O and C(=O)OH)

Relative areas covered by the peaks in the four regions are presented in Table 7. The sewage-derived humic acids have very low aromatic carbon content (8-10%) as compared to soil and aquatic humic acids (Hatcher et al., 1981). The significance of the high C=O (region VI) peak will be discussed later in connection with data on carboxyl groups (-COOH) and amino acids.

Table 7
Solid state ^{13}C-NMR spectra of humic substances.

	Percent area covered by			
	Region I	II	III	IV
Source*	δ0 - 50	50 - 110	110 - 160	160 - 190
ASE-HA	43	26	8	23
ASCE-HA	52	18	10	20

*See Tables 2 and 3 for abbreviations.

3.4.4 Acidic Properties

An important characteristic of humic and fulvic acids, in general, is their behavior as weakly acidic polyelectrolytes. Therefore, in order to characterize the sewage derived humic material we determined their acidic properties.

Carboxyl Acidity

The term carboxyl acidity is used here in a strictly operational sense. Most efforts to subdivide the total acidity of humic material into carboxyl and other acidic components have generally been based on pKa dependent methods

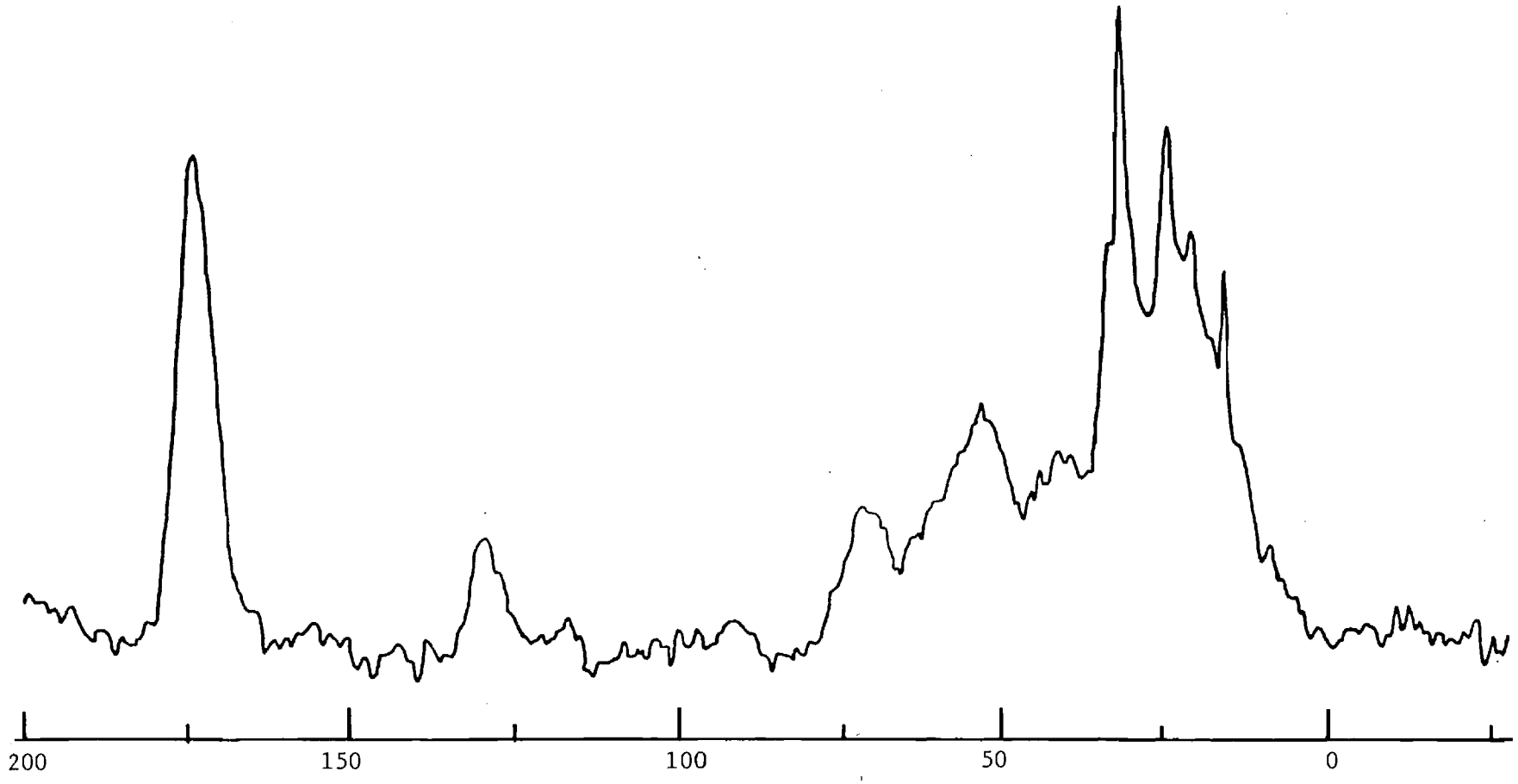


Figure 13. ^{13}C NMR spectrum of humic acid from effluent (ASE-HA)

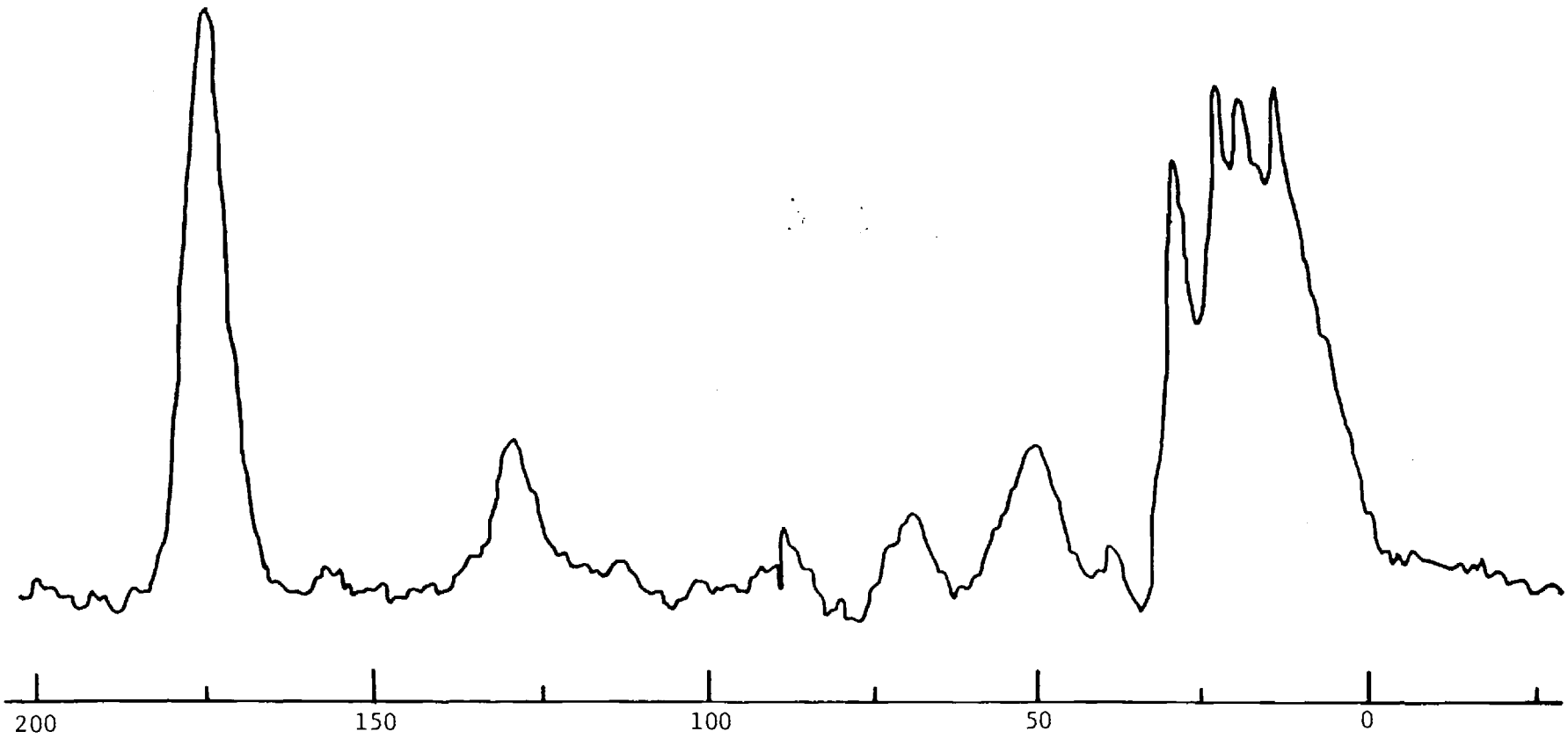


Figure 14. ^{13}C NMR spectrum of humic acid from chlorinated effluent (ASCE-HA)

other acidic components have generally been based on pKa dependent methods rather than on structure specific methods. As clearly pointed out by Dubach et al. (1964) it must be assumed that a complex mixture of polyfunctional acids will exhibit a wide range of pKa values for each class of acidic functional groups and that the pKa ranges of two classes of acidic functional groups may overlap considerably. Analytical methods based on pKa cannot distinguish, with certainty, between carboxyl and other types of acidic functional groups in complex polyelectrolyte mixtures such as humic substances, except in an operational sense. To be generally applicable, any analytical method which is used to determine an operationally defined quantity must be capable of yielding consistent results on a given sample and always measuring essentially the same quantity on different samples. The indirect titration method for carboxyl group determination, as previously described, meets these criteria and yields reproducible values of operationally defined "carboxyl contents" for the sewage derived humic substances (SDHS) (see Table 8).

Table 8
Operational carboxyl content.

Humic Substance*	-COOH (meq/gm)
ASI-HA	1.3
ASI-FA	
ASE-HA	1.6
ASE-FA	2.6
ASCE-HA	1.5
ASCE-FA	2.0
TFE-HA	2.6

*See Tables 2 and 3 for abbreviations

The carboxyl contents of SDHS are quite low compared with those reported for other humic substances. The average carboxyl content of soil humic acids is 3.6 meq/gm or more than twice that found in the SDHS. (Schnitzer and Khan, 1972).

Similar analyses in this laboratory have shown that humic substances from soils and from water have typical carboxyl contents of 3.0 and 5.3 meq/gm, respectively. The SDHS are characterized, therefore, by rather low acidity.

The carboxyl contents of the activated sludge effluent and the chlorinated effluent HS are essentially equal and approximately 20% higher than those of humic substances isolated from the influent sewage.

As expected, the SDHS - fulvic acid fractions are more acidic than the corresponding humic acid fractions but still substantially less so than soil and even aquatic fulvic acids.

The carboxyl content of the TFE-HA is 62% greater than that of ASE-HA. The higher acidity of the trickling filter derived humic material is most likely related to differences in the biological environment of the treatment processes which lead to the formation of humic material. The details of these differences remain to be determined.

Total Acidity

Most workers agree that essentially all acidic hydrogens in humic substances react with 0.1M $Ba(OH)_2$ in the total acidity procedure described by Schnitzer and Gupta (1965) and by Schnitzer and Khan (1972). Furthermore, the phenolic hydroxyl content of humic substances is often calculated as the difference between this total acidity and the carboxyl content. The accuracy of the distribution of acidic functional groups is, therefore, inherently dependent on the accuracy of the methods used for "carboxyl content" and total acidity determination.

Although the $Ba(OH)_2$ method has been used by this laboratory to give reasonable estimates of the total acidity of river water and swamp soil derived humic substances, serious experimental problems resulted when the method was applied to the SDHS. After reacting with $Ba(OH)_2$ the suspension was filtered (under N_2) with a 0.45μ filter prior to back titration with HCl. In comparison to suspensions

with river water and swamp soil derived humus which filtered very readily, filtration of the suspension with sewage derived humus was extremely difficult as filter binding was severe. Titration of the filtrate yielded lower "total acidity" values than the previously determined carboxyl content. Essentially equivalent titrant was necessary to reach the pH 8.4 endpoint for the sample filtrate and the reagent blank thus yielding a "zero" total acidity. This problem was persistent for all trials on humic samples isolated from the activated sludge process. The filter-binding was not as prevalent when applying the total acidity method to humus isolated from the trickling filter process.

The $Ba(OH)_2$ method was repeated with filtration through Whatman #42 under nitrogen blanket. The filtrates were consistently cloudy. This procedure moreover, yielded extremely high total acidity values. For examples, the total acidity of ASE-HA was determined to be 8.9 meq/gm. The subtraction of 1.6 meq/gm of COOH (Table 8) results in an apparent phenolic -OH content of 7.3 meq/gm, which is more than four times higher than the COOH content. Calculations of theoretical upper limits for phenol content, based on elemental, NMR and amino acid data, show that a phenolic -OH content of this magnitude is highly unlikely.

Because of experimental problems encountered in the ration of the suspension, the $Ba(OH)_2$ method is not reliable for determining the total acidity of humic substances derived from activated sludge processes.

Direct Titration

"Net titration curves" (Figure 15), were derived by subtracting reagent blank titration from the original potentiometric titration data of the sample. This procedure eliminates the effect of titratable blank acidity and excess base dilution from the titration curves so that only actual base consumption by titratable acidic matter in the sample is displayed. The variable

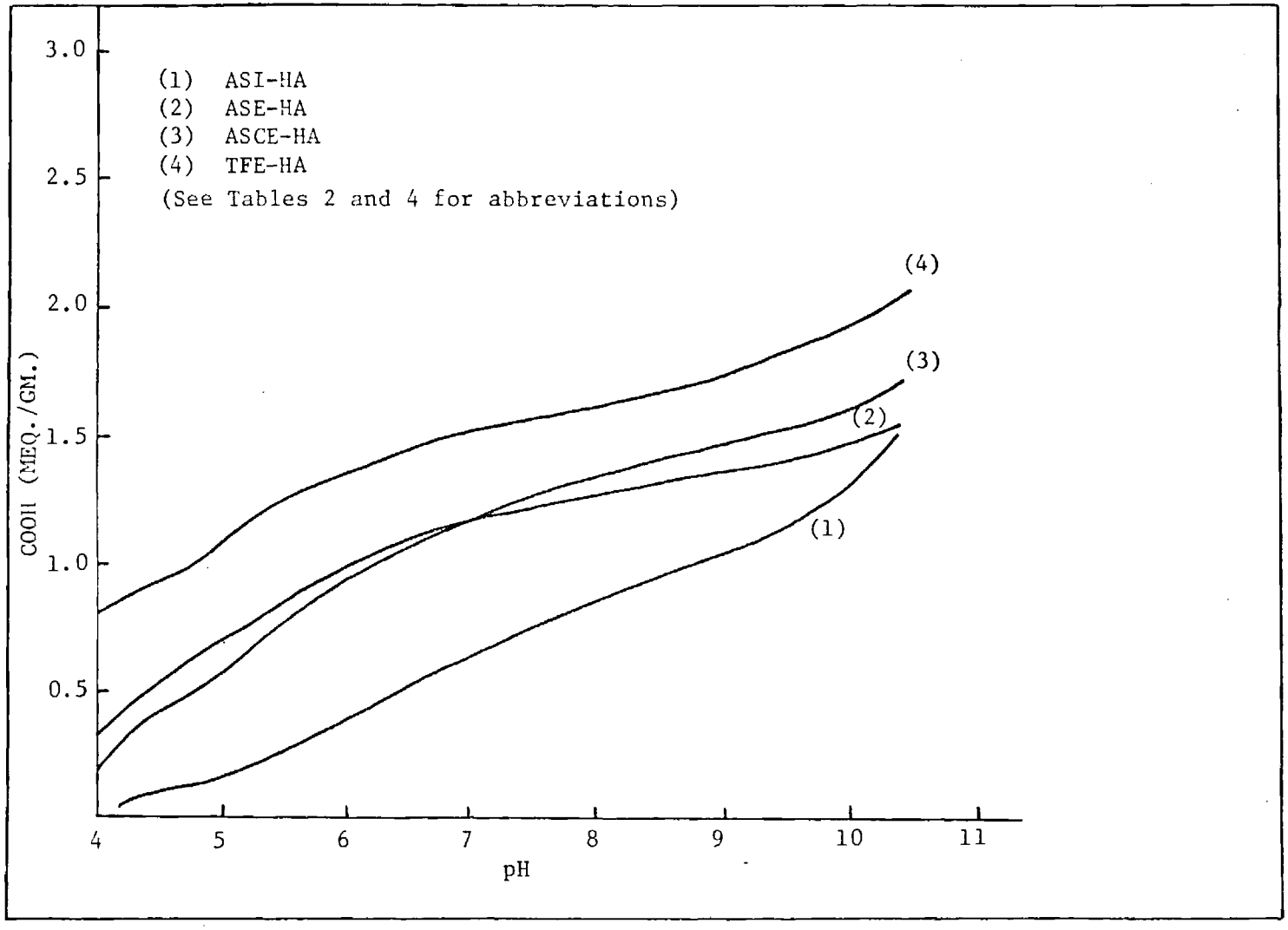


Figure 15. Net titration curves of humic acids.

plotted on the ordinate of these graphs (in meq/gm) is:

$$Y = \frac{(OH_a - OH) - (OH_a^* - OH^*)}{W}$$

where OH is the meq. of OH^- in solution after addition of OH_a meq. of base, W is the grams of humic substance, and (*) refers to the reagent blank titration. Direct potentiometric titrations of the SDHS with NaOH were conducted in 0.1M NaCl.

From the shape of the "net titration curves", it is apparent that acidic functional groups are continuously titrated even beyond pH 10. In none of the curves does the slope reach zero, thus precluding a quantitative evaluation of carboxyl content. Theoretically, the hydrolysis of carboxylate anions should result in an equivalence point at pH < 7. Carboxyl concentrations read directly from the titration curve at pH 7 (Fig. 15) are $\geq$ 1.1 meq/gm for ASE-HA and ASCE-HA, the carboxyl content is $\geq$ 1.5 meq/gm at pH 7. These values compare reasonably well with the "carboxyl content" determined by indirect titration (Table 8).

The direct titrations also indicate that less than 2.0 meq/gm of acidic functional groups have been titrated at pH 10.5. It is unreasonable to believe that 7.3 meq/gm of phenolic -OH groups, as determined by $Ba(OH)_2$ method on ASE-HA, remain to be titrated above this pH. The results from direct titrations strongly suggest the unreliability of the $Ba(OH)_2$ total acidity method for use with SDHS.

Calculations of a Theoretical Upper Limit for Acidic Functional Groups

Utilizing data from elemental, NMR and amino acid analyses it is possible to determine a theoretical upper limit of acidic functional groups in the SDHS (Perdue, E. M., Personal Communication).

The following example is based on data for the sample ASE-HA.

The O-content (%w/w) of sample ASE-HA is 28%. This is equivalent to 17.5 mmol O/gm ASE-HA. On the average, amino acids contain 2.8 mmol O/mmol amino acid. The sample (ASE-HA) contains an average of 4.3 mmol amino acid/gm. Therefore, approximately 4.3 x 2.8 = 12 mmol O is associated with amino acid moieties.

This leaves a maximum of 17.5 - 12 = 5.5 mmols O/gm ASE-HA available for COOH groups, which gives 2.74 mmol/gm ASE-HA as a maximal value.

These calculations establish an upper limit to the content of acidic functional groups in SDHS. A significant fraction of the oxygen is most likely associated with carbohydrate moieties, so that the upper limit of carboxyl groups can be better defined after the completion of the sugar analysis. The calculations indicate that, if the direct titration result of 1.6 meq/gm -COOH is reasonable, then 59% (0.95 meq/gm) of the -COOH group can be accounted for in aspartic and glutamic acid moieties. This leaves approximately 4.1 mmol/gm of oxygen available for carbohydrates, etc. This could yield as much as 0.8 mmol carbohydrate/gm of ASE-HA (or 13% w/w).

^{13}C-NMR data allow the calculation of an upper limit of phenolic -OH. It appears that the aromatic carbon concentration is too small to allow a significant concentration of phenolic hydroxyl groups.

3.4.5 Apparent Molecular Weight Distribution (AMW)

Elution patterns of UF retentates of humic and fulvic acids (isolated from influent, secondary effluent and chlorinated effluent) from Sephadex G-75 column are presented in Figure 16. While the elution patterns of hymic and fulvic acids from the influent and the effluent are almost identical, that of humic acid from the chlorinated effluent has a comparatively larger retention volume, indicative of smaller molecules.

TOC for the three fractions (see Ch. 2, Section 2.6.4) in three molecular weight ranges are presented in Tables 9 and 10. The fractions are I (MW > 50,000), II (MW 1000 - 50,000) and III (MW < 1000). 76% of humic

acid from the chlorinated effluent has AMW between 1,000 - 50,000 (see Fraction 2, Table 9) as opposed to the majority (>50%) of humic acid from the influent and effluent having AMW >50,000 (Fraction 1, Table 9). It appears that chlorination breaks the bigger molecules down to form intermediate sizes (Fraction 2, Table 9) and that Fraction 2 is reduced, while Fraction 3 increases(< 1000) (Table 9). The fulvic acid samples show an even distribution of the three fractions. These results are similar those published by Dewalle and Chian (1977).

Considerable amounts of humic substances were eliminated by the activated sludge process as evidenced by the decrease of humic substances (from 20.8 to 2.3 mg/L of carbon, Table 10) and to some extent by the chlorination process (from 2.3 to 1.1 mg/L of carbon, Table 10). The removal of humic substances during the activated sludge process was mainly due to the removal of high-molecular-weight humic substances (MW > 50,000) which decreased from 12.4% in the influent to 3.9% in the effluent by the activated sludge process (Table 10). According to Dewalle and Chian (1977) high-molecular-weight organic materials in effluent of aeration basin were responsible for the bioflocculation in clarifiers. The possible mechanism of removal of humic substances during activated sludge process may be flocculation and precipitation of the high-molecular-weight fraction (Fraction 1, Table 9) in final clarifiers, where the secondary effluent samples were collected.

Removal of carbon during chlorination may in part be due to oxidation to CO_2. In chlorination experiments of humic substnaces, it has been observed (see Section 2.11) that the product yields are very poor. This may partially be caused by loss of carbon as carbon dioxide due to oxidation. The other contributing factor may be the formation of halogenated compounds (like haloforms) which are lost by volatilization.

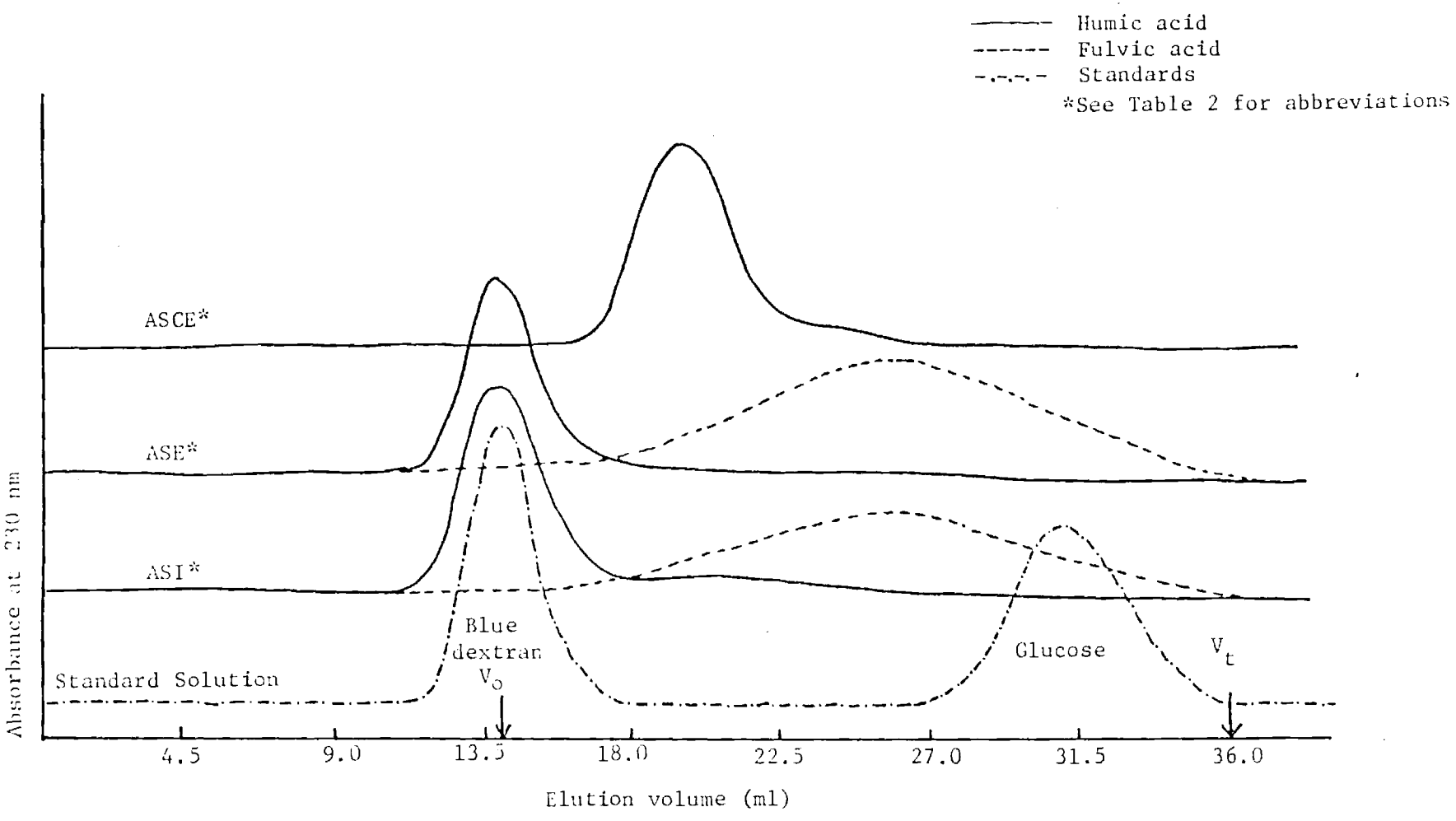


Figure 16. Elution patterns of humic and fulvic acids from influent, secondary effluent, chlorinated secondary effluent and standards.

Table 10

Apparent-molecular-weight (AMW) distribution of humic/fulvic acid from influent, secondary effluent, and chlorinated secondary effluent.

		MW Distribution (mg/l TOC)			
		MW>50,000	50,000>MW>1,000	MW<1.000	Total
Influent	HA	11.0	3.2	4.7	18.9
	FA	-	0.6	1.3	1.9
	Total	11.0	3.8	6.0	20.8
		(12.4)	(4.4)	(6.8)	(23.6)
Secondary Effluent	HA	0.7	0.4	0.3	1.4
	FA	-	0.4	0.5	0.9
	Total	0.7	0.8	0.8	2.3
		(3.9)	(4.0)	(4.1)	(12.0)
Chlorinated secondary effluent	HA	-	0.7	0.2	0.9
	FA**				0.2
	Total				1.1
					(11.0)

*Numbers in parenthesis indicate the percentage distribution of each fraction in influent, secondary effluent, and chlorinated secondary effluent.
**Not analyzed due to isolation of meagre quantity.

Table 9

Molecular-weight distribution of humic substances in influent, secondary effluent, and chlorinated secondary effluent as function of TOC.

		AMW Distribution (% of TOC)			
		GPC			UF
		Fraction 1 (>50,000)	Fraction 2 (50,000-1,000)	Fraction 3 (1,000-500)	Permeate (<500)
Influent	HA	58	17	3	22
	FA	-	32	27	41
Secondary effluent	HA	53	29	<1	17
	HA*	46	26	16	12
	FA	-	40	27	33
Chlorinated secondary effluent	HA*	<1	76	23	<1
	FA**				

*Done at pH=7.4 otherwise buffered at pH = 10.0.
**Not done due to isolation of meagre quantity.

3.4.6 Thermogravimetric Analysis

Plots of the rate of weight loss against temperature are presented for the humic acids in Figures 17 to 19. The principal peak stretches between 140 - 430°C, with two maxima (near 240° and 320°C) in each case. The majority of this broad peak lies below 400°C and is believe to be caused by the elimination of functional groups of aliphatic substances. The influent humic acid has a substantial peak with a maximum at 690°C.

^{13}C-NMR spectra indicate that the effluent and the chlorinated effluent contain about 10% aromatic carbon. The thermogravimetric analysis supports the ^{13}C-NMR data, because the weight loss above 400°C is small in comparison to that below 400°C. According to Schnitzer and Khan (1972, the main reactions governing the pyrolysis of humic and fulvic acids are (1)dehydration up to 200°C; (2) elimination of functional groups between 250° - 280°C; and (3) decomposition of the nuclei at a high temperature maximum (>400°C).

3.5 Amino Acids

The SDHS were analyzed for hydrolyzable acid content (see Table 11).

In the influent HA and both the effluent and chlorinated effluent HA, over 50% of the residues can be accounted for by five amino acids. The relative abundance in each type of HA is:

ASI-HA: Asp> Glu> Ala> Gly> Leu

ASE-HA: Asp> Phe> Gly> Ala> Glu

ASCE-HA: Phe> Asp> Ala> Glu> Gly

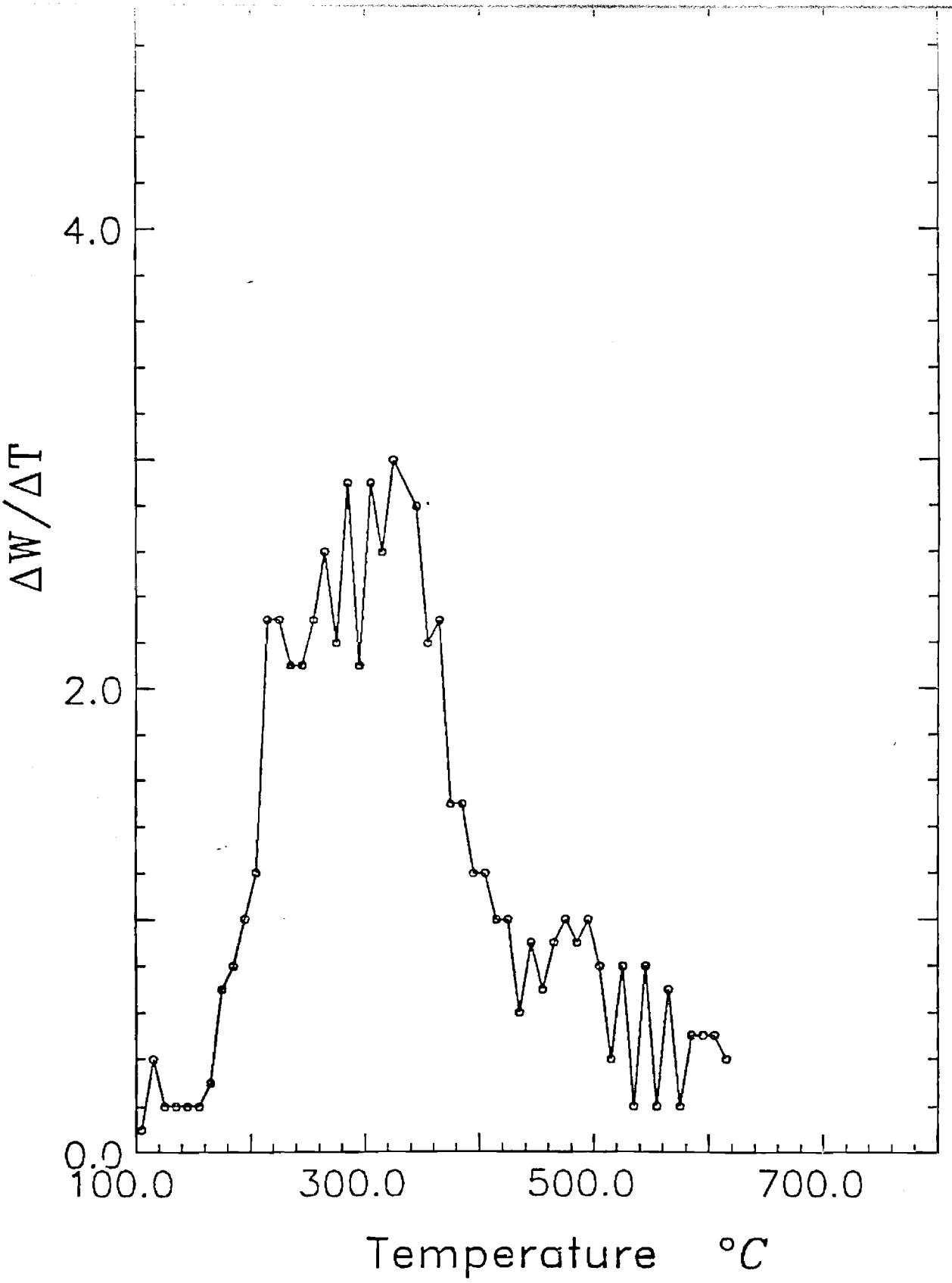


Figure 17. Marginal weight loss of effluent humic acid (TFE-HA) with temperature increasing at 10°C/min.

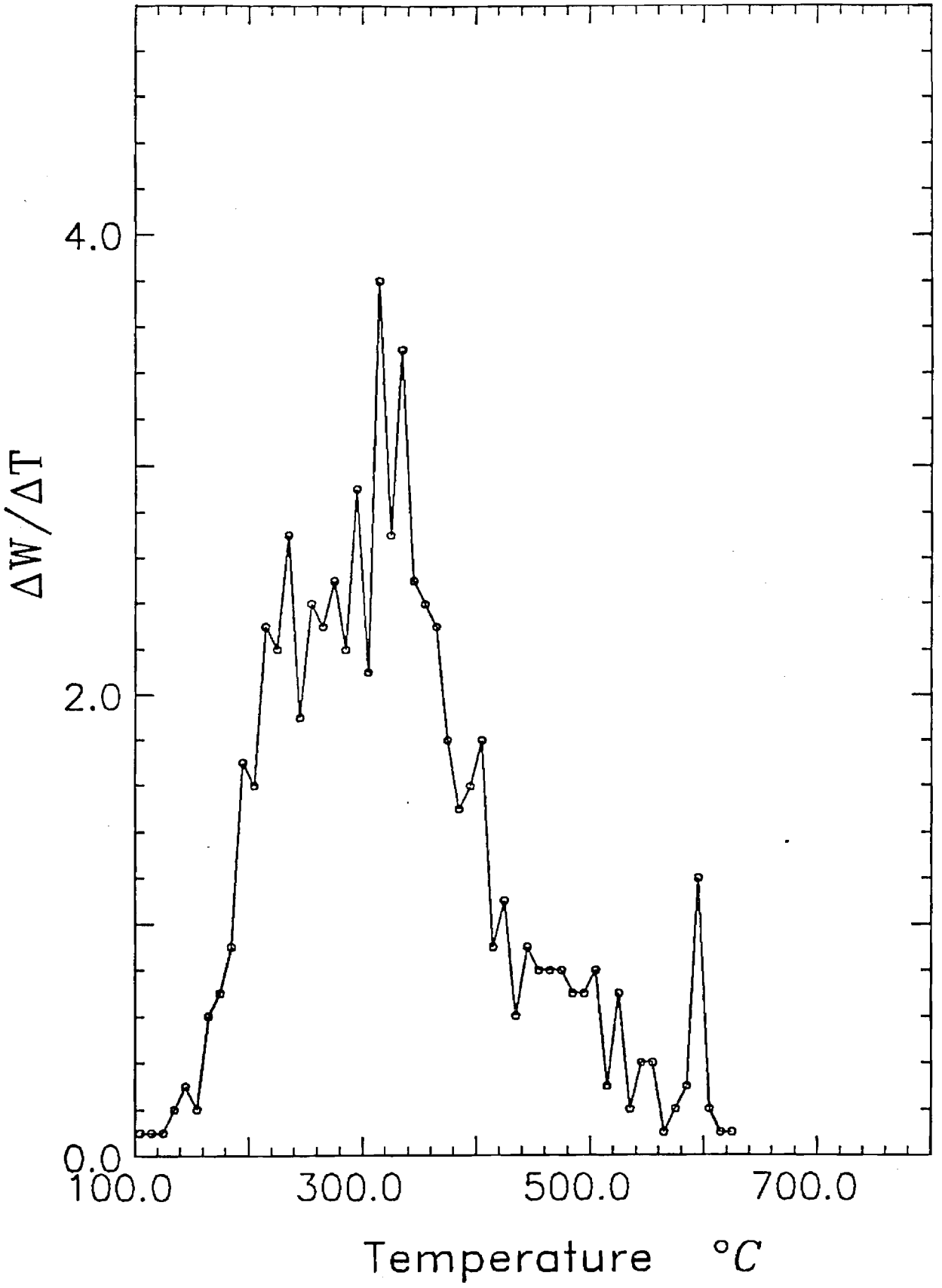


Figure 18. Marginal weight loss of effluent humic acid (ASCE-HA) with temperature increasing at 10°C/min.

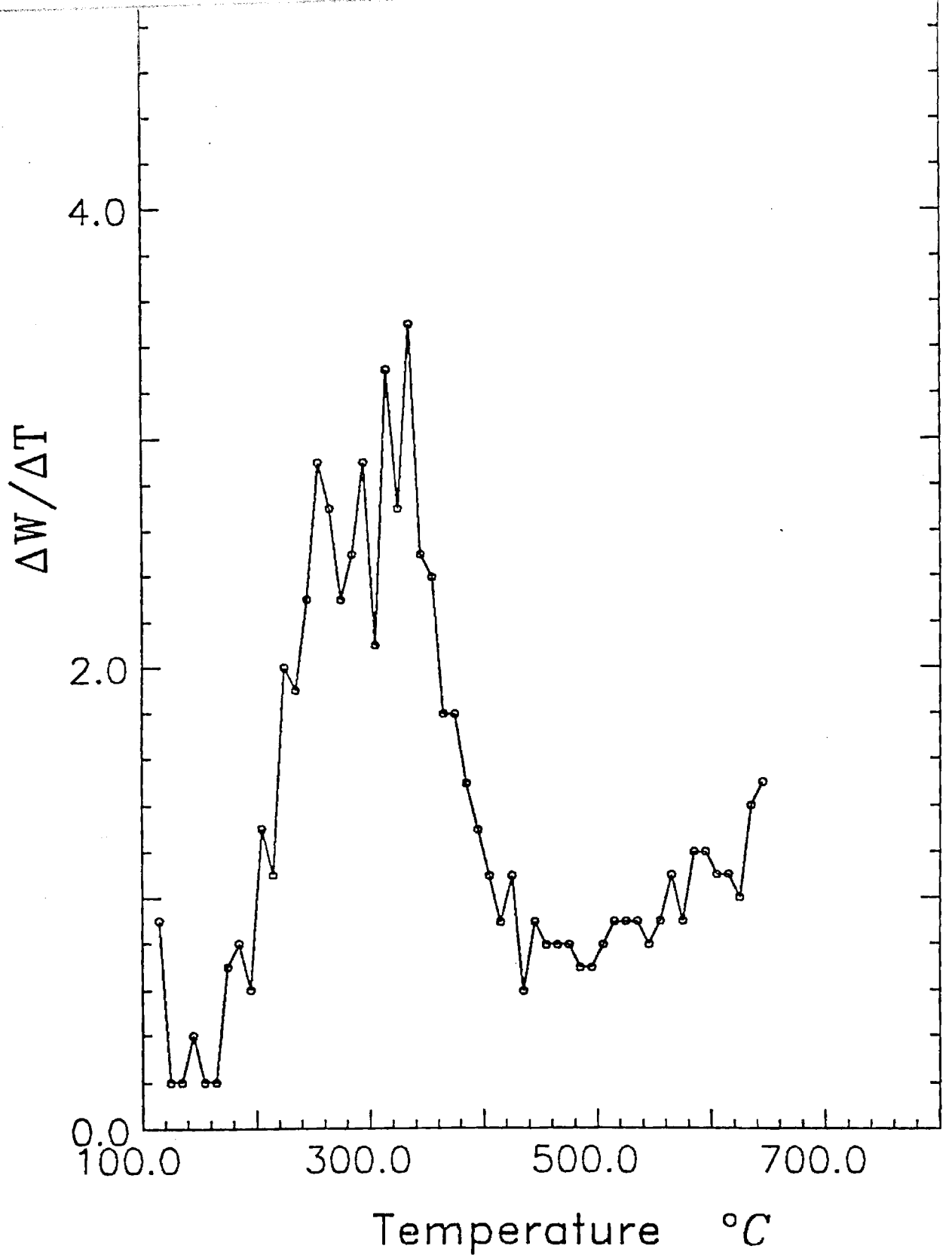


Figure I 9. Marginal weight loss of chlorinated effluent humic acid (ASE-HA) with temperature increasing at 10°C/min.

Table 11

Amino acid moieties in humic material residues/1000 residues

	ASE-HA*	ASCE-HA*	ASI-HA	ASI-FA
Lysine	42	41	55	10
Histidine	10	11	13	0
Arginine	36	42	39	0
Aspartic acid	121	114	115	112
Threonine	66	56	62	30
Serine	53	47	58	87
Glutamic acid	84	97	111	240
Proline	26	30	49	57
Glycine	110	96	98	187
Alanine	108	109	109	74
Valine	70	73	77	64
Methionine	14	4	0	0
Isosleucyne	44	50	54	48
Leucyne	72	82	88	65
Tyrosine	25	20	31	4
Phenylalanine	119	128	41	22

*Average of duplicate samples.

Glycine, alanine, aspartic acid and glutamic acid are often the dominant amino acids in bacterial cells and are expected to occur in SDHS. The substantial amount of phenylalanine in the two effluent derived HA is striking. The presence of this amino acid in the effluent HA is probably indicative of the flourishing biological activity that takes place in the activated sludge process. Amino acid analysis of hydrolyzed biomass from the aeration system is planned. This should indicate whether phenylalinine is an important amino acid in this biological system.

Only thirteen amino acids were detected in the hydrolysate of the ASI fulvic acid. Over 50% of the residues were distributed among only three amino acids Glu>Gly>Asp.

Table 12

Amino acid content in mg/gm of humic substances

Amino Acid	Sample			
	ASI-HA	ASI-FA	ASE-HA*	ASCE-HA*
Lys	19.1	0.5	28.1	27.0
His	5.0	-	6.6	7.4
Arg	16.2	-	28.4	32.3
Asp	36.3	5.5	75.7	68.8
Thr	17.6	1.3	35.8	29.6
Ser	14.6	3.4	25.5	22.4
Glu	35.2	11.7	50.8	57.8
Pro	13.6	2.4	13.6	15.4
Gly	17.7	5.2	38.2	32.0
Ala	23.2	2.5	44.4	43.6
Val	21.5	2.8	37.6	38.3
Met	-	-	9.2	3.1
ILeu	17.1	2.3	26.9	29.0
Leu	27.6	3.2	43.2	48.0
Tyr	13.4	0.3	20.9	15.9
Phe	16.2	1.3	90.8	96.4
Total mg/gm	294.9	42.4	575.7	567.0
Total %	29.5	4.2	57.6	56.7
**% of Total N in HS is AA Nitrogen	63.6	18.1	85.7	96.7

* Average of duplicate runs. Average difference between the two runs is less than 2%.
** Assumed average N content of all the amino acids is 13.8%.

HS = Humic Substances HA = Humic Acid FA = Fulvic Acid

Table 12 presents the amino acid content of the SDHS as mg/gm of HS. Approximately 57% (by weight) of the effluent HA can be accounted for as amino acid residues. In contrast only 29%(w/w) of the influent can be accounted for as HA hydrolyzable amino acid residues. It appears likely that the biological activity of the treatment process is the source of the proteinaceous material found in the humic substances. The ASI fulvic acid contains much less proteinaceous material. Only about 4 (w/w) of the ASI-FA consist of hydrolyzable amino acid moieties.

As previously indicated, elemental analysis shows that the effluent HA contains a rather high percentage of nitrogen. Of this nitrogen about 86% and 97% can be accounted for as amino acid nitrogen in the ASE-HA and ASCE-HA, respectively, whereas about 64% of the nitrogen in the influent HA is amino acid nitrogen.

Amino acid analyses of the SDHS hydrolysates show an important difference between influent and effluent humic acids. It is apparently the biological activity of the activated sludge treatment process that is responsible for the large fraction of proteinaceous material in the effluent humic materials.

Aromatic Carbon and Phenolic -OH Content of ASE-HA

From ^{13}C-NMR spectrometry (Table 7), it is estimated that 8% of the carbon is ASE-HA is aromatic (C_{ar}). Since 53% (w/w) of ASE-HA is carbon, it follows that there are 3.5 mmol C_{ar}/gm ASE-HA. The phenylalanine content of ASE-HA accounts for 0.55 mmol/gm ASE-HA, and the tyrosine content for 0.11 mmol/gm ASE-HA. The total of 0.66 mmol/gm of (Phe + Tyr) contains 3.96 mmol C_{ar}/gm ASE-HA. This means that 100% of the C_{ar} estimated by ^{13}C-NMR can be accounted for in the two amino acids residues Phe and Tyr. Therefore, an upper limit for phenolic -OH content in ASE-HA is that associated with the Tyr moiety (0.11 mmol/gm).

-COOH Content

The carboxyl content in aspartic acid and glutamic acid residues account for

3.7 Acidic Fraction

A number of aliphatic and aromatic acids, including three phthalates (priority pollutants) were detected in the influent of the activated sludge plant. These are listed in Table 13. Tables 14 and 15 present the acids detected in the effluent and the chlorinated effluent of the activated sludge plant. All of them contain bis(2-ethylhexyl)phthalate, which is the most abundant pollutant. Only two other long chain acids were detected in the effluent and the chlorinated effluent.

Table 13 .
List of organic acids detected in the acid fraction (III) of AS influent.

Aliphatic acids
Hexanedioic acid, methyl,ethylester
Octanedioic acid, dimethylester
Nonanedioic acid, dimethylester
Tetradecanoic acid, methylester
Undecanoic acid, trimethyl, methylester
Pentadecanoic acid, methyl, methylester
Hexadecanoic acid, methylester
Aromatic Acids
Benzeneacetic acid, methylester
Benzeneacetic acid, ethylester
Benzoic acid, methoxy, methylester
Benzenepropanoic acid, methoxy, methylester
Benzoic acid, dimethyoxy, methylester
Dimethylphthalate
Dibutylphthalate
bis(2-Ethylhexyl)phthalate

Table 14.
List of organic acids detected in the
acid fraction (III) of AS effluent.

1. Methyl undecanoate
2. Unknown
3. bis(2-Ethylhexyl)phthalate

Table 15.
List of organic acids detected in the
acid fraction (III) of AS chlorinated effluent.

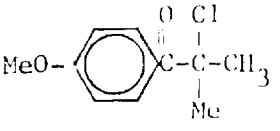
1. Pentadecanoic acid, 14-methyl-, methylester
2. Heptadecanoic acid, 16-methyl-, methylester
3. bis(2-Ethylhexyl)phthalate

3.8 Neutral Fraction

The compounds present in the neutral fraction of the secondary effluent and chlorinated effluent can be divided mainly into three chemical groups:

1. Hydrocarbons
2. Alcohols
3. Methylesters

It is interesting to note that all the compounds present in the secondary effluent are also present in the chlorinated effluent. In addition, the latter contains a host of other compounds including a chlorinated ketone



(1-(4-methoxyphenyl)-2-methyl-2-chloropropanone-1). All identifications are tentative on the basis of mass spectrum. Lists of compounds identified in the effluent and the chlorinated effluent are presented in Tables 16 and 17, respectively.

Table 16.
List of neutral organic compounds detected in the neutral fraction (IV) of the AS effluent.

Hydrocarbons:

*Cyclopentane, 2-ethyl-1,1-dimethyl
Scan 719

*Docosane

Alcohols:

*Heptadecanol

Esters:

Dodecanoic acid, methylester
Tetradecanoic acid, 2-methyl-,methylester
*bis(2-Ethylhexyl)phthalate

Amide

*Dodecanamide

*5 largest peaks

Table 17.
List of neutral organic compounds detected in the neutral fraction (IV) of the AS chlorinated effluent

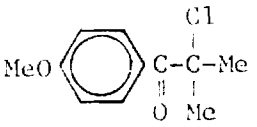
Hydrocarbons:
- Pentane,2,2,3,4-tetramethyl
- *Cyclopentane, 2-ethyl-1,1-dimethyl
- Scan 719
- *Docosane

Alcohols:
- Isooctanol
- (4-methylphenyl)octanol
- Scan 650
- *Heptadecanol

Esters:
- Dodecanoic acid, methylester
- Diethylphthalate
- Tetradecanoic acid, methylester
- Tetradecanoic acid, 2-methyl-,methylester
- *Pentadecanoic acid, 14-methyl-,methylester
- *bis(2-Ethylhexyl)phthalate

Amide:
- Dodecanamide

Chlorinated compound:
- 1-Propanone,2-chloro-1-(4-methoxyphenyl)-2-methyl



*5 largest peaks.

0.95 mmoles COOH/g ASE-HA, out of 1.1 mmol/g found by direct filtration.

3.9 Characteristics of Chlorinated River Water Humus

The infrared spectrum and differential thermal analysis (DTA) curves of the chlorinated river water humus is presented in Figures 20 and 21, respectively, along with those of river water humus and a sample of humus isolated from drinking water supply of Philadelphia (Philly 1).

Infrared Spectral Analysis The prominent absorption in the 1600 cm^{-1} region in river water humus is absent in the drinking water sample (Philly 1). Chlorination definitely causes the infrared pattern to change towards a pattern seen in Philly 1, mainly due to the reduction of adsorption in the 1600 cm^{-1} region (as is enhanced and the adsorption bands in the fingerpring region (1500-900 cm^{-1}) have become remarkably similar to Philly 1.

Differential Thermal Analysis

The chlorinated sample has exotherm maxima at nearly the same temperatures as exhibited by Philly 1. Both profiles are different from that exhibited by the river water humus. This observation suggests that the chlorination reaction changes the structure of the aquatic humus to a comparable structure seen in Philly 1.

3.10 Metals

To determine the total metal content in the influent and effluent streams, two 500 ml samples of the influent, effluent and chlorinated effluent, collected at the activated sludge facility, and centrifuged to remove particles greater than 0.45μ diameter were evaporated to near dryness on the rotary evaporator. One sample was digested by Method I, the other by method II. Table 18 presents the maximum metal concentration in μg/l as determined by either of the two digestion methods. For the influent and effluents the metals occur in the following order of abundances: Al Fe Cu An, with low levels of Cr and Pb in all samples. Removal of Al and Fe through the treatment system is very efficient, i.e. 97% and 93%, respectively, whereas the removal of both Cu and Zn is 44%

Table 18

Total Metal Concentraton (µg/l)

	Al	Fe	Cu	Zn	Cd	Cr	Pb
Influent	4600	1210	88	41	0.05	4.4	2.2
Effluent	1440	340	49	17	0.04	2.2	2.2
Chlorinated Effluent	140	90	48	23	0.03	2.4	0.6

Table 19

Metal Concentration of Ultrafilter Permeate (µg/l)

	Al	Fe	Cu	Zn	Cd	Cr	Pb
Influent	70.00	16.00	0.33	18.33	0.03	0.66	1.50
Effluent	84.19	27.20	0.38	49.22	0.01	1.16	1.29
Chlorinated Effluent	137.50	22.50	0.62	26.25	0.10	1.75	0.62

Table 20

Metal Content of Isolated Humic Acids (mg/gm)

	Al	Fe	Cu	Zn	Cd	Cr	Pb
AS1-HA	1.73	3.76	0.07	0.16	<0.01	0.01	0.05
ASE-HA	0.72	10.66	0.16	0.12	<0.01	0.03	0.04
ASCE-HA	0.92	23.27	0.05	<0.01	<0.01	0.01	0.02

This suggests that two different removal pathways are at work. A majority of the Al and Fe is probably lost with the removal of clay colloids, while the removal pathways for Cu and An appears to be more associated with organic matter.

To estimate the fraction of metal content which is not associated with colloids or higher molecular weight organic matter, a given volume of sample (300-400ℓ) was passed through a UM-2 (Amicon Corp., Lexington, MA) ultrafilter (molecular weight cut off of 1000 Dalton) after adjusting to pH 6-7; under 75 psi of nitrogen pressure. A measured volume of permeate was evaporated in a rotary evaporator. The final volume was adjusted to 50 ml. Metal analyses were done on the solutions without preliminary digestion. The results are given in Table 19. In the influent about 98% Al, 99% Fe and 99+% Cu appear to be complexed withcolloidal or organic material with molecular weight greater than 1000 Dalton. In contrast only 56% of the zinc in the influent is similarly complexed. In the effluent 94% Al, 92% Fe, and 98% Cu are complexed with colloids or higher molecular weight compounds. An anomalously high concentration of zinc was found in the ultrafilter permeate of the effluent.

Chlorination has an apparent effect on aluminum speciation. Essentially all of the aluminum in the chlorinated effluent sample passed through the ultrafilter, showing that, in contrast to the unchlorinated effluent, none of the aluminum is complexed. Chlorination has a similar but less profound effect (92% - 75%) on iron and essentially no effect on copper. Therefore, in the final plant effluent (chlorinated) approximately 75% of the iron and 99% of the copper are complexed with colloids and/or higher molecular weight organic material. Aluminum and zinc, in contrast, do show similar behavior.

It is not possible to determine to what degree the metals are partitioned between clay colloid and organic matter complexes. However, to estimate the metal which is "tightly" bound to the SDHS, approximately 25 mg of the isolated humic acid samples were digested by both Methods I and II. Table 20 presents the maximum metal concentration from either of the two methods in mg metal/gm HA.

Since the isolated HA has been acidified, extracted, and desalted by cation exchange resin, only the very "tightly" complexed metals are detected. The most abundant metal found in all three HA's is iron. The second most abundant metal is Al, whereas, Cu and Zn are present in approximately equal but lesser amounts in comparison to the UF permeate.

The seven metals analyzed in this study make up, by weight, 0.6%, 1.2% and 2.4% of the ASI-HA, ASE-HA and ASCE-HA, respectively. When related to the ash contents presented in the elemental analysis, it follows that 6% of the ASI-HA ash, 33% of ASE-HA ash, and 60% of the ASCE-HA ash consist of these metals.

The observation that aluminum and iron is substantially concentrated in the effluent after chlorination suggests that the chlorination reaction preferentially attacks portions of the HA which are relatively unimportant in forming strong metal complexes.

Based on the amount of HA isolated from the chlorinated effluent sample, the approximate concentration of ASCE-HA is 700 mg/l. If 2.4% of this HA is metal then 16.8mg/l or about 5.5% of the total metal concentration in the chlorinated effluent is strongly complexed with the HA. Of course it is obvious that a great majority of the metal associated with the HA is iron. About 24% of the total iron in the chlorinated effluent which does not pass the UM-2 filter is complexed with the HA. Therefore, 76% of the iron and essentially all of the other metals which do not pass through the UM-2 filter are complexed with clay colloid and/or "loosely" complexed with humic material and subsequently lost in the HA isolation and deslating procedures.

5. CONCLUSIONS

5. CONCLUSIONS

1. The fulvic acids isolated from the ASE and ASCE streams were in liquid form. Even after repeated purification, the small amount of solids obtained showed high ash, sulfur and halogen contents, while carbon was found to be low in comparison to soil and aquatic humic substances.

2. The sewage derived himic substances (SDHS) show lower carboxyl contents than soil and aquatic humic substances. Determination of total acidity by the $Ba(OH)_2$ method did not provide meaningful results.

3. The SDHS are lower in aromatic carbon as evidenced by ^{13}C-NMR spectra and supported by (TGA).

4. Approximately 64%, 86% and 97% of nitrogen content respectively in ASI, ASE and ASCE humic acids can be accounted for as amino acid nitrogen. The biological activity of the activated sludge treatment process leads to an effluent richer in proteinaceous material than the influent humic acid. It indicates a substantial incorporation of amino acid moieties into humic substances during the process.

5. Chlorination is accompanied by loss of carbon and breakdown of the higher apparent-molecular-weight (AMW) fractions to lower molecular weights.

6. The treatment process eliminates most of the humic substances in the influent. However, it appears that there is a process of simultaneous destruction of the influent humic substances and recreation or modification of it during the process, because the structural characteristics of the influent HA and the effluent HA are not identical.

7. Metals are "tightly" complexed in the SDHS with iron being the predominant specie followed by aluminum and lower amounts of copper, zinc, chromium and lead. Although iron has approximately 20% of the total complexed iron "tightly" bound to HA, essentially all of the other bound metals are complexed

with clay colloids or "loosely" complexed with HA and removed during isolation and desalting procedures.

8. Chlorination is accompanied by an increase in the relative concentration of metal, especially iron, in the HA. This suggests that chlorination reactions preferentially attack portions of HA which are relatively unimportant forming "tight" metal complexes.

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