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Title: RECYCLING OF IRON AND CONDITIONING OF SEA WATER BY A MARINE DINOFLAGELLATE

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It is becoming evident that short term recycling of nutrients is important in the growth of phytoplankton in natural waters. Experiments in which a cell homogenate was added to iron-limited cultures of the marine dinoflagellate Amphidinium carteri demonstrated that iron can be recycled. The process was shown to be greatly accelerated by exposure of metabolized iron to a low pH. It is suggested that digestive processes of herbivores may play an important role in making metabolized iron quickly available to the phytoplankton.

Laboratory experiments were performed to investigate possible interactive effects of iron and dissolved organic material on the growth of A. carteri. Treatment of sea water with activated carbon to remove dissolved organics was found to lower the growth rate and final population density in cultures of A. carteri. Final population levels could be restored by addition of $FeCl_3$ or FeEDTA, indicating

that some form of available iron was removed by activated carbon. Iron addition did not restore the growth rate, nor did treatment with EDTA. The growth rate was restored only by the growth of A. carteri itself in the activated carbon-treated media. It is suggested that A. carteri can contribute to the media some dissolved organic material which promotes its own growth.

Recycling of Iron and Conditioning of Sea Water by a Marine Dinoflagellate

by

Bruce Edward Frey

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RECYCLING OF IRON AND CONDITIONING OF SEA WATER BY A MARINE DINOFLAGELLATE

INTRODUCTION

Two of the least understood areas of phytoplankton ecology are the roles of trace metals and the roles of dissolved organics in the growth patterns of phytoplankton. There is a considerable body of evidence that these areas are interrelated, as abundance and form of trace metals may be strongly influenced by dissolved organic molecules acting as ligands and chelators (Duursma and Sevenhuysen, 1966; Goldberg, 1957; Saunders, 1957).

Of the trace metals required for plant growth, iron is required in the greatest amounts and has often been shown to be a factor limiting growth (Davies, 1970; Goldberg, 1952; Hayward, 1968; Price and Caroll, 1964; Ryther and Kramer, 1961). Because it is required in relatively large quantities, problems of contamination and the establishment of iron-deficient media for laboratory experiments are less than with other essential trace metals. Because of its importance in natural systems and its relative ease of manipulation in the laboratory, iron was selected for investigation in this study.

Growth in a nutrient-controlled system is a function of the rate at which cells can take up and assimilate controlling nutrients. The availability of a controlling nutrient will be strongly influenced by the environmental nutrient concentrations (Caperon, 1967). The

physico-chemical form of the nutrient will also influence the availability of nutrients to the cells. Shapiro (1966) reported that $FeCl_3$, Fe-citrate, and FeEDTA were not equally available to phytoplankton, and that the relative availability varied from species to species. Particulate iron was reported by Goldberg (1952) to be available as an iron source for Asterionella japonica, while organic complexes were not. Competing uptake sites in the environment, such as ligands, may influence the availability of nutrients to the phytoplankton. This effect may be complex, for not only may chelators tie up a certain portion of the nutrient pool, but they may affect the form of the nutrient and help it to remain in the environment (Saunders, 1957). The availability of a controlling nutrient might in some cases be a simple function of the rate at which that nutrient is liberated from organic matter.

It is becoming evident that phytoplankton growth in the sea does not always follow a simple pattern in which there is rapid growth until a limiting nutrient is exhausted, after which cells containing the nutrients are lost to the system through sinking and grazing. Even when one or more essential nutrients are below levels of detectability in the environment, rapid recycling of nutrients within the system might maintain phytoplankton populations in a state of health and sustained growth (Pomeroy, 1970).

Dugdale and Goering (1967) have shown that nitrogen can be

rapidly recycled as ammonia. Johannes (1968) has suggested that protozoans are largely responsible for rapid regeneration of phosphate in the ocean. If rapid regeneration of nitrogen and phosphorus is an important characteristic of natural phytoplankton systems, it would appear that any other nutrient not present in great excess and which was not recycled as fast as nitrogen and phosphorus would become severely limiting to growth. Such a nutrient would become available only as new supplies could diffuse into the system, and would be lost at the rate at which cells died or were eaten. The net rate of loss from the system would thus be more rapid than for nitrogen and phosphorus, and, if availability of the particular nutrient were to be maintained in the system, the rate of resupply by diffusion would necessarily have to be greater than the rate of resupply of "new" nitrogen and phosphorus. The rate of diffusion of the limiting element into the system would be expected to control growth within the system. It thus becomes evident that the recycling of a physiologically important and sometimes limiting nutrient such as iron may be of some importance.

Davies (1970) found that production of chlorophyll *a* in cultures of *Dunaliella tertiolecta* was dependent mostly on iron residual in sea water, after storage and filtration of the water. Iron added as ferric chloride was far less effective in stimulating production of chlorophyll *a*. From this result Davies speculated that organo-iron

complexes might be required for chlorophyll production in phytoplankton. It is possible that stable organo-iron complexes might constitute a nutritional substance somewhat different from uncomplexed iron. Membrane permeability of the complexes would certainly be different from the uncomplexed forms, and perhaps they might follow different metabolic paths in the cell. Possibly some forms of iron have thermodynamic advantages over other forms, although there seems to be no information on this possibility in sea water.

Barber et al. (1971) found that newly upwelled water, although rich in nutrients, is sometimes unable to support rapid phytoplankton growth. They found that addition of EDTA and/or trace metals (including iron) brought production rates up to those of highly productive upwelled waters. They found further, that treatment of highly productive upwelled water with activated carbon, a treatment which removes much of the organic material, reduced the productivity of that water to levels similar to those of the poorly productive upwelled water. The carbon-treated water could be restored to high productivity by additions of trace metals and/or EDTA. Barber hypothesized a biological conditioning required for sea water to become fully productive.

The above studies indicate that organically complexed trace metals such as iron might play an important role in the primary

productivity of the oceans. We may speculate that a significant portion of the iron in natural waters has been cycled through the biomass. When the cell is broken down and iron exits the biomass, the question arises as to what form it is in: particulate hydroxide or phosphate, dissolved ions, or organic complexes? Is it indeed available for biological uptake? As is apparent, this field is little understood.

My study is intended as a preliminary set of probes into the dynamics of iron and dissolved organics in phytoplankton systems. Activated carbon was utilized to remove substances from sea water, and the effects on log growth rates and final population densities of the test organisms were observed. Treatment necessary to overcome the effects of activated carbon were investigated in order to determine the modes of action of the activated carbon. Finally, the recycling of metabolized iron was investigated using bioassay techniques.

MATERIALS AND METHODS

General Procedures

The marine dinoflagellate Amphidinium carteri was grown at 15.5° C under 0.035 langleys per minute illumination during the illuminated portion of a 14 hour - 10 hour light-dark cycle. 1500 ml cultures were grown in 2-liter Erlenmeyer flasks in a plexiglass water bath which maintained the temperature within ±0.5° C. Illumination came from four cool-white high-intensity fluorescent tubes located beneath the cultures.

Due to the nature of these experiments, care was taken to minimize contamination of cultures with trace metals and organics. All glassware was scrubbed with detergent and hot tap water, then rinsed twice with hot tap water and twice with distilled water. It was soaked in 10% NaOH for at least one day and rinsed three times with distilled water. Then it was soaked in 3N HCl for at least one day and rinsed three times with distilled water and three times with Pyrex-distilled water. The glassware was then autoclaved for 20 minutes at 15 psi.

Culture media was prepared from sea water collected offshore as described later. This sea water was filtered through 0.8 μm pore-size membrane filters into a clean Pyrex 20-liter carboy.

When the experimental procedure called for treatment with

activated carbon, 1 gram of Darco G-7 activated carbon was added for each liter of sea water. A large, clean Teflon magnetic stirring bar was used to maintain the carbon in suspension for one week with the temperature at 17° C. The carbon was then allowed to settle out and the sea water was filtered into a clean Pyrex 20-liter carboy through a 0.8 um membrane filter.

The filtered sea water was enriched with the nutrients shown in Table I. The media was poured into clean culture flasks and additional nutrient treatments of iron and/or EDTA were made when required by the particular experimental procedure.

When the treatment called for the addition of iron, it was added in the form of $FeCl_3 \cdot 6H_2O$, to give a concentration of 5.8 µg-at Fe per liter, well above the iron saturation value for A. carteri found by Ryther and Kramer (1961). When the treatment called for the addition of EDTA, it was added as disodium ethylendiaminetetraacetate, to give a concentration of 4.9 moles per liter. Iron added to senescent cultures was given as FeEDTA, to yield a concentrarion of 0.6 µg-at Fe per liter.

The media was allowed to equilibrate at 15.5° C before inoculation with A. carteri. Inoculations were made at the beginning of the dark cycle by pipetting with a clean pipet the appropriate number of cells to give an initial population density of approximately 350 cells per ml. The inoculum culture was maintained under the same

Table 1. Culture Medium

Millipore filtered sea water	1 liter	
$NaNO_3$	75 mg	(0.88 mg at N)
$NaH_2PO_4 \cdot H_2O$	5 mg	(36.3 μg at P)
$NaHCO_3$	0.1 g	
Vitamin mix		
Thiamin · HCl	0.1 mg	
Biotin	0.5 μg	
B_{12}	0.5 μg	
Trace metal mix		
$CuSO_4 \cdot 5H_2O$	0.010 mg	(0.039 μg at Cu)
$ZnSO_4 \cdot 7H_2O$	0.022 mg	(0.008 μg at Zn)
$CoCl_2 \cdot 6H_2O$	0.010 mg	(0.042 μg at Co)
$MnCl_2 \cdot 4H_2O$	0.180 mg	(0.91 μg at Mn)
$Na_2MoO_4 \cdot 2H_2O$	0.007 μg	(0.027 μg at Mo)

conditions as the experimental cultures.

Monitoring of Growth

Cell density was monitored by periodic counts with a Model B Coulter Counter, fitted with a 200 μm apreture. Three counts of 2 ml each were made for each sample, giving a counting variability of approximately ± 10%. Samples were diluted when necessary with membrane-filtered sea water to keep cell concentrations under 3, 500 cells per ml. This dilution was done to avoid counting errors which occur when two or more particles pass through the aperture simultaneously.

Sampling was done by swirling the culture several times, then pouring a sample into a 20 ml calibrated sample tube. If dilution was necessary, a sample was poured into a beaker and an appropriate amount of sample was pipetted into a 20 ml sample tube. It was then diluted in the tube with membrane-filtered sea water. Sampling variability was less than ± 10%.

In Experiments IV and V, cultures were monitored by fluorometry in addition to the usual particle counts. A Turner fluorometer fitted with a high-sensitivity chamber was used for this work.

Collection of Sea Water

Experiments I and II

Sea water was collected on August 28, 1972 at Station NH-40, 40 miles off Newport, Oregon (44° 45'N). PVC Van Dorn bottles were used to collect water from 165 meters depth. The water was stored in two clean 20-liter Pyrex carboys at 17° C for one day before filtering and treatment with activated carbon.

Experiment III

Sea water was collected on March 17, 1972 from the surface at Station NH-40. It was stored at 17° C in a 13-gallon polyethylene carboy for one week before filtering and treatment with activated carbon.

Experiments IV and V

Sea water was collected on October 30, 1972 from the surface at Station G-7, 26 miles off Newport, Oregon (44° 55'N). It was stored at room temperature in a 13-gallon polyethylene carboy for three months before filtering and treatment with activated carbon.

Description of Experiments

Experiment I

Experiment I was designed to establish general growth characteristics of A. carteri. There were eight different treatments, consisting of various combinations of iron, EDTA, or activated-carbon treatment (Table 2). Two replications of each treatment were made. Cultures were inoculated with iron-limited senescent cells. After cultures had reached senescence, nutrients in Table 1 at one-half strength plus iron were added to one replicate of each treatment. Only iron was added to the other.

Experiment II

Experiment II was designed to investigate the effects of autometabolites on the growth of A. carteri. Sea water was carbon-treated and enriched with full nutrients plus iron. The media was inoculated with log-growth cells and the population was allowed to grow to senescence. The cultures were then filtered through fired glass-fiber filters. Full nutrients including iron were again added, and the media was inoculated with log-growth cells. Two replicates were made.

Experiment III

Experiment III was designed to test the suitability of senescent cultures of A. carteri for use as bioassays for available iron. Culture media was prepared as usual except the trace metal mix was deleted. Six different initial treatments were made, consisting of various combinations of treatment with activated carbon, iron, and EDTA. Three replications of each treatment were made. Cultures were allowed to grow to senescence. Additions of either iron, trace metals (Table 1), or a nitrate-phosphate-vitamin mix were made to each of the three replicates of each treatment, to determine if any of these nutrients would stimulate growth. Illumination during this experiment was lower than illumination in other experiments.

Experiment IV

Experiment IV was designed to test the availability of metabolized iron released by lysed A. carteri cells. Eight cultures of A. carteri were grown to a state of iron limitation by growing them to senescence in iron-deficient media. Various additions were then made to these cultures as described below.

Twenty liters of iron-deficient media were prepared in a clean Pyrex carboy. The media was inoculated with A. carteri and grown at 17° C with constant illumination from three circular fluorescent tubes.

The culture was gently stirred by a large Teflon magnetic stirring bar suspended by a Teflon tape to avoid grinding cells against the bottom of the carboy. When the culture had reached an asymtote (80,000 cells per ml), it was centrifuged in clean 250 ml polycarbonate centrifuge tubes at 8,000 RPM for 15 minutes. The cell concentrate (about 4 ml) was then thoroughly ground in a ground-glass tissue grinder. Microscope checks showed that virtually all cells were destroyed.

Cell homogenate volumes corresponding to original culture volumes of 1, 2, and 4 liters of the senescent culture in the carboy were added to three different senescent, iron-limited cultures. Two distilled water controls and one FeEDTA control were made. In addition, cell homogenate corresponding to 1 and 4 liters of culture were added along with FeEDTA to two separate senescent, iron-limited cultures. This treatment was to determine if stimulatory effects of iron (added as FeEDTA) would be masked by toxic or inhibitory factors in the cell homogenate.

Three weeks following the first homogenate additions, further additions were made to several cultures that had shown no response. A cell homogenate volume corresponding to an original culture volume of 6 liters was added to the culture which had previously received homogenate corresponding to 4 liters of culture. In addition, one distilled-water control was treated with 5.6 mg of humic extract,

and the culture which had previously received homogenate equivalent to 1 liter of culture was treated with 28 mg of humic extract. FeEDTA was added to the culture which had received cell homogenate from 2 liters of culture in order to determine if the cultures were still viable.

The humic extract was prepared as follows. Soil collected in the Willamette Valley (100 gm) was washed twice with 100 ml portions of 1N HCl and then centrifuged. The supernatant was discarded. The soil was then leached for 24 hours with 200 ml of 10% NaOH and centrifuged. The supernatant, containing the dissolved humic extract, was decanted off. The humic extract was then reprecipitated with HCl and centrifuged. The supernatant was discarded, and the precipitate was redissolved in NaOH. This process was repeated five times, and the residue was washed twice with Pyrex-distilled water. It was then redissolved and stored at about pH 8. The ash weight of the humic extract preparation was 3% of the dry weight.

Experiment V

Experiment V was designed to test the availability of metabolized iron released by lysed, acid-treated A. carteri cells. It was similar to Experiment IV except for the acid treatment.

Twenty liters of culture were grown and concentrated by centrigugation as described for Experiment IV. One ml of 6N HCl was added to the cell concentrate prior to grinding. The ground, acidified

cell homogenate was allowed to sit for one week at room temperature. The HCl was then evaporated off at a temperature of 65° C and the homogenate was neutralized to pH 8 with the addition of 2 ml of 10% NaOH. Two senescent, iron-limited cultures were then treated with additions of cell homogenate corresponding to 0.5 liter and 4 liters of original culture.

A distilled water control received the same acid-base treatment as the cell homogenate. The HCl contained as much as 0.0001% iron contaminant, and the NaOH contained as much as 0.0001% iron contaminant in 10% solution. Maximum computed levels of iron contamination were as follows:

Distilled water control	.07 μg/ml
0.5 liter HCl-treated homogenate	.005 μg/ml
4 liter HCl-treated homogenate	.04 μg/ml

RESULTS

Maximum Population Densities

Cultures receiving iron in some form, regardless of other treatments, reached higher maximum densities than those not receiving iron (Table 2). This result showed that iron present in the stored and filtered sea water plus iron entering the system as contamination was not sufficient to promote maximum population levels of Amphidinium carteri.

Among the cultures which had not received iron, those which received treatment with activated carbon developed a lower maximum cell density than those which were not carbon-treated. Among cultures which had received an excess of iron, there was no significant difference (at $p = 0.05$) between maximum population densities attained in cultures which received carbon treatment and those which had not received carbon treatment. These results indicated that treatment with activated carbon removes some available iron, but that iron availability can be restored through additions of ionic or EDTA-chelated iron.

No enhancement of maximum population density was given by addition of EDTA, either alone or in combination with iron. This is consistent with findings of Lewin (1973), who reported that EDTA enhanced growth in freshly collected sea water, but not in sea water

that had been in containers for several days.

Rate of Growth

Specific growth rates, as cells produced/cells present/day (b), were computed by a least squares fit to the equation $N_t = N_0 e^{bt}$, where t is time (in days), and N_0 and N_t are cell numbers at t = 0 and time t, respectively. Values obtained in Experiment I ranged from 0.35 to 0.43 cell/cell/day (Table 2). Treatment of sea water with activated carbon resulted in decreased growth rates (mean = 0.36 cell/cell/day) relative to no carbon treatment (mean = 0.41 cell/cell/day).

Variability among replicates of the same treatment was exceedingly low. Additions of iron and/or EDTA appeared to have little or no effect on growth rates. The additions were unable to restore growth rates in carbon-treated water to those achieved in non-carbon-treated water.

Treatment in Experiment II was effective in restoring higher growth rates to carbon-treated media (Table 2). In this experiment cultures were first grown to senescence in carbon-treated media, the growth rate being 0.35 doublings/day (the same as in carbon-treated cultures in Experiment I). Cells were then filtered out of the media, and the filtrate was re-enriched with all nutrients. This filtrate was then reinoculated with log-growth cells. These cells

Table 2. Growth Rates and Final Populations in Experiments I and II.

Treatment	Specific Growth Rate (cell/cell/day) $\bar{x}$	σ	Asymptote (10^3 cells/ml $\bar{x}$	σ
Experiment I				
Non-carbon treated	0.40	± 0.02	71	± 0.0
Non-carbon treated + EDTA	0.43	± 0.00	72	± 3.2
Non-carbon treated + Fe	0.42	± 0.00	134	± 3.6
Non-carbon treated + FeEDTA	0.39	± 0.00	168	± 88.4
Carbon treated	0.36	± 0.00	31	± 9.9
Carbon treated + EDTA	0.36	± 0.01	29	± 13.8
Carbon treated + Fe	0.35	± 0.01	170	± 45.9
Carbon treated + FeEDTA	0.36	± 0.01	150	± 24.8
Experiment II				
Carbon treated	0.35	± 0.02		
Filtered and re-enriched	0.40	± 0.01		

achieved a growth rate of 0.40 cell/cell/day, higher than any other growth rate in carbon-treated sea water, and equal to growth rates in the non-carbon-treated water.

Bioassays for Presence of Available Iron

Additions of iron to senescent cultures which initially received no iron or trace metals (Exp. III) produced renewed growth after a short lag period, while additions of other trace metals or of a nitrate-phosphate-vitamin mix had no stimulatory effect (Fig. 1). Senescent cultures to which iron was added grew to cell densities as high as those of cultures treated initially with iron. Reactions to additions were the same regardless of initial treatment with EDTA or activated carbon (not shown).

Addition of FeEDTA, either alone or in combination with cell homogenate (Exp. IV), resulted in renewal of growth in senescent cultures (Fig. 2). This result demonstrated that the cell homogenate was not toxic or inhibitory to the growth of A. carteri, so that the stimulatory effect of available iron was not masked by any effects of the homogenate. Cell counts and fluorometric determinations were highly correlated, indicating that iron enrichment did not preferentially affect pigment synthesis over cell division.

The senescent culture which received cell homogenate from 10 liters showed a gradual but definite increase in population density

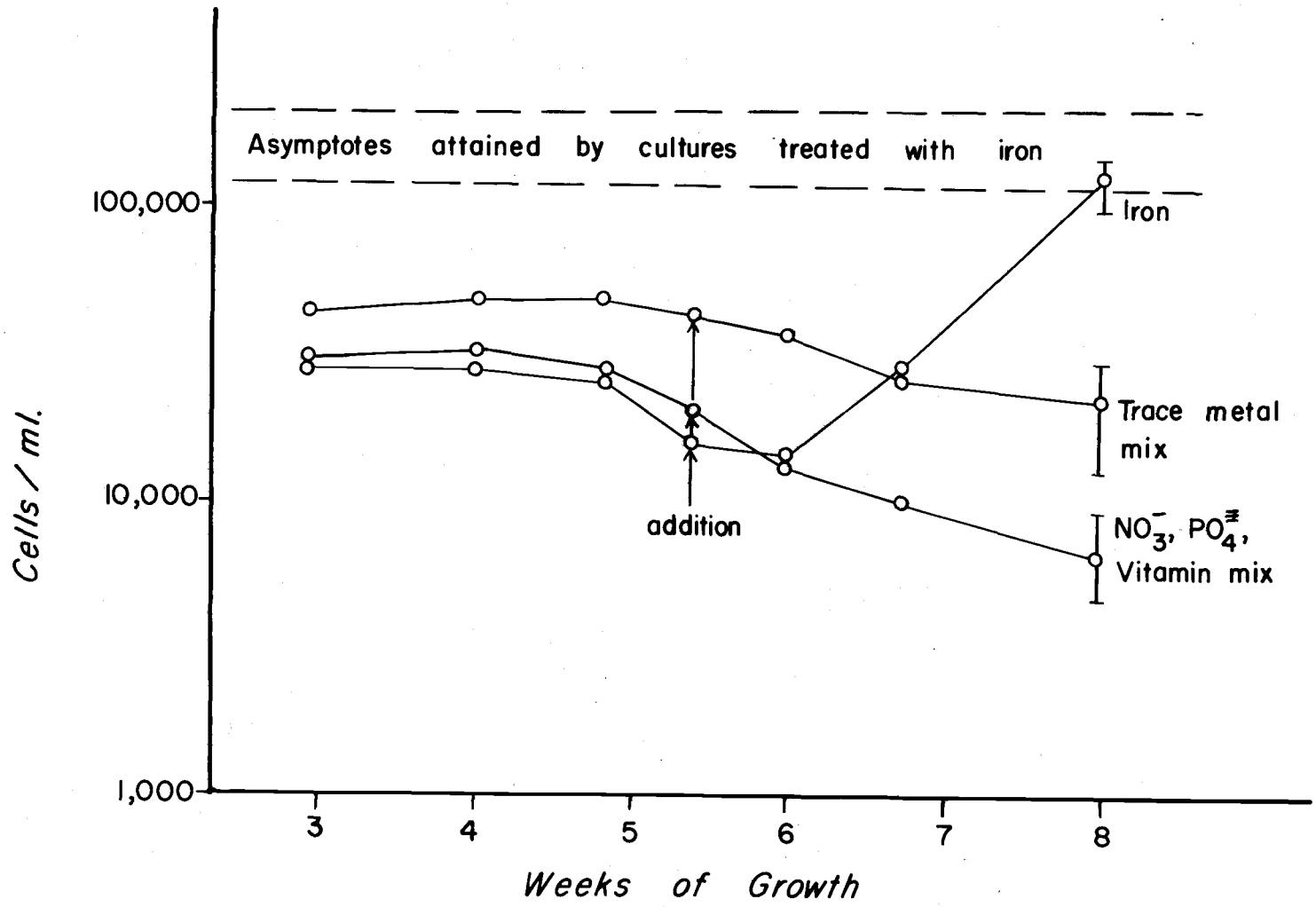

FIGURE 1. Experiment I. Effects of nutrient additions to iron-limited, senescent cultures of *A. carterae*. Points indicate means of three replicates. Final ranges of values are indicated by brackets.

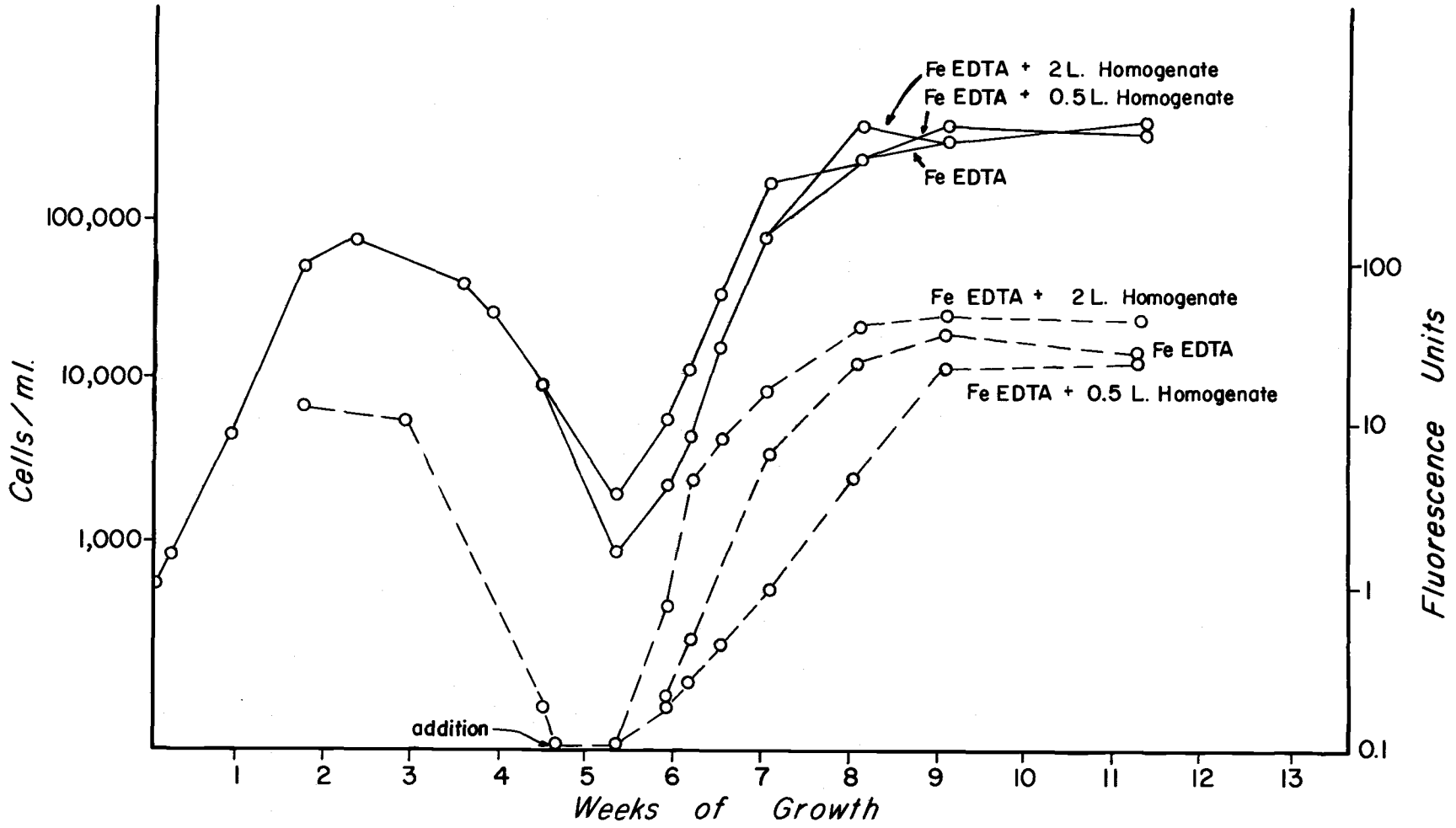

FIGURE 2. Experiment IV. Effect of cell homogenate on growth stimulation by iron.

Solid line = Cell counts; Dashed line = Fluorometer readings

and fluorescence (Fig. 3), but the rate of increase did not match that achieved with the FeEDTA addition. Addition of humic acids in two concentrations gave renewed growth equal to that with FeEDTA alone (Fig. 4). Again, pigment increases paralleled increases in cell counts. The culture which received HCl-treated cell homogenate from 4 liters (Exp. V) showed rapid growth similar to that of the FeEDTA control (Fig. 5). The HCl-treated homogenate from 0.5 liter gave no demonstrable stimulation of growth.

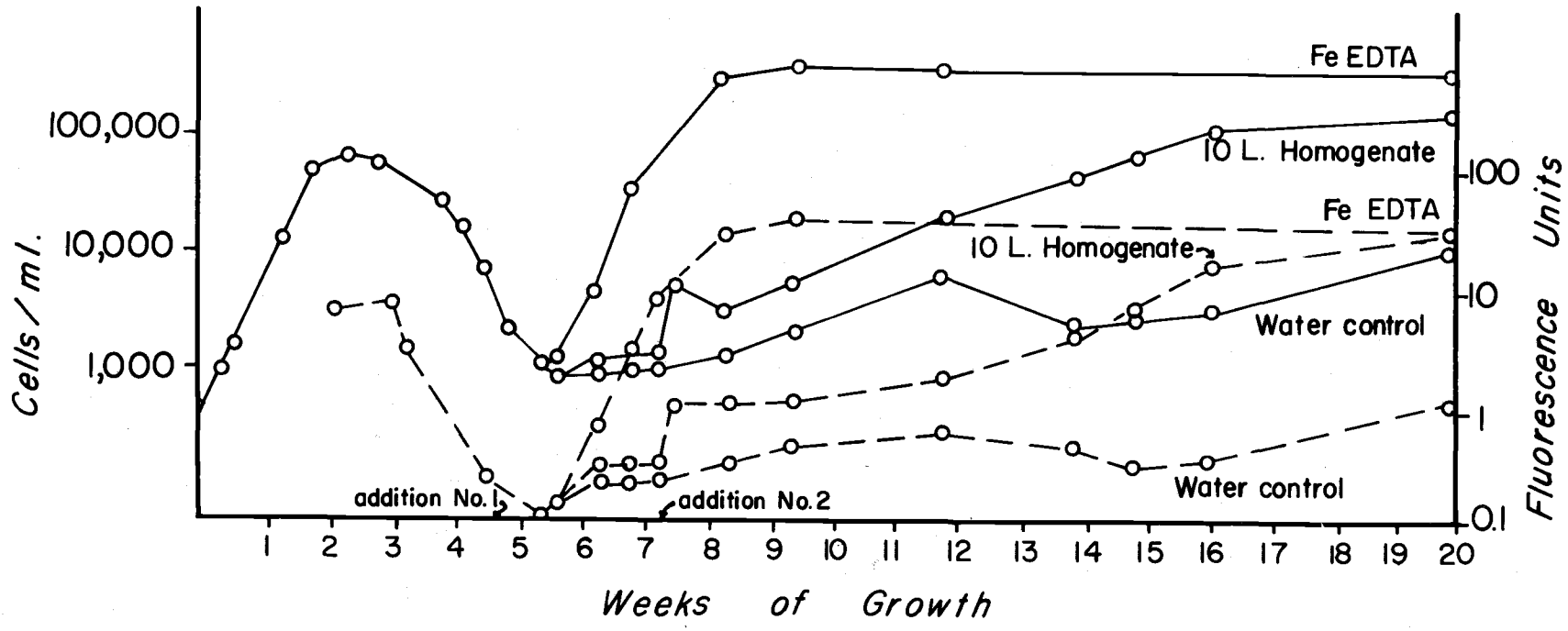

FIGURE 3. Experiment IV. Effect of cell homogenate additions on populations of *A. carteri*. Solid line = Cell counts; Dashed line = Fluorometer readings.

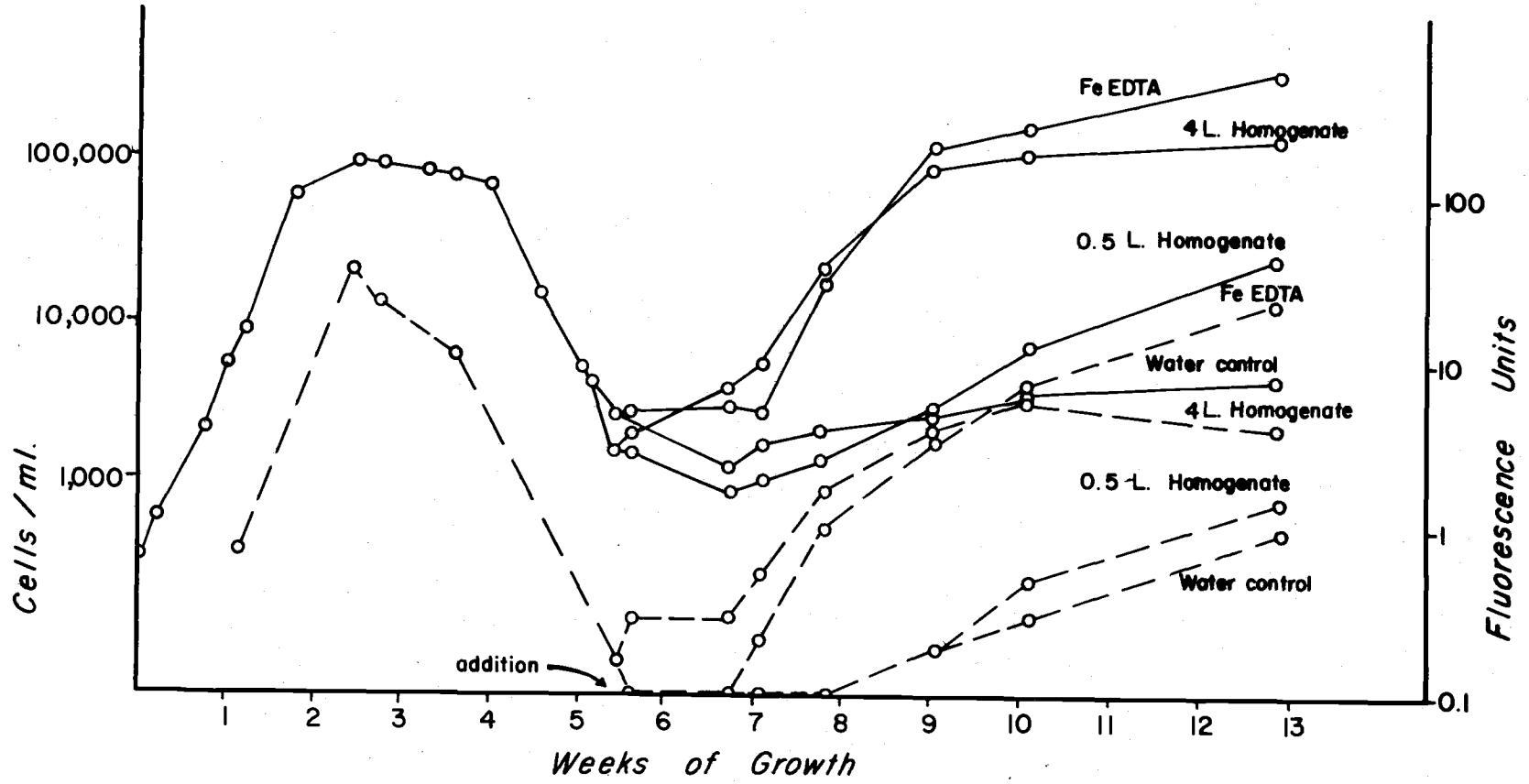

FIGURE 5. Experiment V. Effect of HCl-treated cell homogenate additions on populations of *A. carteri*. Solid line = Cell counts; Dashed line = Fluorometer readings.

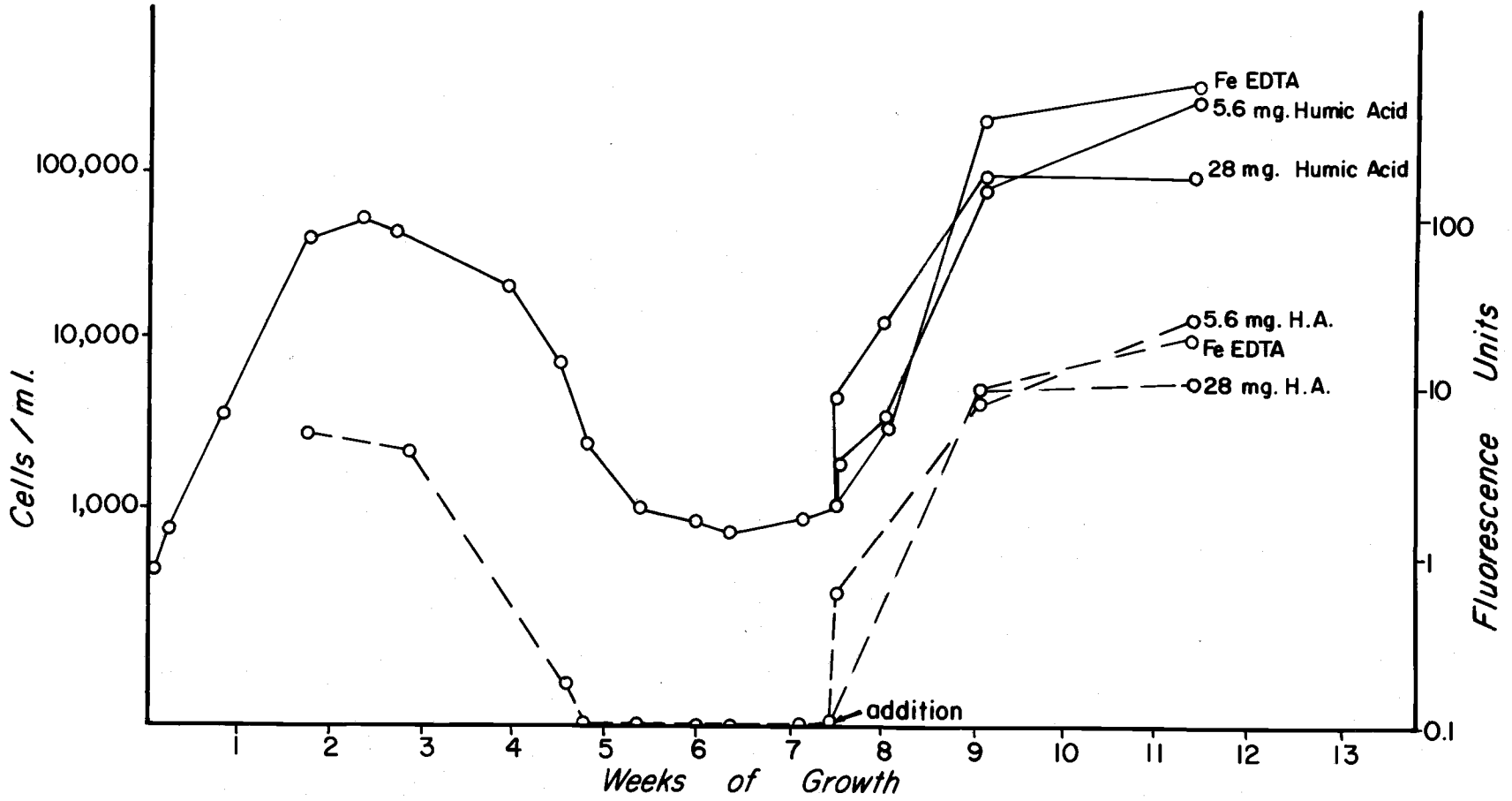

FIGURE 4. Experiment IV. Effect of Humic Acid additions on populations of *A. carteri*.

Solid line = Cell counts; Dashed line = Fluorometer readings.

DISCUSSION

Two discernible effects of treatment with activated carbon were observed. One effect was a lowering of the maximum population density attained, and the other effect was a lowering of the growth rate during exponential growth phase. That these two effects were not completely coupled was shown by the fact that different treatments were required to overcome the different effects of activated carbon.

The lowering of maximum population density was overcome by the addition of either ionic or chelated iron, making it clear that one effect of carbon treatment was to remove some available iron. Whether this removed iron was particulate, ionic, or an organic complex, is so far unknown. Lewin and Chen (1973) have shown that a certain fraction of the dissolved iron (defined as that iron fraction which will pass through a 0.45 μm pore-size membrane filter) in sea water rapidly particulates when the water is contained, and the remaining dissolved iron stays dissolved for long periods of time. This dissolved fraction may be in the form of ionic iron, organo-iron complexes, or very small iron hydroxide particles. Because the sea water in my experimentes was stored and then filtered before treatment with activated carbon, it must be some part of this stable dissolved fraction which the activated carbon is removing.

The lowering of the exponential growth rate was overcome by

the growth of A. carteri in the carbon-treated media. It appears that some factor or factors necessary for growth at full potential is removed from sea water by activated carbon, and that the factor can be resupplied to the sea water through the growth of A. carteri itself. The "biological conditioning" of low-organic-content sea water, proposed by Barber and Ryther (1969), is strongly suggested. Because the growth rate in carbon-treated cultures did not increase with time during the log phase of growth, it appears that the water did not become conditioned during log growth. If in fact there was a conditioner or growth factor added to the water in Experiment II, it must have been added during senescence, when cells are known to exude materials into the water (Guillard and Wangersky, 1958), or during filtering, when the contents of some lysed cells might have passed through the filter.

An alternative hypothesis for reduced growth rate in carbon-treated water might be that the carbon initially adds a growth-repressing substance to the water, but this substance becomes inactivated by increasing cell growth. Because the capacity of the water to support full growth potential does not seem to be continually changing during log growth, however, this explanation does not appear to be a reasonable one. Also, one might expect a substantial lag period in the growth curve if an inhibitor was added to the culture medium (Fogg, 1966). No such lag was observed (not shown).

Experiments IV and V have shown that metabolized iron can become available iron when cells are broken apart by mechanical grinding. Growth induced by the cell homogenate was quite slow compared to FeEDTA controls, and seemed to be a function of the concentration of the homogenate in the water. This result indicates that the iron becomes available slowly, with the rate of availability limiting the rate of increase in cell density. The iron in the cell homogenate might be tied up in subcellular structures from which it is slowly released by bacterial action, or perhaps it is bound to organic molecules which slowly release the iron.

Treatment of cell homogenate with HCl resulted in a high growth rate, indicating that a low pH was effective in rapidly releasing bound iron from the homogenate. This result suggests that the digestive processes of herbivores, involving acidic digestive juices, might be important in the recycling of iron in natural plankton systems. We can as yet say little about rates and exact processes involved, only that iron can be recycled on a fairly short time scale.

Humic extracts have long been known to stimulate plant growth (Olsen, 1930; Waksman, 1936). Burk, et al. (1933) gave evidence which indicated that stimulatory properties of humic acids were due to available iron in the humic preparation. Since additions of humic extract in Experiment IV gave results similar to the FeEDTA control, this provides additional evidence that at least some of the stimulatory

effects of humic extracts can be attributed to the contribution of iron to the system. Goldberg (1952) reported that use of humic extract as a source of iron did not promote growth in cultures of Asterionella japonica. This may indicate that iron in humic extract complexes is not equally available to all species of phytoplankton.

It was observed that when cultures with an excess of iron reached a maximum population density, the density remained near that maximum (the iron-control populations in Figs. 3 and 5, for example). Cultures not receiving an excess of iron would reach a maximum and then drop to a lower population density which would be reasonably maintained for long periods of time (the water-control populations in Figs. 3 and 5, for example). It would seem that senescent cultures of A. carterae remain dynamic systems, and require a certain nutrient supply in order to remain at some population level. These observations suggest that iron-starved cultures are forced to reduce population densities until they reach a cell concentration at which dead cells release enough iron to meet the needs of the remaining viable population. This stable, steady-state system can maintain itself for long periods of time. Such a nutrient-controlled system is a very useful one for bioassay, as was shown in Experiments III, IV and V. The technique of utilizing steady-state,

senescent populations for bioassay, rather than populations in exponential growth phase, probably can be utilized in work with any of the major nutrients.

The excellent correspondence between fluorescence and cell numbers indicates that these are both effective methods for monitoring the cell populations. I have found that low-density, nutrient-controlled populations of A. carteri can be maintained in small fluorometer tubes, and growth can be monitored directly by measuring chlorophyll fluorescence directly from undisturbed cells in the tubes. This technique makes the handling of large numbers of assay treatments quite manageable.

CONCLUSIONS

1. Activated carbon removes some form of available iron from sea water.
2. Activated carbon appears to remove some growth factor from sea water, and this factor can be resupplied to the water by old Amphidinium carteri cells.
3. Senescent, iron-limited cultures of A. carteri constitute a stable, steady-state system which may be used effectively as a bioassay for available iron.
4. Humic extract contains available iron.
5. Metabolized iron in lysed A. carteri cells is slowly made available to viable A. carteri cells.
6. The release of available iron from lysed cells is greatly accelerated by exposure of the dead cells to a low pH.

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