

APPARENT MOLECULAR WEIGHTS OF ORGANICS IN
SECONDARY TREATED WASTEWATER EFFLUENTS

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ABSTRACT

A study regarding the composition of secondary treated sewage effluents based on their apparent molecular weight (AMW) was conducted. Effluents from two domestic wastewater treatment facilities, Lake George, NY (a trickling filter system) and Clifton Knolls, NY (a contact stabilization system), were collected for investigation. The sewage effluents were membrane filtered (0.45 μ pore size) and then concentrated by freeze-drying. Sephadex gels G-10, G-15, and G-25 were employed for chromatographic separation. The efficiency of organic carbon recovery for the three Sephadex columns was 85.1 to 97.8%. There was neither sorption of organic carbon on the column nor any dissolution of dextran fractions from the gels. An apparent molecular weight was assigned to each organic fraction. In the concentrated effluents approximately 60% of the organics were of an AMW < 700 and 25% of the organics were of an AMW > 5000.

INTRODUCTION

Organic nutrients in secondary treated sewage effluents can provide a mode for accelerated biostimulation in receiving waters. Little has been reported regarding chemical composition or characterization of organics in sewage effluent from primary and secondary wastewater treatment plants. Bunch, et al., (1961), analyzed secondary treated effluents from two trickling filter plants and three conventional activated sludge treatment facilities. Sixty-five percent of the soluble organics were unidentified. The identified fractions were labelled as ether extractable matter, proteins, carbohydrates, polysaccharides, tannins and lignins, and alkyl benzene sulfonates. Hunter and Heukelekian (1965) analyzed raw sewage and separated the organic, into two fractions; particulate and soluble fractions. The soluble fraction was further divided into ethyl ether extractable matter (mostly organic acids) and ether insoluble matter containing amino acids. Rebhun and Manka (1971) studied the composition of soluble organics in effluents from a high rate trickling filter plant and reported the following component makeup: ether extractable ~ 8.3%, anionic detergents ~ 13.9%, carbohydrates ~ 11.5%, proteins ~ 22.4%, tannins ~ 1.7%, and humic substances 40-50%. Zuckerman and Molof (1970) reported that the effluent from an activated sludge treatment plant contained soluble organic material only of molecular weight greater than 1200. The identification of various organics in treated wastewater effluents with respect to their molecular weights has not received adequate attention. This study has attempted to identify the organics in treated effluents from trickling filter

and contact stabilization plants according to their apparent molecular weights.

METHODS OF PROCEDURE

Effluents were collected from the Lake George (New York) Water Pollution Control Plant (a trickling filter treatment system) and the Clifton Knolls (New York) Water Pollution Control Plant (a contact stabilization system). These two conventional biological treatment facilities operate primarily for the treatment of domestic sewage. Approximately 120 liters of effluent, collected from each facility, were membrane filtered (0.45 μ pore size, Millipore Corporation, Bedford, Mass.) to rid the samples of as much abiotic and biotic particulate matter as possible.

The filtered samples were concentrated by freeze-drying to obtain a sufficient amount of organics for chromatographic separations. A Virtis mechanical freeze-drying unit (Model No. 10-146 MR-BA; Virtis Company, Inc., Gardiner, New York) was used employing methyl alcohol as a bath fluid. No artificial heat was supplied. The primary object was to maintain the integrity of the sample during concentration. Certain methods (e.g., solvent extraction, vacuum evaporation, distillation, etc.) which involved contact with other chemicals or required elevated temperatures, were considered incompatible with research objectives.

Sephadex gels G-10, G-15, and G-25 were employed for chromatographic separations of the concentrate. A fraction collector (Radirac Model 3401 B, LKB Instruments, Inc., Rockville, Md.) was employed to collect 5 ml fractions. The number of fractions to be collected were

calculated based on the bed volume of each column. A Beckman Model 315 Carbonaceous Analyzer was used to determine organic carbon concentrations in the samples examined. The Sephadex columns were standardized with solutions containing a mixture of dextran blue (M.W. ca. 2×10^6) raffinose (M.W. 594.5), dextrose (M.W. 180.0) and methanol (M.W. 32.0). Apparent molecular weights (AMW) of various organic fractions from each column were calculated from the standard curves of that column. Details of these methods are explained elsewhere (Sachdev, 1973).

RESULTS AND DISCUSSION

Concentration by Freeze-Drying - The results of effluent concentration from both the Lake George and Clifton Knolls sewage treatment facilities are seen in Table I.

The organic carbon recovery of the freeze-dried samples was 92.3% and 96.2%. These values are in close agreement with those of others (McDonald, 1971; Kumar, 1971; and Greene, 1972). Since the concentrated effluent contained some insoluble material, they were membrane filtered and the organic carbon content of the filtrate and the retentate were determined. The data are presented in Table II.

The organic carbon in the retentate accounts for 24.4% and 26.9% in the two concentrated effluents. The loss of organic carbon is 3.2% (Lake George) and 2.3% (Clifton Knolls). This loss might have occurred during drying of the retentate. The soluble organics in the concentrated effluents were 72.4% and 70.8% respectively (Table II). McDonald (1971)

TABLE I

ORGANIC CARBON RECOVERY OF THE FREEZE-DRYING METHOD FOR CONCENTRATION OF
EFFLUENTS

Item	Lake George Sewage Treatment Plant	Clifton Knolls Sewage Treatment Plant
Volume of Effluent	121.5 liters	120.0 liters
Organic Carbon in Total Effluents	1051.6 mg	1337.3 mg
Volume of Concentrate	1177.0 ml	1072.0 ml
Organic Carbon in Concentrate	971.0 mg	1286.4 mg
Recovery of Organic Carbon	92.3 %	96.2 %
Concentration factor	103	112

TABLE II

ORGANIC CARBON IN FILTERED CONCENTRATE AND SOLID MASS

Facility	Organic Carbon in Concentrated Effluent mg	Organic Carbon in Membrane Filtered Concentrated Effluent mg	Organic Carbon in Solid Mass Retained on Filters mg	Percent Recovery	Percent Loss
Lake George Sewage Treatment Facility	971.0 (1177 ml)	703.7 mg (1091 ml) (72.4%)	236.6 mg (24.4%)	96.8%	3.2%
Clifton Knolls Sewage Treatment Facility	1286.40 mg (1072 ml)	911.40 mg (980 ml) (70.8%)	346.1 mg (26.9%)	97.7%	2.3%

and Kumar (1971) reported their percentages to be 79 to 92% and 70.5% respectively. Gjessing and Lee (1967) concentrated lake waters by evaporation under reduced pressure and reported recoveries of 60 to 95% of the organics in the concentrate. It should be noted that method of concentration of the sample and the concentration factor would affect the percentage of the solid mass in a concentrate.

Gel Permeation Chromatography - A number of runs through each chromatographic column were performed to ensure reproducibility of the results.

The effectivity of each column was determined by calculating the equivalent height of one theoretical plate (EHTP), i. e., column height divided by the number of theoretical plates. The EHTP values for the columns were:

Sephadex G-10 column, 0.77 mm; Sephadex G-15 column, 0.64 mm, and Sephadex G-25 column, 0.99 mm. Hall (1970) reported an EHTP for Sephadex G-25 column as 1.19 mm. Determann (1968) reported EHTP values, for Sephadex G-25 column, obtained by various researchers as 0.39 mm to 5.49 mm depending upon the test substance and flow rates. EHTP is a measure of the effectivity of a column and the lower this value is the more efficient the column will be. An AMW was assigned to each component collected from the Sephadex column by using the calibration curve developed for that column.

Fractionation of the Concentrated Effluents

Sephadex G-10 Column: Concentrated Lake George sewage effluent was applied to a Sephadex G-10 column. Five ml fractions were collected and analyzed for organic carbon. The resulting chromatogram containing

these data is shown in Figure 1. Four such separate runs were conducted. In each case five well-defined peaks were obtained which were similar and the elution volume of a peak in each of the four runs did not differ by more than one fraction number. Although not reported herein, removal of the gel from the column and repacking the column with the same gel did not result in any modifications of the results shown. One of the several evaluations regarding experimental reproducibility consisted of examining the possible sorption of organic compounds on the column packing or elution of some soluble fractions of the dextran by eluant; the organic carbon recovery from the Sephadex G-10 column was calculated for the four runs. As seen in Table III the organic carbon recovery from this column varied from 91.1% to 97.8%.

Similarly, the concentrated Clifton Knolls sewage effluent was fractionated on a Sephadex G-10 column. Once again five well-defined peaks (Figure 2) were obtained and the organic carbon recovery varied from 92.5% to 96.8%. In both these instances the organic carbon recovery values strongly suggest that there was neither any sorption of these organic compounds, present in the concentrates, on the Sephadex G-10 column, nor was there any elution of any soluble fractions from the Sephadex gel.

In order to obtain a sufficient amount of each organic fraction, a number of chromatographic separations were made of each concentrated effluent. These organic fractions were then concentrated via freeze-drying for further research.

In order to establish that concentration had not affected the stability

TABLE III
 ORGANIC CARBON RECOVERY OF SEPHADEX G-10 COLUMN FOR
 THE CONCENTRATED LAKE GEORGE EFFLUENT

Run No.	Organic Carbon Applied (mg/l)	Organic Carbon in all the Fractions (mg/l)	Percent Recovery of Organic Carbon
1	4.50	4.3	95.6%
2	4.50	4.4	97.8%
3	4.50	4.1	91.1%
4	4.50	4.1	91.1%

of the organic fractions, the concentrated frontal fraction (G-10-1) of each effluent was rechromatographed on the Sephadex G-10 column. In each case only one peak was obtained thus indicating that the contents had not been affected.

Depending upon their elution volumes an AMW was assigned to each organic fraction (see Table IV). The proportion of each organic fraction as a percentage of the total effluent was calculated based on the area under the fraction in the chromatogram. These values are displayed in Table IV. No AMW was assigned to organic fraction G-10-IV and G-10-V as the elution volume of these organic fractions was greater than the elution volume of methanol (the lowest molecular weight standard; M.W. 32). McDonald (1971) obtained five fractions on a Sephadex G-10 column. No AMW was assigned by him to fraction IIIA, IIIB and IV.

Sephadex G-15 Column. The concentrated frontal peaks (G-10-I) from the Lake George and Clifton Knolls effluents were separately applied to a Sephadex G-15 column. The resulting chromatograms are shown in Figures 3 and 4, respectively. In each case, two organic fractions were obtained and an appropriate AMW was assigned to each organic fraction (Table IV).

Also, the organic carbon recoveries from this column were determined for both these effluents. These varied from 92.5% to 97.0% for the Lake George frontal peak (G-10-I) and 96.6% to 104.7% for the Clifton Knolls frontal peak (G-10-I). The proportion of each organic fraction as percentage of the whole effluent was calculated as explained earlier (Table IV).

TABLE IV

SUMMARY OF RESULTS

(Apparent Molecular Weight and Effluent Percentage of Each Organic Fraction)

Sephadex Gel	Organic Fraction Number	<u>Lake George Concentrated Effluent</u>		<u>Clifton Knolls Concentrated Effluent</u>	
		Percent of Fraction in Concentrated Effluent %	Apparent Molecular Weight	Percent of Fraction in Concentrated Effluent %	Apparent Molecular Weight
Sephadex G-10	I	37.8	> 700	41.5	> 700
	II	33.7	430	31.6	310
	III	7.1	117	6.3	100
	IV	7.3	Undefined	14.6	Undefined
	V	6.7	Undefined	1.6	Undefined
Sephadex G-15	I	20.4	> 1500	28.8	> 1500
	II	17.4	1200	12.7	750
Sephadex G-25	I	<u>20.4</u>	> 5000	<u>28.8</u>	> 5000
	TOTAL	92.6		95.6	

Sephadex G-25 Column. The Sephadex G-15 frontal peaks (G-15-I) from Lake George and Clifton Knolls effluents were concentrated by freeze-drying and then separately applied to a Sephadex G-25 column. Figures 5 and 6 show the resulting chromatograms. In each case only one organic fraction was obtained. An AMW was assigned to this fraction and its proportion as a percentage of the whole effluent was determined (Table IV). The organic carbon recovery was 86.6% to 93.6% (Lake George) and 85.1% to 89.5% (Clifton Knolls).

The gel filtration research showed that the organic carbon recovery for the three Sephadex columns varied from 85.1% to 97.8% except in one run on a G-15 column wherein the recovery for the Clifton Knolls G-10 frontal peak was 104.7%. These values indicated that there was neither sorption of organic carbon on the column (because the recovery values were approximately 90%), nor was there any dissolution of dextran fractions from the gel (there being no increase in organic carbon recovery). The increase in organic carbon recovery in only one run may be explained due to instrumental error (carbon analysis) or personal error in calculating areas under each peak. There is no possibility of dissolution of dextran fractions as this phenomenon was never observed in this column or any other column. Hall (1970) reported high recovery values (101% to 114.7%) for Sephadex G-75 columns and noted that the high values could be due to leaching of soluble dextrans from the column but possibly were the result of cumulative error in analyzing 25 to 30 fractions. Gjessing and Lee (1967) reported 87 to 95% organic recovery from G-75 column and 100% recovery from other columns

(G-200, G-100, G-25, and G-10). Greene (1972) reported organic carbon recovery of 90 to 107% from Sephadex G-15 columns.

As indicated in Table IV, the secondary treated sewage effluents consisted of groups of compounds of different apparent molecular weights varying from less than 100 to more than 5000. The organic fraction (G-25-1) with an AMW greater than 5000 was not further fractionated and presumably contained compounds of varying molecular weights (all greater than 5000). These compounds (with AMW > 5000) formed 20.4% and 28.8% of the dissolved organic matter in the Lake George (a trickling filter system) and Clifton Knolls (a contact stabilization system) concentrated effluents respectively. Organic compounds with AMW less than 700 made up 62.2% (Lake George) and 58.8% (Clifton Knolls) of the soluble organics in the effluents. McDonald (1971) reported that 69% of the organic carbon in the sewage effluent from an activated sludge plant he investigated, consisted of organic compounds with AMW less than 700 whereas 18% of the organic compounds were of AMW greater than 30,000. Bunch, et al, (1961) analyzed effluents from different treatment plants and concluded that only 40% of the compounds were of high molecular weight. Greene (1972) noted that more than 90% of the soluble organics in municipal wastewater had AMW less than 1500. He further stated that after chemical treatment and activated carbon adsorption the soluble organic component was primarily of low molecular weight (less than 1500). Kumar (1971) reported that 18% of the organic compounds in the effluent from a contact stabilization plant were with AMW greater than 1500 and 59% were with AMW less than 500. Thus, observation

reported herein are in accord with their data.

However, Zuckerman and Molof (1970) reported that the effluent from an activated sludge treatment plant contained organic material having molecular weight greater than 1200. These results are in conflict with those obtained in this study as well as the others mentioned. The measurement of organics was performed by Zuckerman and Molof (1970) using a low level automated C.O.D. analysis technique. Hall (1970) compared the results of organic carbon (measured by a Leco Carbon Analyzer) and C.O.D. (measured by Technicon Autoanalyzer) in various fractions from a Sephadex G-75 column and reported that the C.O.D. procedure measured 60 to 70% of the carbon in these fractions. Also, he measured C.O.D. values by the procedure recommended by Standard Methods (1965) and by the autoanalyzer. The values obtained by using procedures outlined in Standard Methods were consistently higher, averaging 1.3 times the autoanalyzer values. Gjessing and Lee (1967) noted that 30 to 40% of the organic carbon present in their fractions was measured by the autoanalyzer C.O.D. procedure. Zuckerman and Molof (1970) used unconcentrated samples for chromatographic separation. The treated wastewater from Lake George had 8.66 mg/l-C organic carbon and that from Clifton Knolls had 11.14 mg/l-C organic carbon. Due to low organic carbon contents in treated sewages and the autoanalyzer reportedly measuring 30 to 70% of the organic carbon, it is possible that full detection of the organic carbon in the fractions collected by Zuckerman and Molof was not achieved. In view of the results by many other investigators, referred to above, which are in essential agreement with this study, the

results of Zuckerman and Molof (1970) do not appear to be tenable.

From Table IV, it is evident that organic compounds with AMW between 1500 (exclusion limits of Sephadex G-15) and 5000 (exclusion limit of G-25) were not existing in any of the two sewage effluents investigated. Also, McDonald (1971) did not report any organic fraction with AMW between 1000 and 5000.

CONCLUSIONS

The results of this investigation have led to the following conclusions:

1. Freeze-drying is an efficient method of concentrating organics in sewage effluents. The organic carbon recovery was 92.3% to 96.2%.
2. Gel permeation chromatography is a powerful tool for fractionation of sewage effluents.
3. In concentrated Lake George sewage effluent 62.2% of the soluble organics were of AMW < 700 and 20.4% > 5000. Similarly in the case of Clifton Knolls sewage effluent the organic compounds of AMW < 700 formed 58.8% of the soluble organics and 28.8% of the organic compounds were of AMW > 5000. In secondary treated domestic effluents most of the organic compounds are of molecular weight less than 700.
4. There were no organic compounds with AMW between 1500 and 5000 in both Lake George and Clifton Knolls concentrated effluents.
5. The organic carbon recovery from the Sephadex gel columns was 85.1% to 97.8%. There was neither sorption of organics on the gels nor was

there any dissolution of dextran fractions from the gels.

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- Figure 1: Chromatogram of Concentrated Lake George Sewage Effluent on Sephadex G-10. The volume of sample used was 7.0 ml (645 mg C/l). Column specifications: 80.1 cm x 2.5 cm; 75 ml/hr.
- Figure 2: Chromatogram of Concentrated Clifton Knolls Sewage Effluent on Sephadex G-10. The volume of sample used was 10.0 ml (930 mg C/l). Column specifications: 80.1 cm x 2.5 cm; 75 ml/hr.
- Figure 3: Chromatogram of Frontal Peak G-10-I of Lake George Effluent on Sephadex G-15. The volume of sample used was 2.4 ml (835 mg C/l). Column specifications: 82.2 cm x 2.5 cm; 67 ml/hr.
- Figure 4: Chromatogram of Frontal Peak G-10-I of Clifton Knolls Effluent on Sephadex G-15. The volume of sample used was 2.0 ml (1050 mg C/l). Column specifications: 82.2 cm x 2.5 cm; 67 ml/hr.
- Figure 5: Chromatogram of Frontal Peak G-15-I of Lake George Effluent on Sephadex G-25. The volume of sample used was 2.0 ml (560 mg C/l). Column specifications: 76.6 cm x 2.5 cm; 80 ml/hr.
- Figure 6: Chromatogram of Frontal Peak G-15-I of Clifton Knolls Effluent on Sephadex G-25. The volume of sample used was 3.0 ml (760 mg C/l). Column specifications: 76.6 cm x 2.5 cm; 88 ml/hr.











