ORGANIC NUTRIENT FACTORS EFFECTING ALGAL GROWTH
- PHASE I PROGRESS REPORT -

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INTRODUCTION

The main purpose of the present study is to ascertain and identify those organic compounds extant in treated secondary waste water effluent which have an effect on algal growth. It was, therefore, of importance to select an effluent for investigation, which would give representative data for secondary waste water effluent. With this in mind, the following criteria were set as guidelines for the selection of the sampling site:

1) Low effluent organic content, i.e., an efficiently operated control facility.

2) A pollution control facility that was utilizing a form of treatment applicable for present as well as future practices.

Three facilities were selected for investigation:

1) Eastern-Western Sewage Treatment Plant - An extended aeration treatment system serving approximately 80 single-family residences located in the County of Albany, New York State.

2) Coxsackie Reformatory Sewage Treatment Plant - A trickling filter plant located at the State Reformatory, Coxsackie, N. Y.

3) City of Batavia Activated Sludge Treatment Plant - A modern conventional activated sludge plant at Batavia, N. Y.

After some deliberation it was decided that the first two of the above mentioned effluents did not best fall within the boundaries of the criteria for the present study so Batavia effluent was selected and is presently used as the main source of a sample supply.

PRELIMINARY TREATMENT

It was realized that in a study of this nature utmost care should be taken to maintain the integrity of the sample under investigation.

The following procedures were devised to limit possible alteration of the sample organic content.

Samples were collected and returned to the laboratory immediately after collection. Upon arrival at the laboratory, the sample was placed directly in a constant temperature room (4°C), and an aliquot of the waste taken for a total organic carbon analysis (Beckman Total Carbon Infrared Analyzer). Periodically, throughout the study the organic carbon of the wastewater was rechecked for possible effects of storage. No change in organic carbon was noted throughout the storage period.

The procedure of continuous centrifugation for separation of particulate and dissolved matter was investigated and found to be less than desirable. It was found that during centrifugation the temperature of the sample was increased significantly and it was felt the increase would be deleterious to efforts at maintaining the effluent in its original state.

As a substitute for the above, a large membrane filter apparatus (142 mm diameter) was employed and the effluent filtered using a 0.45 μ (avg. pore size) membrane filter. Filtration was carried out at a rate of approximately 20 liters/hour. Once filtered the effluent was again placed in the cold room.

After separation of particulate (0.45 μ) and soluble forms, a concentration step followed. At first thin film rotary evaporation procedures were attempted. The experimental apparatus included a Cal-Lab Thin Film Evaporator which utilized an ethylene glycol cooled vapor trap. The ethylene glycol was maintained at or below 10°C with the aid of a Forma

cold finger. However, this procedure was soon discontinued. The apparatus required continuous monitoring to insure proper operation, especially the constant sample delivery accessory. When left unattended the system was erratic and on several occasions, overnight concentration attempts resulted in complete filling of the apparatus including the high vacuum pump with sample water. In addition to operational difficulties it was necessary to raise the temperature of the sample to approximately 34°C to ensure reasonable evaporation rates. It was again felt that these elevated temperatures would be deleterious to sample composition. It was felt that under these circumstances another more reliable and less troublesome concentration procedure should be sought.

The second and present concentration procedure utilized was freeze-drying. A Virtis 10-liter freeze-dryer has been purchased fitted with 16 special 3/4" inlet ports to allow a greater rate of sublimation. The apparatus as used is capable of concentrating 8 liters of effluent during one run. However, it was found that for best operation 5 liters was the optimum load on the system. The average rate of concentration using the above system with 10 sample flasks was 5 liters per 24 hours.

The concentration procedure as presently developed is as follows: first, the effluent to be concentrated is filtered through a 0.45 μ membrane filter and transferred to ten 1200 ml freeze-drying flasks. Each flask contains a measured 500 ml volume of sample. The sample flasks are then sequentially shell-frozen in a dry-ice-acetone bath (-61°C) and placed on the ports of the concentration apparatus. A McLeod gage is integrated into the system to give an overall value for the vacuum of

the system. Using a high volume Welch Duo-Seal Vacuum pump we were able to maintain a vacuum of 10 μ mercury throughout the operation except during the one-minute equilibration period following sample application when a maximum of 100 μ mercury was noted. Initially some difficulty was encountered with the system due to a problem with the flask glass connectors and the Virtis filters supplied with the flask caps. The connectors were of thick pyrex glass and in concert with the filters constricted the water vapor mean free path. This defect caused a melt-back phenomenon which essentially ruined the experiment. The substitution of larger inside diameter connectors and the deletion of the filters eliminated the difficulty and no further problems have arisen with the system.

Once the 500 ml samples had been reduced to approximately 50-75 ml, another 500 ml of wastewater was added to each flask and the composite sample shell-frozen and concentrated. This sequence was repeated until a sufficient volume of effluent (30.5 liters) had been concentrated. TABLE I gives a representative sampling of recovery for freeze-drying versus thin-film rotary evaporation.

After termination of the concentration step, the samples were composited and the soluble fraction (0.45 μ) separated from the particulate via membrane filtration. The particulate fraction was then acidified with 0.1 N HCl. This fraction was labeled acid-soluble organic component.

PROCEDURES AND INITIAL INVESTIGATIONS

The original methods of choice for the fractionation of the organic matter in the effluent were (1) separation of the dissolved organics from

the inorganic components in the effluent via ion exchange resins, and (2) fractionation of the organic components of the concentrate by gel permeation chromatography.

Preliminary experimentation with the ion exchange procedure proved unsatisfactory in that, although, removal of the inorganic fraction was successful a major portion of the effluent organics was also lost due to sorption into the resin. Therefore, the ion exchange step was eliminated and the fractionation procedures narrowed to gel permeation chromatography.

A standard chromatographic column, 100 cm x 2.5 cm, fitted with upward flow adaptors (Pharmacia Fine Chemicals) was used to optimize the resolution of the fractionation procedure. During the present phase of investigation, Sephadex G-10, G-25, and G-50, manufactured by Pharmacia Fine Chemicals, Upsula, Sweden, have been employed. Preliminary investigation showed that the use of another gel, Bio gel, an acrylic base polymer would be unsatisfactory.

The technique established for sample application and fractionation with gel permeation chromatography was as follows: each column, fitted with specially designed upward flow adaptors, has the lower, or bottom adaptor connected to a 3-way stopcock which allows the addition of either, 0 to 10 ml. of sample, or eluent (glass distilled water). The column effluent was collected in 5 ml fractions with the aid of an LBK Radi-Rac fraction collector. The organic carbon content of the fractions was monitored using a Beckman Carbonaceous Analyzer. Examples of

typical chromatograms of the organic concentrate using this procedure are shown in Figures 1, 2 and 3. Additional data regarding the composite fractions obtained through chromatographs are also given in TABLE II.

Concurrent with the organic fractionation portion of this study has been the establishment of an algal growth culture room. Sufficient equipment has been purchased and built to allow the simultaneous growth of 96 continuously stirred batch cultures and 48 bubbling tubes. Two irradiance values are in use for the cultures (550 ft.-candles and 150 ft.-candles). Presently the three organisms prescribed in the Provisional Algal Assay Procedure are being monitored using a modification of the Basic ASM media.

TABLE I

Effluent	Concentration Method	Total Organic Carbon In Orig. Sample (mg.)	Total Organic Carbon In Soluble Organic Component (mg.)	% Recovery Soluble Organic Component	Total Organic Carbon In Acid Soluble Component	% Recovery Total
Eastern-Western STP	Thin-film Rotary Evaporation	84	57	68	---	---
Coxsackie Reformatory STP	Thin film Rotary Evaporation	33	20.1	61	---	---
Batavia STP (1-22-69)	Freeze-Drying	213.5	190	94.5	25	100%
Batavia STP (3-17-69)	Freeze-Drying	360	284	79	14	83
Batavia STP (12-14-68)	Freeze-Drying	42	38	92	2.5	97

TABLE II

Fraction	Organic Carbon Conc. mg/l	pH	Conductivity mho/cm	Percent of Original Effluent Organic Carbon Content
G-10 I	120	5.6	850	30
G-10 II	100	8.2	2,400	35
G-10 III A	60	7.1	20,000	11
G-10 III B	30	7.7	380	5
G-10 IV	17	6.3	34	3
G-25 I	64	7.1	56	22
G-25 II	39	6.4	5.7	5.1
G-50 I	23	6.4	8.5	19

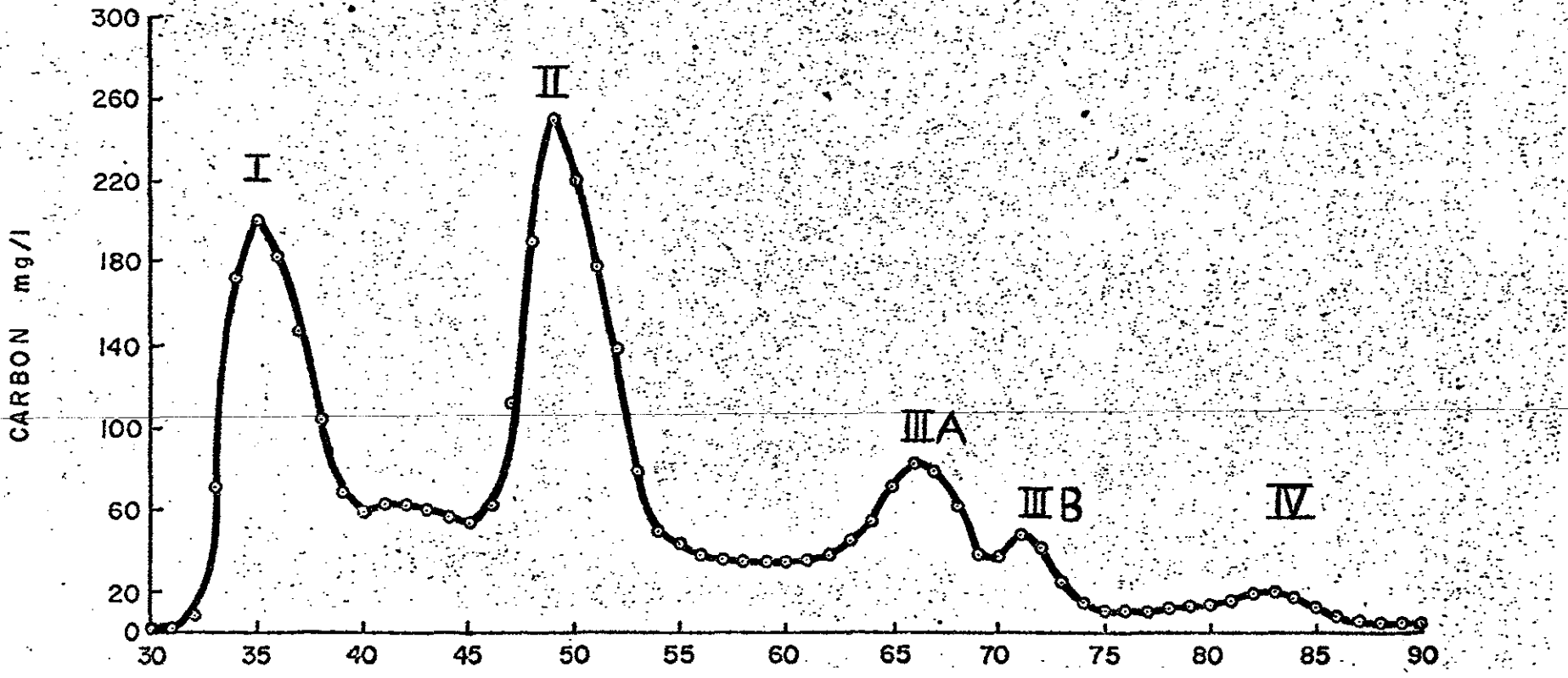

Figure 1. Fractionation of Batavia (1-22-69) Concentrate on Sephadex G-10. Sample Volume: 10 ml, Bed Dimensions: 2.5x85 cm, Flow Rate: 50 ml/hr, Eluant: Glass Distilled Water.

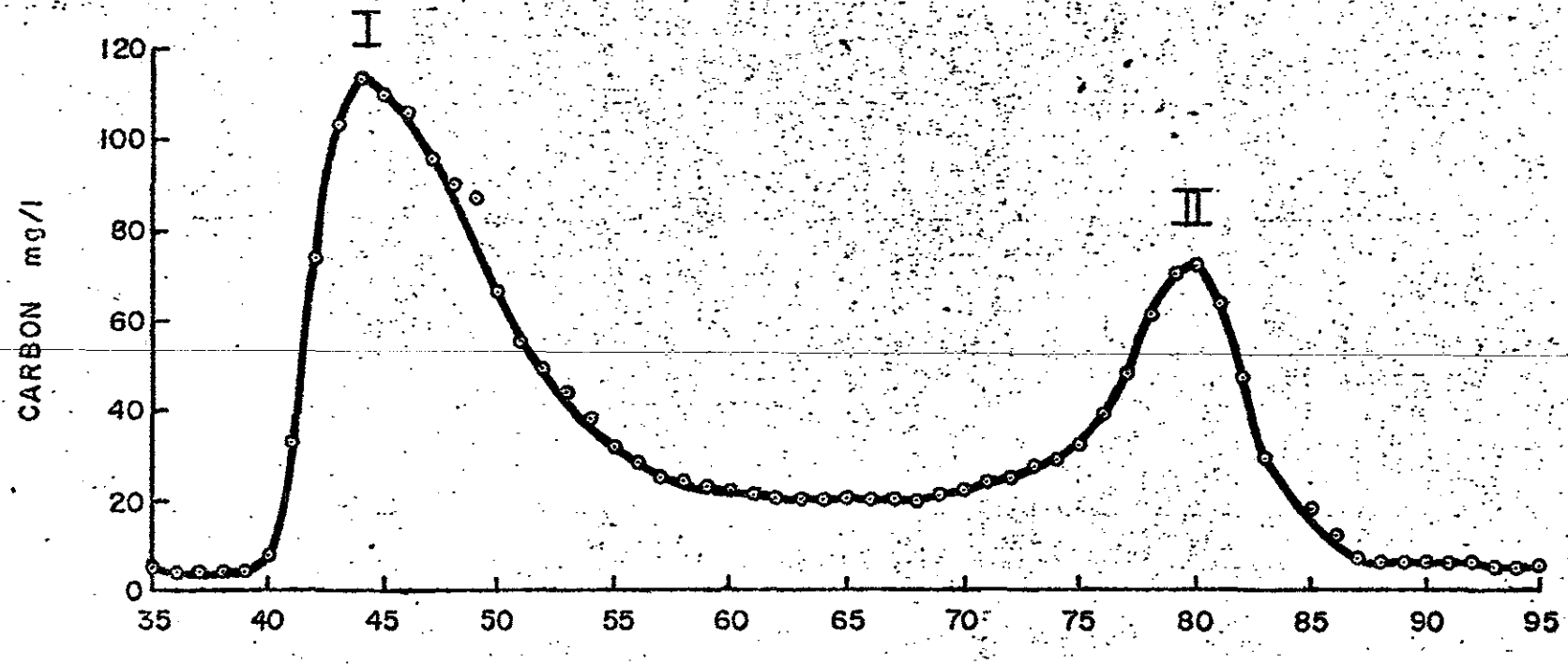

Figure 2. Fractionation of Composite Fraction G-10-I on Sephadex G-25. Sample Volume: 10 ml, Bed Dimensions: 2.5x90 cm, Flow Rate: 135 ml/hr, Eluant: Glass Distilled Water.

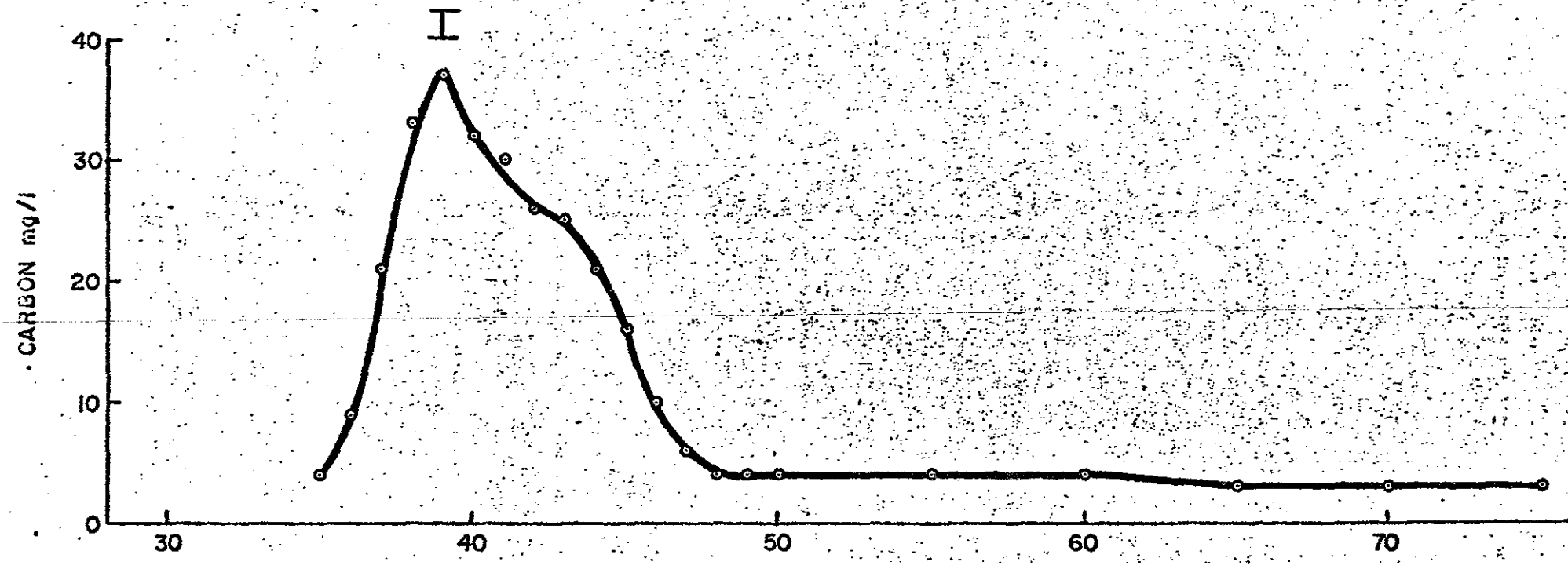

Figure 3. Fractionation of Composite Fraction G-25-I on Sephadex G-50. Bed Dimensions: 2.5x90 cm, Flow Rate: 120 ml/hr, Sample Volume: 5 ml, Eluant: Glass Distilled Water.