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27. EFFECT OF WASTEWATER ORGANIC FRACTIONS ON THE GROWTH OF SELECTED ALGAE

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With the rise of the Industrial Revolution the accelerated aging of the natural water resource has become increasingly evident. This phenomenon has, in part, been attributed to the unchecked discharge of man-made wastes.[1-5] The agents within such wastes responsible for the increased algal productivity, indicative of such phenomena, have been investigated extensively. Nitrogen and phosphorus have received the main attention, but the results elicited to date have not fully explained the occurrences of increased productivity solely in light of these entities.[6-11] The investigations have produced grounds for speculation and hypotheses requiring further serious study. To date, a specific aspect of wastewaters, namely, the possible stimulatory effects produced by organic components, has received little attention. The work outlined herein describes an attempt to determine the existence and extent of algal growth enhancement brought about by the addition of wastewater organic fractions to representative algal cultures.

MATERIALS AND METHODS

The objective of this investigation was to ascertain the effects of organic wastewater fractions on the growth rate of selected algae. To this end a sample of effluent from a conventional

activated sludge facility located at Batavia, New York was subjected to fractionation using gel chromatographic techniques. Prior to such fractionation the sample was membrane filtered (0.45 μ) and concentrated by freeze drying to insure its usefulness for further experimentation. The latter technique was carried out using a 10 liter Virtis large port freeze dryer specially equipped with sixteen 3/4" ports to allow for an increased rate of sublimation (Virtis Co., Gardiner, New York). Preliminary studies with locally available wastewater clearly demonstrated that this concentration technique did not cause a change in the basic character of the wastewater, *i.e.*, did not result in selective organic carbon losses. Figure 122 displays chromatographic data relative to one such study.

Subsequent to filtration and freeze drying, the concentrated wastewater was separated into organic fractions through the use of gel chromatography. The chromatographic apparatus used was utilized in a 4° C cold room. The gel columns, 2.5 by 100 cm (K25/100 Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey), were fitted with up-flow adapters to optimize the resolution of the separation procedure. Five ml eluent fractions were collected and analyzed for total organic carbon (TOC) content with a Beckman infra-red carbonaceous analyzer, model 315.

The gels used for separation of the organic fractions were Sephadex G-10, G-25 and G-50 (Pharmacia Fine Chemicals, Inc.). Separation ranges for such gels, in terms of molecular weight are 0-700 (G-10), 1000-5000 (G-25) and 1000-30,000 (G-50). Before introducing the wastewater concentrate the columns were standardized with compounds of known molecular weight. Based on these standardizations apparent molecular weights (AMW) were assigned to the fractions from the wastewater concentrate.[12-14]

The chromatographic procedure involved the application of the wastewater concentrate to the G-10 column in 10 ml passes and the subsequent compositing of similar fractions produced in each of the runs. The composite G-10 frontal peak (AMW > 700) was then reconcentrated using the freeze drying technique and applied to the G-25 column. The fractions resulting from this operation were composited in the same manner as the G-10 fractions.

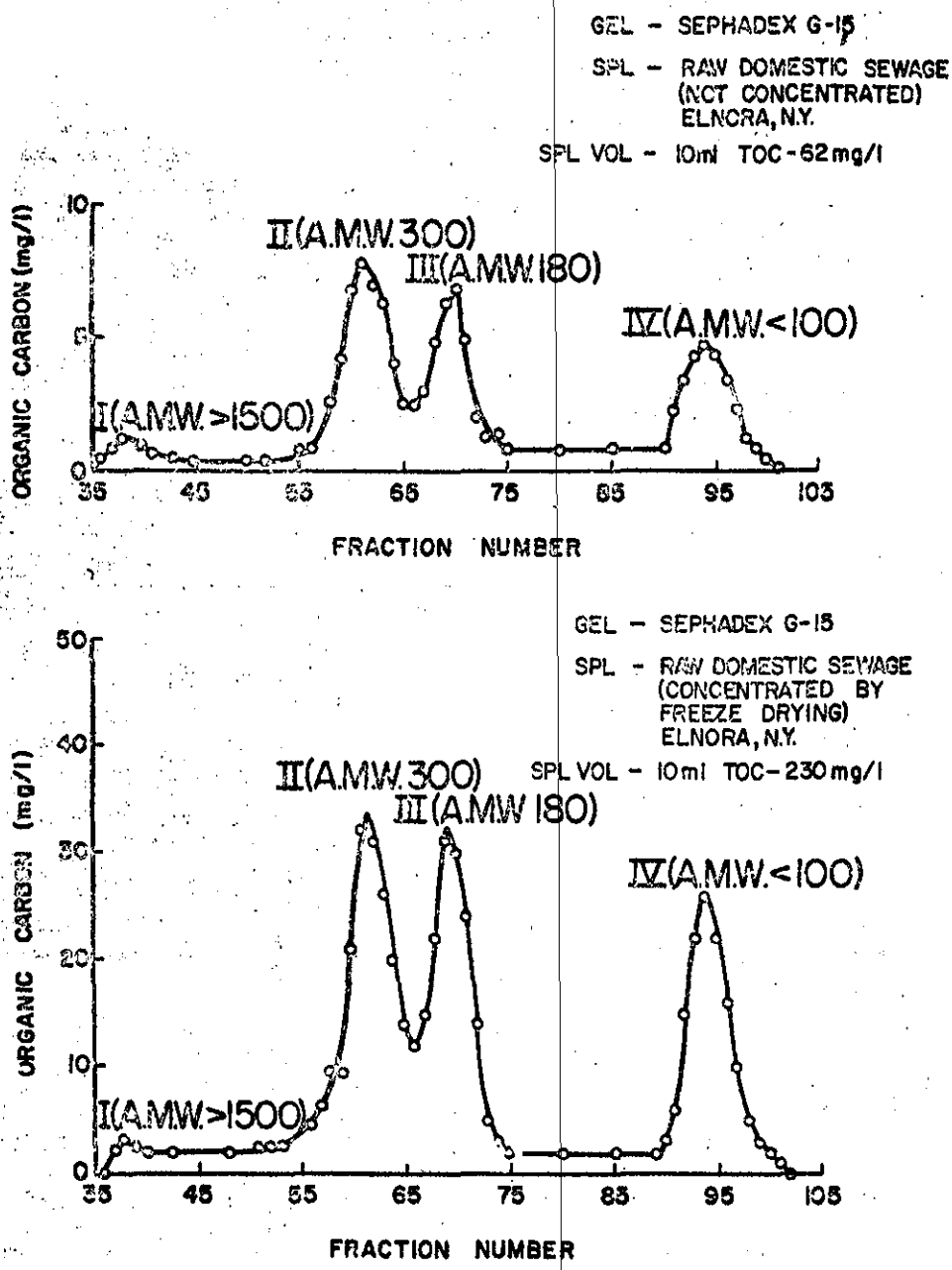

Figure 122. Elution diagrams of the fractionation of unconcentrated and concentrated samples of raw domestic sewage from Elnora, New York on Sephadex G-15.

The G-25 composite frontal peak was in turn concentrated and used as the G-50 column sample. Displayed in Figures 123, 124, 125 are typical chromatograms produced during these chromatographic procedures. The composite fractions were examined for selected physical and chemical properties. The data relative to such examinations are as shown in Table 65. Conductivity measurements were of special interest as they would afford a means of assessing whether or not the causative factor for any possible growth enhancement was organic in nature.

Table 65

Selected Parameters for Wastewater Fractions

Fraction	*Total Organic Carbon Concentration mg/l*	*pH*	*Conductivity µmhos/cm*	*Apparent Molecular Weight*
G-10 I	120	5.6	850	> 700
G-10 II	100	8.2	2400	250
G-10 IIIa	60	7.1	20,000	*
G-10 IIIb	30	7.7	380	*
G-10 IV	17	6.3	34	*
G-25 I	64	7.1	56	> 5,000
G-25 II	39	6.4	5.7	1,000
G-50 I	23	6.4	8.5	>30,000

* indefinable

Algae selected for use in the study were *Selenastrum capricornutum* and *Anabaena flos-aquae* obtained from the Pacific Northwest Water Laboratory, Environmental Protection Agency, Corvallis, Oregon. Both species were maintained as continuously stirred cultures in 100 ml of Basic ASM medium in 250 ml Erlenmeyer flasks fitted with plastic foam plugs. This medium was the modification of the ASM of McLachlan and Gorham[16] originally proposed for use in the Provisional Algal Assay Procedure. The medium was modified[15] through a reduction in the concentration of K_2HPO_4 from 17.4 mg/l to 3.48 mg/l,

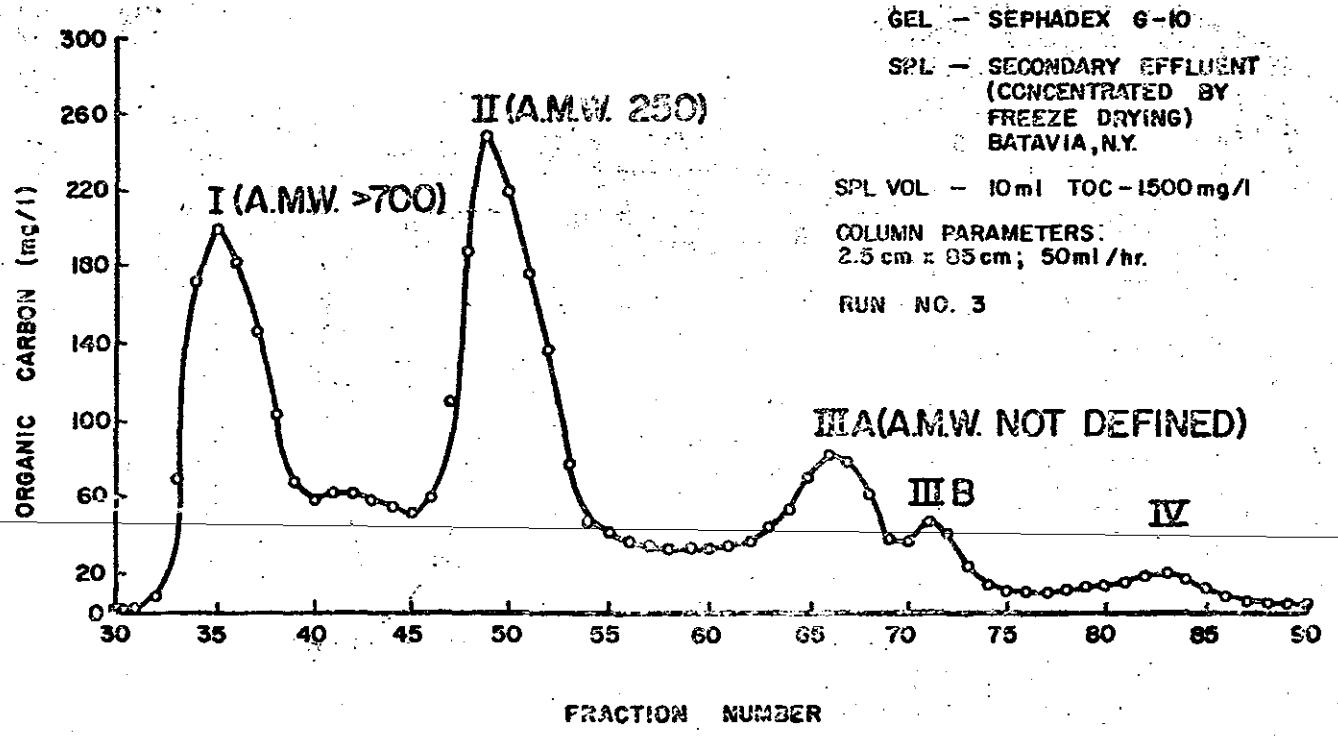

Figure 123. Elution diagram of the fractionation of concentrated Batavia effluent on Sephadex G-10.

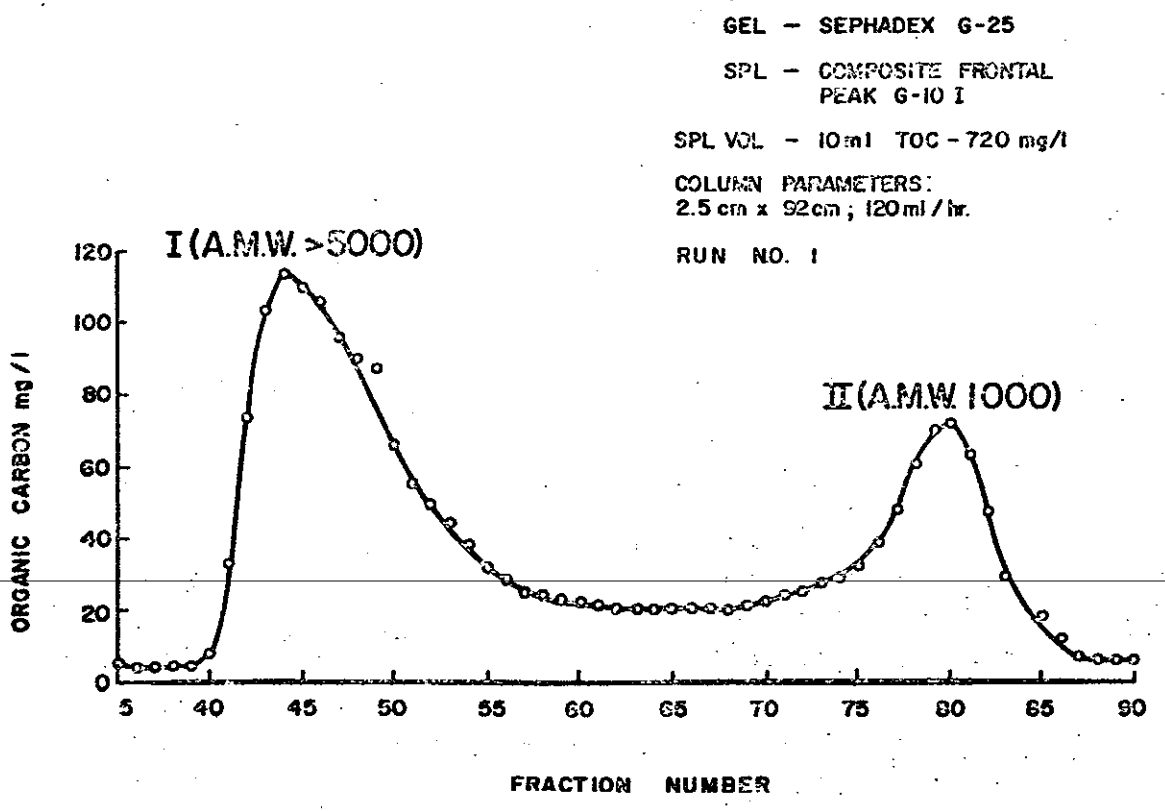

Figure 124. *Elution diagram of the fractionation of concentrated composite frontal peak G-10 I on Sephadex G-25.*

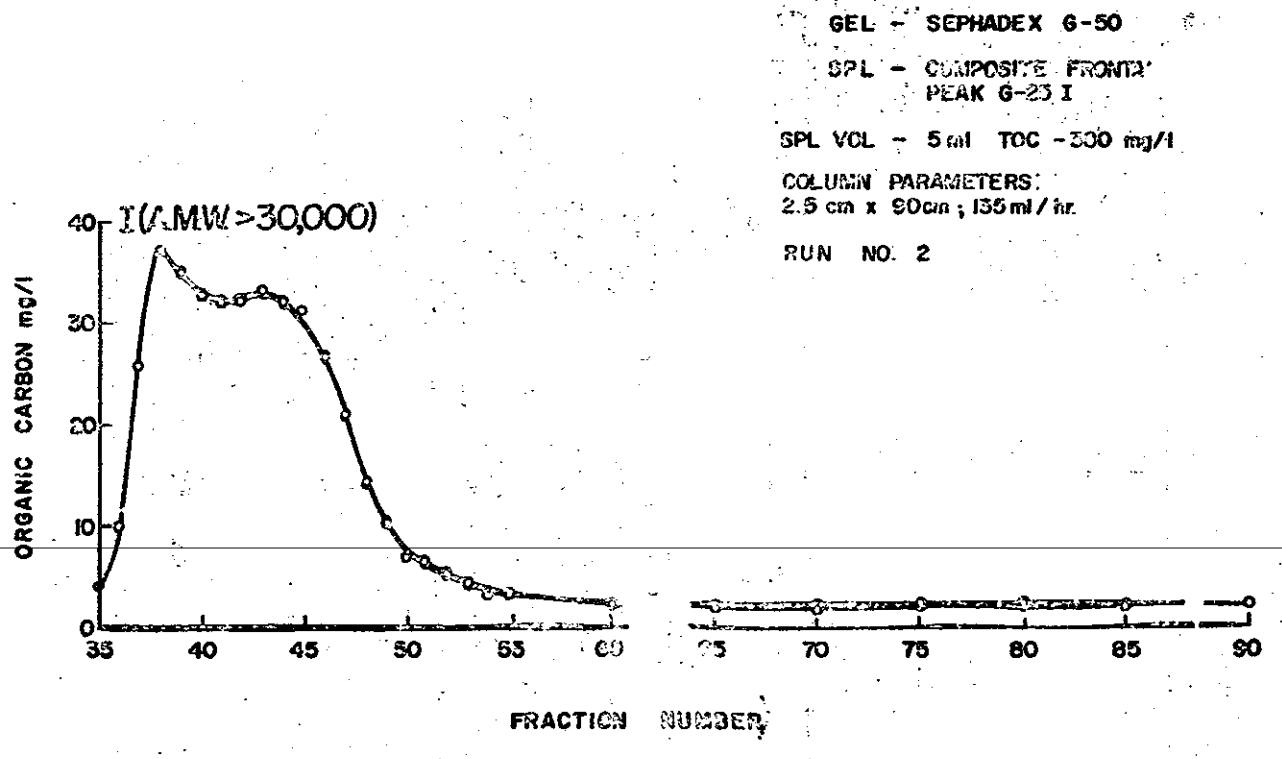

Figure 125. Elution diagram of the fractionation of concentrated composite frontal peak G-25 I on Sephadex G-50.

of Na_2EDTA from 7.4 mg/l to 1.0 mg/l, and the addition of 50 mg/l Na_2CO_3 to insure adequate buffering capacity. Trace metals were not added to the medium. The constituents are shown in Table 66.

Table 66

Basic ASM Medium

Compound	*Stock Solution (g/l)*	*For 1 Liter of Solution Use (ml)*	*Final Concentration in Medium (mg/l)*
$NaNO_3$	8.50	10	85.0
K_2HPO_4	0.348	10	3.48
$CaCl_2 \cdot 2H_2O$	1.47	10	14.7
Na_2CO_3	5.00	10	50.0
$MgSO_4 \cdot 7H_2O$	4.90	10	49.0
$MgCl_2$	1.90	10	19.0
$FeCl_3$	0.032	10	0.32
Na_2·EDTA	0.100	10	1.0

Glass Distilled Water - Dilute to 1 Liter

The culture room temperature was kept at 23 ± 1° C and illumination levels provided were 550 ft-candles[17,18] for *Selenastrum capricornutum* and 150 ft-candles for *Anabaena flos-aquae*. Prior to inoculation, the Erlenmeyer flasks and medium were sterilized by autoclaving. Inocula for both species were transferred from stock cultures to the control and test flasks at set periods. The stock cultures were maintained under the same conditions outlined for the test cultures.

Five-day-old cultures were used as inocula for *Selenastrum capricornutum* and seven-day-old cultures as inocula for *Anabaena flos-aquae*. In the case of *Selenastrum capricornutum* this transfer schedule produced an initial cell concentration of 35,000 cells/ml in the test cultures.

Growth of both species was determined by absorbance measurements at 750 nm with a Beckman DU-2 spectrophotometer.[19] The procedure had previously been correlated with cell count for

Selenastrum capricornutum and dry weight determinations for *Anabaena flos-aquae*. For each culture those points on the growth curve delineating the log phase of growth were subjected to a regression analysis from which the growth rate constant (K_{10}) was calculated. Growth rate constants from similar cultures were then averaged and a mean growth rate constant obtained for a specific set of cultures.

RESULT

Significant and marked effects on the growth rate of the algal forms were noted upon addition of several of the organic wastewater fractions to the respective algal cultures.

Data relative to the effects on *Selenastrum capricornutum* are shown in Table 67. Analysis of these data indicates that a marked increased growth rate response was noted on addition of fraction G-50 I (AMW > 30,000) and the concentrated effluent. Fractions G-25 II (AMW = 1000) and G-10 IIIb produced somewhat lesser responses. The concentrated effluent and G-50 I produced growth rate constants of 0.96 and 0.72, respectively, as opposed

Table 67

Effect of Organic Fractions on the Growth Rate of Selenastrum capricornutum

Fraction	Concentration of Fraction mg/l Organic Carbon	Mean Growth Rate K_{10} (day^{-1})	95% Confidence Interval
Control*	---	0.43	0.42 - 0.44
G-10 II	2.0	0.43	0.41 - 0.45
G-10 IIIa	0.6	0.42	0.40 - 0.44
G-10 IIIb	0.3	0.49	0.48 - 0.50
G-10 IV	0.2	0.43	0.39 - 0.47
G-25 II	0.4	0.50	0.49 - 0.51
G-50 I	1.3	0.72	0.70 - 0.74
Concentrated Effluent	7.0	0.96	0.89 - 1.03

*Basic ASM medium, without fraction addition

to 0.43 for the control. The data also indicate that the responses noted were selective, in that no correlation between increased growth rate and the organic carbon concentration in the culture vessel is evident. The organic carbon concentrations of the fractions noted in Table 67 are those, calculated on the basis of chromatographic data, that would be contained in the original wastewater effluent.

Reduced concentrations of the active fractions were also investigated. The data developed in these experiments are shown in Table 68. It is evident that, in order to eliminate growth rate enhancement, the strongly active G-50 I fraction and the concentrated effluent must be reduced to a point where their respective concentrations are only 10% of those existing in the original effluent; whereas a 50% reduction in the concentrations of fractions G-25 II and G-10 IIIb eliminates all effects.

Table 68

Effect of Organic Fractions in Reduced Concentrations on the Growth Rate of Selenastrum capricornutum

Fraction	*Concentration of Fraction mg/l Organic Carbon*	*Mean Growth Rate K_{10} (day^{-1})*	*95% Confidence Interval*
Control	---	0.43	0.42 - 0.44
G-10 IIIb	0.2	0.43	0.39 - 0.47
G-25 II	0.2	0.45	0.40 - 0.50
G-50 I	1.3	0.72	0.70 - 0.74
	0.7	0.63	0.60 - 0.66
	0.1	0.43	0.40 - 0.46
Concentrate	3.5	0.86	0.79 - 0.93
	0.7	0.43	0.40 - 0.46

Data were also developed regarding the possible interactive effects of the fractions. The addition, in concert, of the three active fractions, G-10 IIIb, G-25 II and G-50 I, to a *Selenastrum* culture allowed for the development of a growth rate

constant of 0.85. Comparison of this constant with that found using only the concentrated effluent (K_{10} = 0.96) suggests that the greater portion of the concentrated effluent response is due principally to these fractions.

Anabaena flos-aquae was not similarly affected by the wastewater fractions. As shown in Table 69, only fraction G-50 I and the concentrated effluent enhanced the growth rate of this alga. This growth rate response, measured as a percent increase, was much less than that exhibited on addition of these same fractions to the *Selenastrum* cultures. Data

Table 69

Effect of Organic Fractions on the Growth Rate of *Anabaena flos-aquae*

Fraction	Concentration of Fraction mg/l Organic Carbon	Mean Growth Rate K_{10} (day^{-1})	95% Confidence Interval
Control	---	0.34	0.32 - 0.36
G-10 II	2.0	0.34	0.30 - 0.38
G-10 IIIa	0.6	0.38	0.34 - 0.42
G-10 IIIb	0.3	0.33	0.29 - 0.37
G-10 IV	0.2	0.34	0.29 - 0.39
G-25 II	0.4	0.37	0.33 - 0.41
G-50 I	1.3	0.41	0.38 - 0.44
Concentrated Effluent	7.0	0.54	0.50 - 0.58

collected during investigations at reduced concentrations, Table 70, show that a 50% reduction in the concentration of G-50 I did not allow for any enhanced growth while a 90% reduction in the concentration of the whole effluent was required to achieve this same end.

Investigations with phosphorus at the level found in the concentrated effluent (1.5 mg/l) indicated growth rate enhancement for *Selenastrum capricornutum* alone, but the growth rate constant achieved (K_{10} = 0.50) was significantly less than

Table 70

Effect of Organic Fractions in Reduced Concentrations on the Growth Rate of Anabaena flos-aquae

Fraction	*Concentration of Fraction mg/l Organic Carbon*	*Mean Growth Rate K_{10} (day^{-1})*	*95% Confidence Interval*
Control	---	0.34	0.32 - 0.36
G-50 I	1.3	0.41	0.38 - 0.44
	0.7	0.37	0.33 - 0.41
	0.1	0.33	0.30 - 0.36
Concentrated Effluent	3.5	0.41	0.39 - 0.43
	0.7	0.33	0.31 - 0.35

that produced by the concentrated effluent or fraction G-50 I, those organic entities exhibiting the greatest effect on the algal species.

Limited information relative to the effect on final cell yield was also developed. Figures 126 and 127 depict typical *Selenastrum* and *Anabaena* growth curves developed from control and concentrated effluent data. Final yield (200 hours) was consistently higher in those cultures spiked with full concentrated effluent than in the control cultures.

DISCUSSION

Reviewing the data in Table 65 relative to the conductivity of each of the organic fractions allows for a preliminary assessment of the cause of the demonstrated effects. In view of this information, it may be assumed that any algal growth rate enhancement produced by fractions G-25 II and G-50 I, with conductivities of 5.7 and 8.5 µmhos/cm respectively, was due either to indirect or direct action of an organic agent. In the case of G-10 IIIb (380 µmhos/cm) such a clear determination is not possible. The high conductivity of this fraction may well indicate that the growth enhancement found resulted from inorganic factors or possibly organic ions within the fraction.

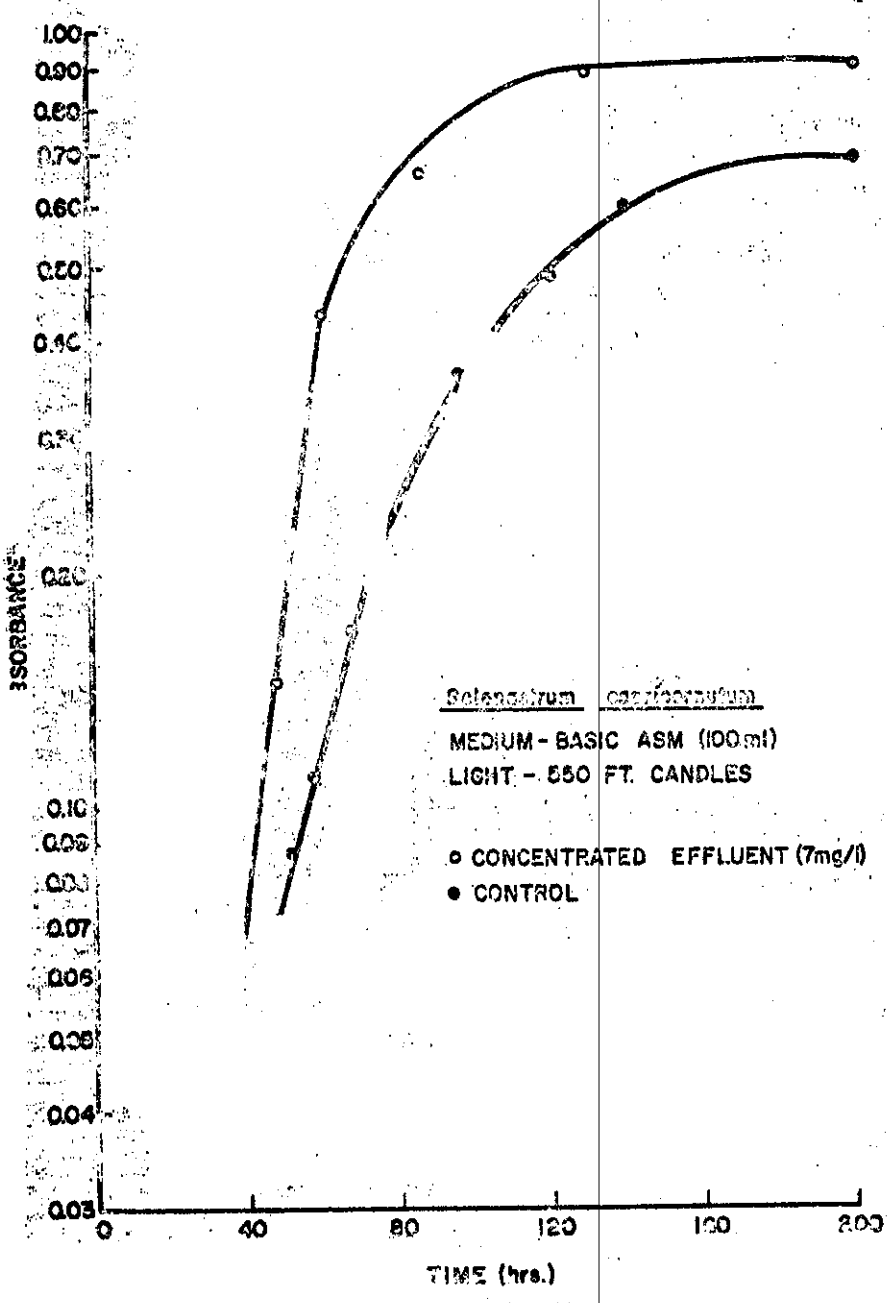

Figure 126. *Selenastrum capricornutum* growth curves.

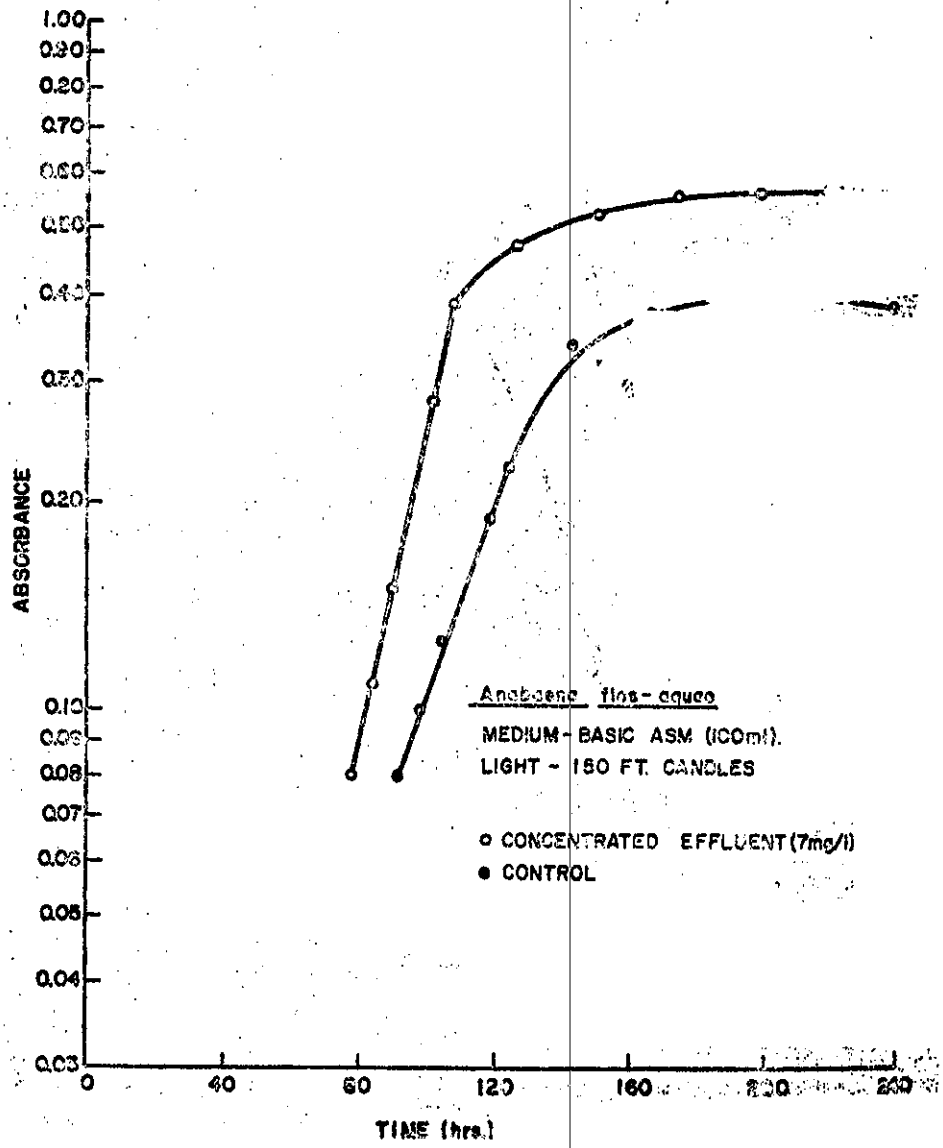

Figure 127. Anabaena flos-aquae growth curves.

Though no direct experimental evidence was produced relative to the question of carbon limitation, the data developed indicates that removal of carbon limitation was not a factor. The specificity of the effects coupled with significant increases in growth rate achieved by the addition of smaller quantities of carbon, *e.g.*, comparing fraction G-10 II to fraction G-50 I, would not be indicative of this explanation. If carbon alone was the operative factor, then the fraction which adds the greatest amount would be expected to produce the largest increase in growth rate.

As noted previously, experiments with phosphorus at the level found in the concentrated effluent (1.5 mg/l) elicited a growth rate increase with *Selenastrum capricornutum* but none with *Anabaena flos-aquae*. However, the growth rate achieved ($K_{10} = 0.50$) was significantly lower than that produced by the concentrated effluent ($K_{10} = 0.96$). These data in combination with the absence of a measureable phosphorus concentration in all fractions, except the concentrated effluent, show that the effects produced upon addition of the fractions cannot be attributed to the action of phosphorus.

Several possible factors may be responsible for the effects exhibited. First among these would be the provision of a required trace element not available in the Basic ASM medium. The conductivity for G-10 IIIb would allow that such trace elements could be provided by this fraction directly. In the case of fractions, G-25 II and G-50 I, based on conductivity measurements, an indirect effect may be suggested. This indirect effect would involve previous chelation by a compound or compounds within such fractions of required trace elements and their availability or release in the culture vessel as a result of bio-degradation.

Another possible explanation geared to the assumption of an organic causative agent would be the provision of an accessory growth factor or substance in the fractions. Examples of each would be the vitamin B_{12} as the factor and 3-indoleacetic acid as the substance. Both entities have been reported as being present in secondary effluents, though only vitamin B_{12} would have a molecular weight to match that of a particular active fraction, namely G-25 II. But it is noted that a vitamin requirement has yet to be

established for *Selenastrum capricornutum* and that *Anabaena flos-aquae* was unaffected by fraction G-25 II. Thus, the relationship between such growth factors and substances and the effects found are tenuous.

An explanation in line with that proposed by Prakash and Rashid[20] for the effect of humic substances on marine algal growth may also be offered. Such explanation supposes that a factor or factors within the active fractions act as sensitizing agents for cellular transport. It is believed that such agent or agents allow for increased rate of cellular transport resulting in enhancement of nutrient uptake from the basal medium and thus leading to increased growth.

Final consideration must be given to the provision of a proteinaceous material resulting from bacterial breakdown of the active fractions. Since the cultures were not maintained in the axenic state, it may be that biological breakdown of organic compounds within the active fractions provides protein degradation products such as amino acids and peptides. Subsequent algal uptake of such materials may allow the organism to bypass normal synthetic processes, resulting in increased growth.

Although the dilution used to obtain the above data would not normally be expected to occur in the natural environment, the effects noted are still important. The Batavia Activated Sludge Plant effluent is not unique. Effluents from activated sludge systems serving similar areas would generally be the same. Thus, if the conventional system were built on a water course not allowing for maximum dilution, problems resulting from algal growth enhancement would be expected. Examples of such receiving waters are lakes and tidal rivers, both of which, under certain circumstances, could allow for a concentration buildup of the organic fractions responsible for accelerated growth rate. Future investigations with other effluents will be undertaken to assess the generality of the results produced in this study.

CONCLUSION

Organic compounds contained in wastewater fractions have been found to exert a growth-enhancing effect on two algae species. The exact nature of the causative factors and the pathway by which they achieve their effects are as yet unknown, and further

investigative efforts are being conducted to assess the generality of these results. This undertaking is important as increased algal productivity may yet continue to occur if such factors are general constituents of wastewater effluents, notwithstanding the removal of nitrogen and phosphorus from such effluents.

ACKNOWLEDGMENT

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