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Quantitative growth relationships of Microcoleus vaginatus (gom.) ex Drouet and Alcaligenes sp. in a sucrose-salts medium

James Rexford Brownell

QUANTITATIVE GROWTH RELATIONSHIPS OF *MICROCOLEUS VAGINATUS* (GOM.) EX DROUET AND *ALCALIGENES SP.* IN A SUCROSE-SALTS MEDIUM

by

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ABSTRACT

The blue-green alga, Microcoleus vaginatus (Gom.) ex Drouet 1968, and a bacterium, Alcaligenes sp., collected from the same habitat, were cultured together and separately in mineral salts media with and without sucrose. Growth of axenic M. vaginatus was stimulated by sucrose. In association with the bacterium in sucrose-containing media algal growth was suppressed, while in inorganic media it was stimulated. Factors involved in this algal-bacterial system probably included competition for the carbon source, changes in pH, and accumulation of cellular waste and lytic products.

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INTRODUCTION

Although Henry (1966) believes that planktonic cyanophytes always are associated with bacteria in a symbiotic relationship, the association needs further consideration, as little evidence to support this hypothesis is to be found in the literature.

Legge and Dingeldein (1970) described the mutual supportive relationship between blue-green algae and bacteria as a symbiosis that dates back to the dawn of life. Bacteria degrade organic matter and produce carbon dioxide that algae use in photosynthesis. Algae liberate oxygen that bacteria use in aerobic respiration.

Silvey and Roach (1964) stated the need for more specific algal-bacterial cultural studies and indicated that more specific identification of the directing forces of microbiotic cycles must be relegated to detailed laboratory investigations. They also deduced from their studies that blue-green algae appear to be the best sources of nutrition for gram-negative heterotrophic bacilli, found often in great numbers within gelatinous algal sheaths.

Lange (1967, 1970, 1971) has reported a stimulatory effect of added carbohydrates to cyanophyte-bacterial systems, in which the algae are autotrophic and fast growing. He has demonstrated that the algae are stimulated in the association by bacterial production of CO_2.

Ward et al. (1964) deduced from their study of algal-bacterial cultures lacking organic nutrients that: "Multiplication of heterotrophic bacteria in association with autotrophic algal cultures in inorganic medium would require the presence of a metabolizable carbon source, presumably of algal origin. The bacteria could obtain sustenance through a parasitic or pathogenic mode released during cell division, or by relying on compounds excreted by algal cells during growth."

Ward also found that the growth of Chlorella sp. was reduced when the alga was cultured with bacteria associated with it in nature. The bacterium that showed the greatest inhibition was a gram-negative bacillus, similar to the isolate used in the present study. In consideration of the works cited previously, some interrelationships between Microcoleus vaginatus and Alcaligenes sp. were investigated.

MATERIALS AND METHODS

I. Experimental organisms and preparation of inoculum.

Microcoleus vaginatus from a stream in Maymont Park, Richmond, Va. was collected and cultured axenically by Falls and Loos (1970). To prepare inoculum for use in experiments, the alga was placed in the center of plates of Allen's medium (Table 16) enriched with 1.5 g/l $NaNO_3$ and solidified with 1.3% agar. Plates were wrapped with four layers of

parafilm around the edge and incubated for one month in a growth chamber equipped with a fan. Ample illumination was found to be overhead and reflected light averaging 20 foot-candles at the level of the cultures. The photoperiod was 15 hours light and nine hours darkness following Lange (1967). The day temperature ranged from 22 to 30C.

The bacterium was isolated from a streak of freshly collected M. vaginatus and maintained on Difco nutrient agar. It may be described as a gram-negative, non-spore forming, motile rod with biochemical properties corresponding closely to those given for the genus Alcaligenes by Breed et al. (1957). Because of time limitations, it was not feasible in this study to identify the bacterium to the species level. The original culture was refrigerated after 48 hours growth at room temperature and subcultures were made monthly.

II. Experimental

A. Media

A stock solution of Allen's medium (Table 16) enriched with 3.0 g/l $NaNO_3$ was prepared. The stock medium was then divided into two portions; to one portion, sucrose was added at the rate of 0.5 g/l, and the remaining portion was used without modifications.

The media were distributed in volumes of 10 ml each to Pyrex culture tubes (15 x 1½cm). The tubes were then stoppered with cotton and sterilized by autoclaving at 121C for 15 minutes.

B. Experimental conditions

The experimental conditions were as follows:

1. the alga alone in sucrose medium (Aw)
2. the bacterium alone in sucrose medium (Bw)
3. the alga and the bacterium together in sucrose medium (ABw)
4. the alga and bacterium together in the sucrose-lacking medium (ABw/o)
5. the alga alone in sucrose-lacking medium (Aw/o)

One hundred tubes of each condition involving the alga and 50 tubes of Bw were inoculated. All inoculations were performed aseptically in an ultra-violet irradiated transfer chamber. Bacterial inoculum was 0.5 ml per tube from a 24 hour culture. The tube of inoculum was agitated between transfers to insure uniformity. Algal inocula were plugs cut from the periphery of agar plate colonies using a five mm cork borer, which was sterilized before opening each sealed plate.

The cultures were incubated one cm apart, in test tube racks under the same conditions as previously described for the preparation of the inoculum of alga.

C. Evaluation of growth

Data concerning the culture were collected on nine days: 4, 8, 19, 25, 32, 39, 46, 64, and 102. The choice of these specific times was based upon observed growth rates of previous cultures. Measurements included dry weights and indices of chlorophyll concentrations of the algal clumps,

turbidimetric measurements of bacteria and pH of all culture fluids. Subjective descriptions of cultures also were noted.

Ten cultures from each of the five experimental conditions were chosen at random at each harvest interval. Five cultures from each condition involving the alga were used for both pH and dry weight determinations, and the remainder for turbidimetric and chlorophyll tests. Bw cultures were used only for pH and turbidimetric analysis.

For pH determinations cultures were mixed by inverting once; the combination electrode of a Corning Model Seven pH Meter was then inserted into the cultures.

Following the pH determinations the supernatant culture fluid was decanted carefully. The algal clump remaining in the test tube was rinsed once with 10 ml of distilled water and transferred to a tared aluminum weighing cup. The algal mats were dried in an oven at 100C for 24 hours, cooled at room temperature for one hour in a glass desiccator and weighed to the nearest tenth of a milligram.

For turbidimetric measurements on the remaining five tubes of each set, the supernatant culture medium was decanted back and forth one time between the culture tube and a pre-rinsed colorimeter cuvette. The spectronic 20 colorimeter was calibrated against distilled water and transmission was measured at 550 mu.

The procedure for chlorophyll determinations followed that of Vishniac (1957). The cultures were first rinsed

carefully one time with 10 ml of distilled water, to remove salts and extracellular products that might interfere with the determination. Next, four ml of methanol was added to each culture. To insure thorough solvent penetration, the algal mats were crushed and scraped down the sides of the tube with a glass rod equipped with a rubber policeman. The mixture was allowed to stand for 10 minutes, after which the supernatant was decanted into a 10 ml graduated cylinder. This process was repeated once, following which the total volume of the extract was brought up to 10 ml with methanol. The percent transmittance of each solution was determined at 650 and 665 mu, using a Spectronic 20 colorimeter that had been calibrated against a methanol blank. Determinations were made immediately following extraction to minimize errors due to photolysis of the chlorophyll. Because of the closely similar readings obtained at both wavelengths, only those at 650 mu were used in analysis of results.

To check for possible bacterial contamination of the Aw and Aw/o cultures one ml of fluid from two tubes of each condition was transferred aseptically to nutrient broth at the time of the first and ninth harvests. No purity checks were run on Bw cultures. As a control, cultures of Alcaligenes sp. were inoculated into several tubes of the test medium without sucrose.

All but three ABw cultures at day 19 were enriched with 0.001 g/l EDTA in anticipation of a temporary revival of

algal growth as found by Lange (1967). At this time also, approximately 0.01 g of $NaNO_3$ was added to the three remaining ABw cultures to determine if a depletion of NO_3 had caused the cessation of algal growth.

Statistical analysis performed following Winer (1962) included two factor analysis of variance, tests for simple effects, listings of standard errors of the means, and the Newman-Keuls tests to determine the differences between ordered means. Arcsin transformations of the percent transmittance data were used in the statistical analyses.

RESULTS

There was substantial growth of Alcaligenes sp. in media in which sucrose was the only substantial organic ingredient, according to low percent transmittance and maintenance of a low pH (ABw & Bw, fig. 1 and 2). The growth peaked and dissipated by day 19. The bacterium did not grow in cultures lacking sucrose (Aw/o, fig. 1 and 2). No growth was evidenced in control cultures of bacteria alone without sucrose.

Algal growth was abundant in Aw, less in ABw/o, still still less in Aw/o (fig. 3 and 4). After day four, only one ABw culture remained viable, as evidenced by the reappearance of green color.

The standard error of the means was found to be low in all tests except in dry weights (Tables 1-4). At the 0.05

level of confidence significant F values were found from a two factor analysis of variance of the growth conditions, C, harvest times, H, and interaction between these factors, CH, in all tests, except for interaction of conditions and harvests in the percent transmittance of culture fluids (Tables 5-8).

Analysis of variance of the simple effects from the data of each quantitative technique with significant interaction gave all highly significant values. Significant differences were found between mean pairs using the Newman-Keuls test of the main effects of the percent transmittance of culture fluids (Tables 12a and b) and also the Newman-Keuls test of the simple effects of the remaining quantitative techniques (Tables 13a-15c).

No revival of growth was evidenced following the addition of either EDTA or $NaNO_3$ to ABw cultures after decline of growth.

Visible growth was produced initially only by the expansion of clumps of inoculum of the alga, which remained at the bottom of the tubes. After three weeks, considerable surface growth of the alga appeared in ABw/o cultures. This was often attached to the bottom clumps by long filaments and extended to attach to the glass about a centimeter above and below the surface of the medium.

As the level of the medium dropped due to evaporation the upper filament portions dried and became colorless. Also,

clumps of inoculum, lifted by bubbles of gas, rose sometimes to the surface.

DISCUSSION AND CONCLUSIONS

The chlorophyll extract and dry weight techniques are quick, simple and use no special equipment. They are especially valuable with Microcoleus vaginatus which because of its clumping nature prohibits the use of other tests.

Dry weights as a direct index of algal growth were not always paralleled by chlorophyll concentrations, because in older cultures the alga may continue to increase in dry weight for a time even after the chlorophyll decomposes. The maximum of $\pm$ 5.45 standard error among dry weight replicates (Table 3) is attributed to variation in algal clump size and to experimental error. The abrupt dry weight peaks evidenced in the ABw curve (fig. 3) represent error due to inadvertent fluctuations of the oven temperature. No dry weights were available for harvest eight because of loss of the cultures during processing. Reproducibility of weighings could have been improved by using glass weighing vials and staggered cooling intervals equalizing moisture uptake. Also, the filtration drying method developed by Ward et al. (1964) might have proved more reliable.

Spectrophotometric analysis of the culture fluids was found to be an adequate index of bacterial growth. The high

significance of Newman-Keuls differences was attributed partially to the use of several different Spectronic 20's from one harvest to another (Table 12b).

Although definite interrelationships were found between the organisms used in this work, no definite symbiotic relationship was demonstrated, possibly because Microcoleus vaginatus grows slowly under laboratory conditions. Lange (1971) related that the growth-enhancing effect of specific organic additives varies with algal species. The greater growth of M. vaginatus in sucrose media probably involved heterotrophic utilization of sugar (fig. 2, 3 and 4).

The alga and bacterium growing together in sucrose medium showed negligible algal growth possibly because of competition for oxygen. The high concentration of bacteria could have combined all available oxygen, including that from photosynthesis, thereby suppressing algal respiration. In Torpey's (1968) studies of the Thames estuary algae were found not to bloom until the bacterial peak of sewage decomposition had passed, making available sufficient dissolved O_2 for algal respiration and thereby utilization of accumulated CO_2.

Also, excretions of the living cells, along with decomposition products of dead algal and bacterial cells when accumulated could have inhibited growth of the alga (Lange 1971).

Rates of algal growth and decline, as based on the concentration of chlorophyll in the cultures, reveal that Aw cultures, although peaking earlier, fell off abruptly to a level below that of Aw/o upon termination of the experiment. The data indicate that the axenic alga utilized the sucrose and that growth therefore declined abruptly upon exhaustion of the sugar (fig. 2, 4). This sharp decline was not apparent in dry weights (fig. 3) because the cells had not yet broken down, even though the chlorophyll content had decreased.

At the time the cultures were terminated the pH of Aw cultures had abruptly dropped to the level of those in Bw, and below that of Aw/o and ABw/o. At this low pH, all cellular growth had stopped. Aw, ABw, and Bw arrived at this condition simultaneously (fig. 2).

The amount of algal growth in ABw/o was greater than Aw/o possibly due to the algal utilization of bacterial end products, even though the bacteria remained at a low concentration. The sharp decline of the alga in ABw/o cultures at the end of the experiment indicates a metabolic dependence upon bacterial end products. As in Aw cultures, ABw/o declined rapidly as the substrate was exhausted (fig. 2, 4).

The pH of ABw/o cultures rose faster and maintained the highest level until near the end of the run, even though growth of the alga was less than in Aw (fig. 2). This response was due probably to the high pH of end products

released from the bacteria and algae under this sub-optimal condition. Lange (1971) refers to an increase of pH in algal cultures.

The bacterium in Bw and ABw cultures grew well in sucrose media containing inorganic salts (fig. 1). The marked difference in bacterial growth rates of Bw and ABw cultures is attributed to the initial competitive action of the alga in ABw cultures for sucrose. Growth of bacteria dropped off sooner in Bw cultures than in ABw because the sucrose was evidently utilized earlier. The optical density became equal in both conditions at day 32, showing likely end points of bacterial metabolism in both media. The Bw peak of bacterial growth, based on percent transmittance (fig. 1) corresponds to the lowest amount of ABw/o algal growth according to dry weight (fig. 3), showing a temporary depression of algal growth caused by rapid bacterial growth. The late rapid growth of ABw/o, evidenced by the increase in pH at day 19 followed by an increase in dry weight was probably made possible when bacterial competition for available oxygen decreased (fig. 2, 3).

In summary, Microcoleus vaginatus was found to be slow growing in all of the experimental conditions. When grown axenically in a sucrose-containing medium growth was increased, apparently by heterotrophic utilization of the sugar. In the same medium in association with Alcaligenes sp., algal

growth was prevented. However, in a completely inorganic medium with the alga and bacterium together early growth of the alga was stimulated, while bacterial growth soon subsided. Other factors, contributed largely by the bacterium, that may have influenced algal growth were pH changes and waste products.

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Figure 1

Percent transmittance of culture fluids at 550 mu. The mean of five cultures plotted for the following conditions:

1. the alga alone in sucrose medium (Aw)
2. the bacterium alone in sucrose medium (Bw)
3. the alga and bacterium together in sucrose medium (ABw)
4. the alga and bacterium together in the sucrose-lacking medium (ABw/o)
5. the alga alone in sucrose-lacking medium (Aw/o)

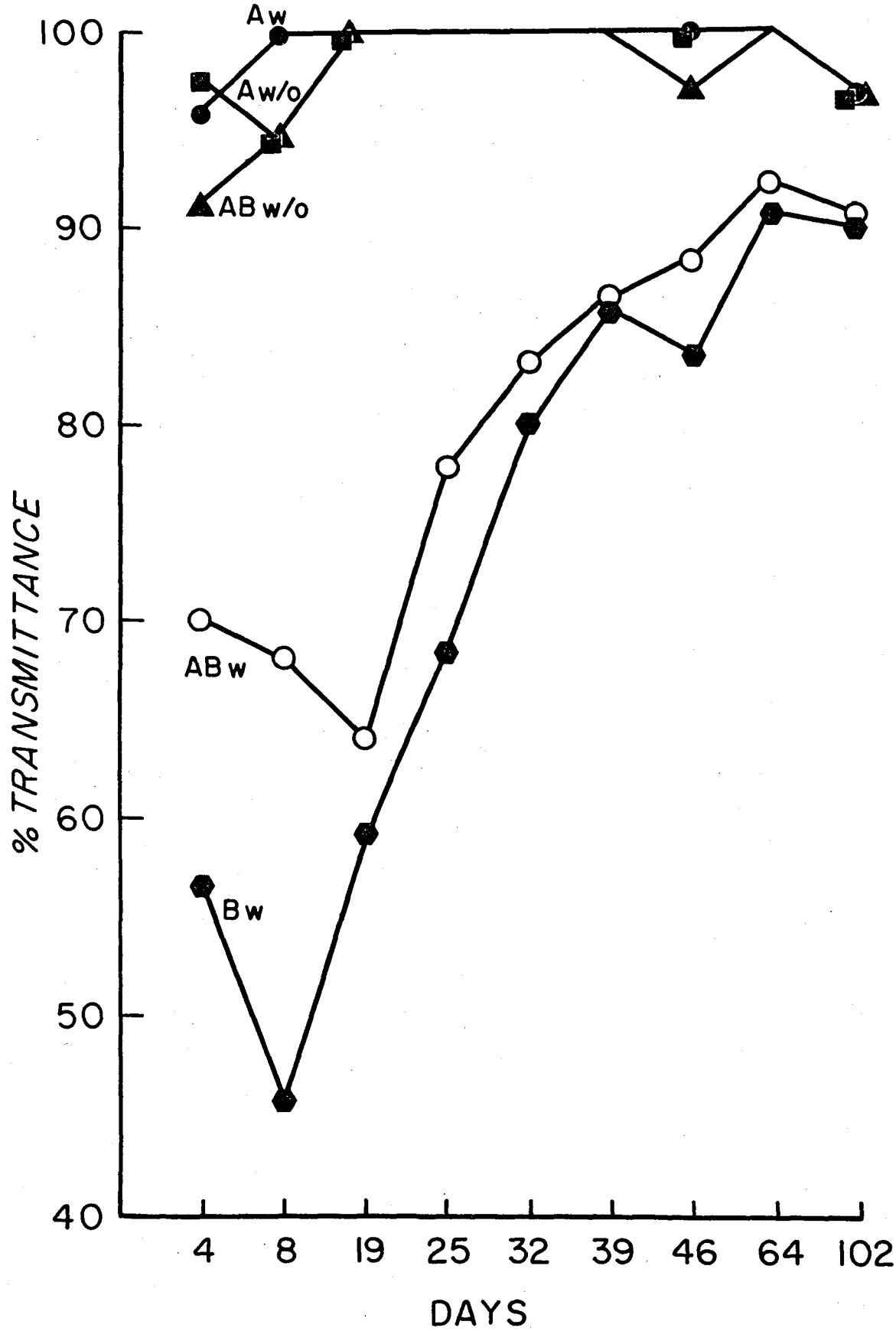
Aw
Aw/o
ABw/o
ABw
Bw
100
90
80
70
60
50
40
% TRANSMITTANCE
4
8
19
25
32
39
46
64
102
DAYS

Figure 2

The pH of culture fluids. The mean of five cultures plotted for the following conditions:

1. the alga alone in sucrose medium (Aw)
2. the bacterium alone in sucrose medium (Bw)
3. the alga and bacterium together in sucrose medium (ABw)
4. the alga and bacterium together in the sucrose-lacking medium (ABw/o)
5. the alga alone in sucrose-lacking medium (Aw/o)

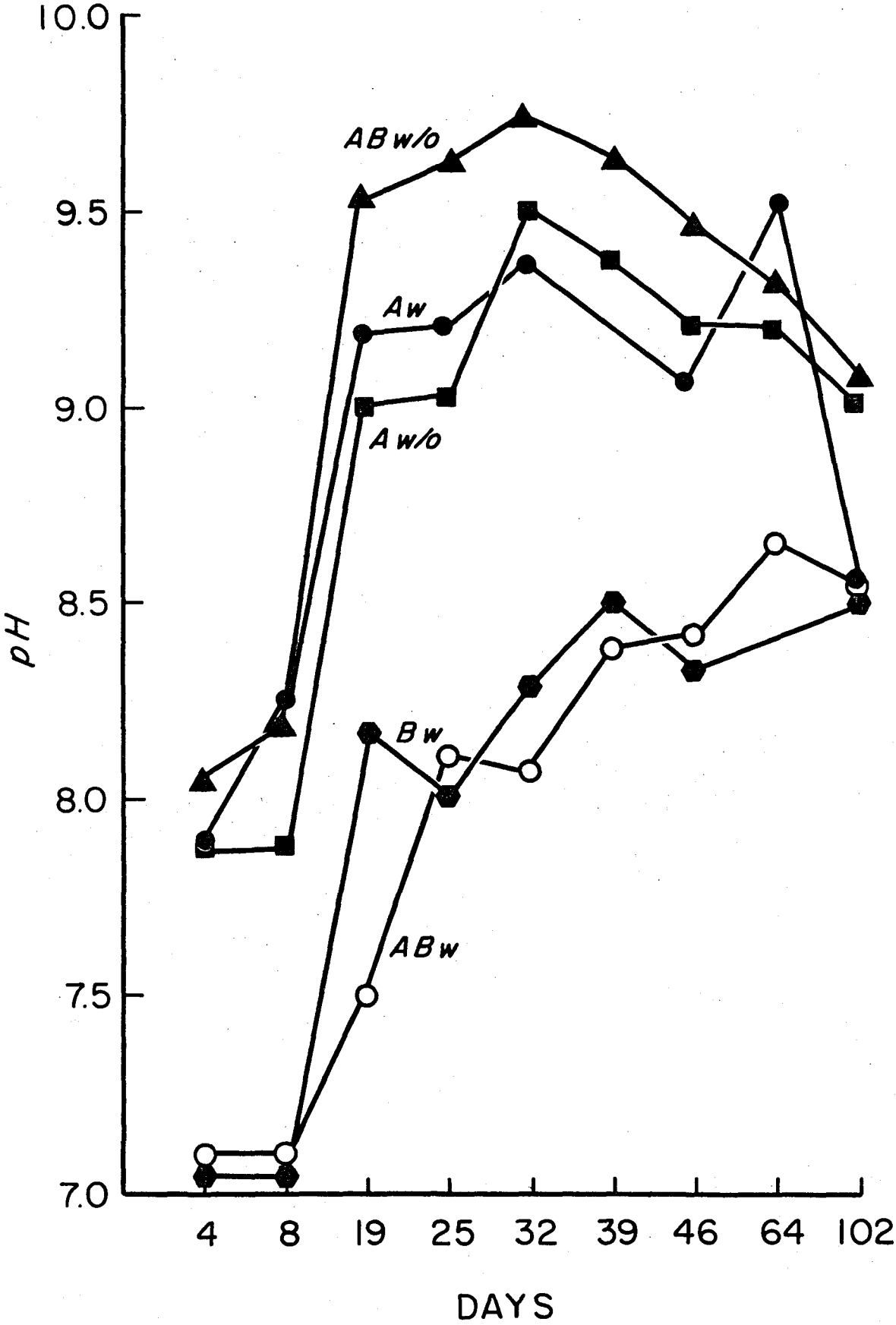
10.0
9.5
9.0
8.5
8.0
7.5
7.0
pH
ABw/o
Aw
Aw/o
Bw
ABw
4
8
19
25
32
39
46
64
102
DAYS

Figure 3

Dry weights in milligrams of algal clumps. The mean of five cultures plotted for the following conditions:

1. the alga alone in sucrose medium (Aw)
2. the alga and bacterium together in sucrose medium (ABw)
3. the alga and bacterium together in the sucrose-lacking medium (ABw/o)
4. the alga alone in sucrose-lacking medium (Aw/o)

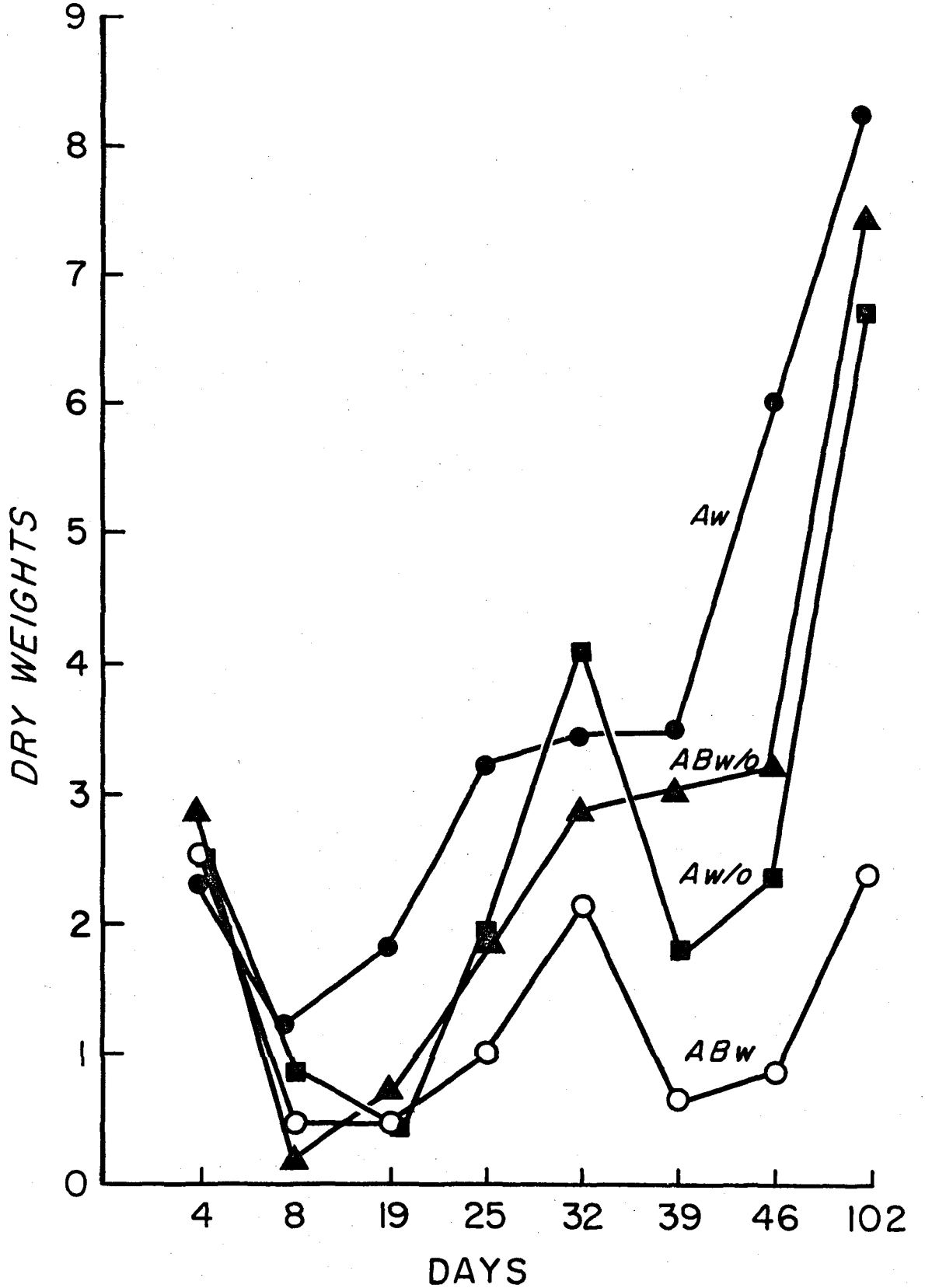
DRY WEIGHTS
9
8
7
6
5
4
3
2
1
0
4
8
19
25
32
39
46
102
DAYS
Aw
ABw/o
Aw/o
ABw

Figure 4

Percent transmittance at 650 mu of methanol extract of algal clumps. The mean of five cultures plotted for the following conditions:

1. the alga alone in sucrose medium (Aw)
2. the alga and bacterium together in sucrose medium (ABw)
3. the alga and bacterium together in the sucrose-lacking medium (ABw/o)
4. the alga alone in sucrose-lacking medium (Aw/o)

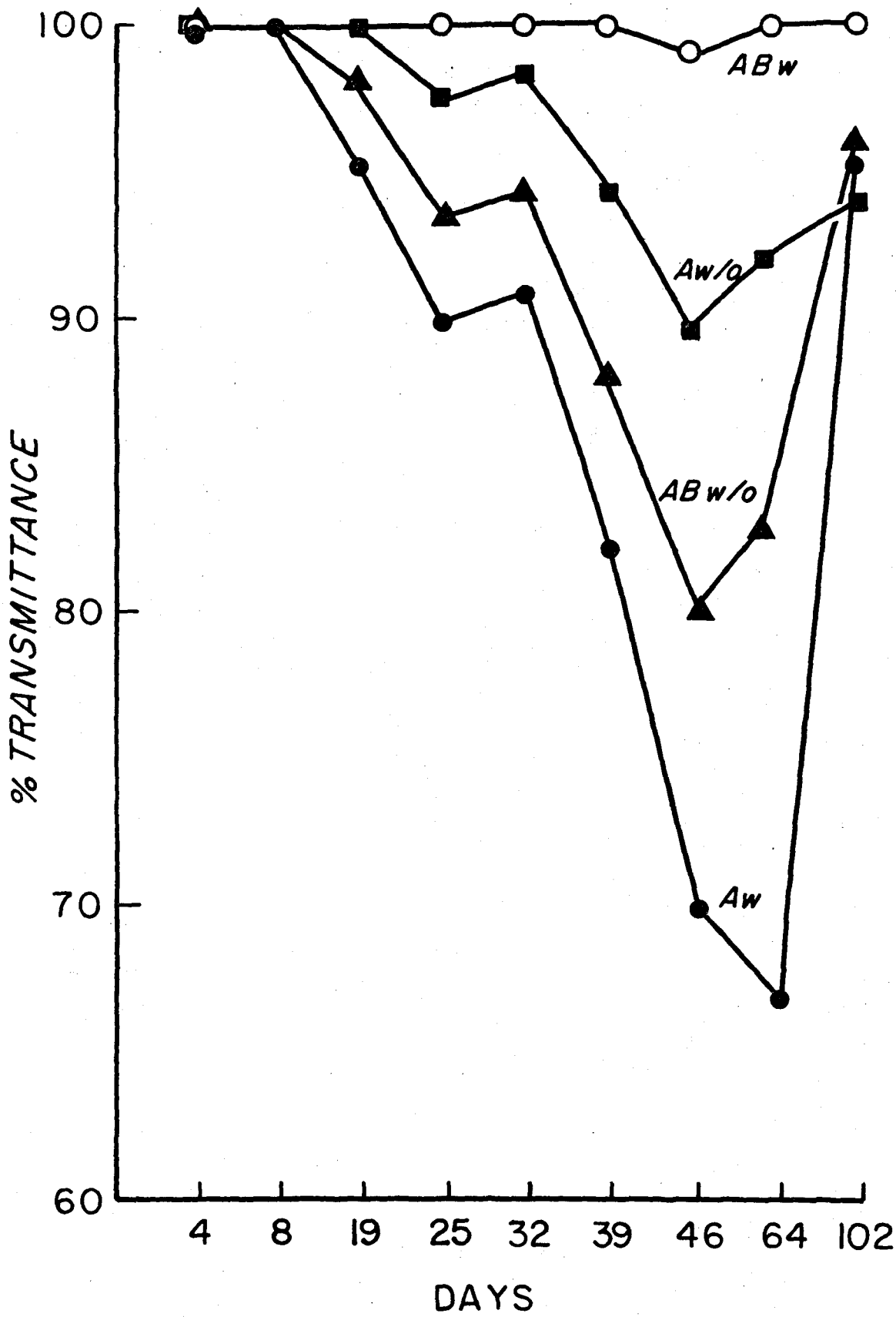
100
90
80
70
60
% TRANSMITTANCE
ABw
Aw/o
ABw/o
Aw
4
8
19
25
32
39
46
64
102
DAYS

Table 1

The mean and standard error of the percent transmittance of culture fluids at 550 mu for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o). Each value represents arcsin transformations of the mean ± the standard error of five cultures.

Conditions	Harvests								
	1	2	3	4	5	6	7	8	9
Aw	2.82 ±.02	2.91 ±.03	2.91 ±.03	2.94 ±0	2.91 ±.02	2.94 ±0	2.94 ±0	2.91 ±.02	2.70 ±.03
ABw	1.98 ±.01	1.95 ±.04	1.93 ±.05	2.07 ±.03	2.27 ±.02	2.39 ±.04	2.41 ±.04	2.47 ±.03	2.40 ±.01
Aw/o	2.83 ±.02	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.74 ±.02
ABw/o	2.64 ±.06	2.80 ±.01	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.86 ±.03	2.94 ±0	2.74 ±.02
Bw	1.79 ±.05	1.63 ±.05	1.78 ±.02	2.10 ±.03	2.26 ±.03	2.34 ±.03	2.33 ±.02	2.43 ±.01	2.41 ±.03

Table 2

The mean and standard error of the pH of culture fluids for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o). Each value represents the mean ± the standard error of five cultures.

Conditions	Harvests								
	1	2	3	4	5	6	7	8	9
Aw	8.04 ±.02	8.58 ±.05	9.16 ±.02	9.22 ±.15	9.28 ±.10	9.38 ±.12	9.40 ±.06	9.36 ±.12	8.60 ±.06
ABw	7.24 ±.02	7.20 ±.09	7.58 ±.08	8.18 ±.02	8.12 ±.04	8.46 ±.02	8.40 ±0	8.70 ±0	8.64 ±.02
Aw/o	7.92 ±.06	8.08 ±.06	9.00 ±.08	9.18 ±.14	9.56 ±.12	9.20 ±.11	9.10 ±.10	9.12 ±.04	8.94 ±.02
ABw/o	8.14 ±.04	8.34 ±.07	9.62 ±.07	9.58 ±.05	9.74 ±.02	9.56 ±.07	9.48 ±.05	9.20 ±.05	8.90 ±0
Bw	7.08 ±.04	7.10 ±.04	8.26 ±.02	8.12 ±.02	8.28 ±.02	8.48 ±.02	8.42 ±.02	8.36 ±.02	8.54 ±.02

Table 3

The mean and standard error of dry weights of algal clumps for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o). Each value represents the mean ± the standard error of five cultures.

Conditions	Harvests							
	1	2	3	4	5	6	7	8
Aw	22.80 ± .86	8.20 ±2.52	15.80 ±1.50	29.20 ±3.79	31.60 ±4.32	34.60 ±3.88	64.00 ±5.17	84.60 ±3.26
ABw	23.00 ±3.51	4.60 ± .68	3.80 ± .66	10.80 ±1.93	18.00 ±2.77	6.60 ±1.50	9.80 ±1.65	23.00 ±2.66
Aw/o	25.00 ±1.14	6.80 ±1.11	4.20 ±1.16	22.40 ±3.50	36.40 ±5.45	18.60 ±3.71	21.60 ±3.06	64.80 ±2.85
ABw/o	25.60 ±1.81	3.20 ± .37	5.00 ±1.00	21.00 ±1.82	25.80 ±1.43	28.60 ±3.70	34.00 ±3.96	74.80 ±3.87

Table 4

The mean and standard error of percent transmittance of methanol extract at 650 mu for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o). Each value represents arcsin transformations of the mean ± the standard error of five cultures.

Conditions	Harvests								
	1	2	3	4	5	6	7	8	9
Aw	2.94 ±0	2.94 ±0	2.74 ±.02	2.50 ±.05	2.56 ±.03	2.25 ±.05	2.20 ±.05	2.04 ±.04	2.80 ±.02
ABw	2.94 ±0	2.91 ±.02	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0	2.94 ±0
Aw/o	2.94 ±0	2.94 ±0	2.84 ±.04	2.77 ±.02	2.86 ±.03	2.56 ±.07	2.64 ±.09	2.54 ±.04	2.67 ±.05
ABw/o	2.94 ±0	2.94 ±0	2.74 ±.04	2.67 ±.09	2.71 ±.04	2.45 ±.03	2.39 ±.01	2.39 ±.03	2.81 ±.08

Table 5

Main effects for the percent transmittance of culture fluids at 550 mu (C), the harvest times (H), and the interaction between the two.

ANOV Summary Table

Source of Variation	SS	df	MS	F
Between conditions	40.13	24		
C	28.41	4	7.10	12.03*
error a	11.72	20	0.59	
Within conditions	23.16	200	0.115	
H	2.83	8	0.35	3.50*
C X H	3.96	32	0.12	1.20*
error b	16.37	160	0.10	
Total	63.29	224		

*$F_{.95}$=2.89 *$F_{.95}$=2.00 *$F_{.95}$=1.54

Table 6

Main effects for the pH of culture fluids (C), the harvest times (H), and the interaction between the two.

ANOV Summary Table

Source of Variation	SS	df	MS	F
Between conditions	0.54	24		
C	0.52	4	0.13	129*
error a	0.03	20	0.001	
Within conditions	0.62	200	0.003	
H	0.51	8	0.06	9.14*
C x H	0.10	32	0.003	4.28*
error b	0.01	160	0.0007	
Total	1.17	224		

*$F_{.95}$=2.87 *$F_{.95}$=2.00 *$F_{.95}$=1.54

Table 7

Main effects for the dry weights of algal clumps (C), the harvest times (H), and the interaction between the two.

ANOV Summary Table

Source of Variation	SS	df	MS	F
Between conditions	12,146	19		
C	11,600	3	3,866.67	113.29*
error a	546	16	34.13	
Within conditions	60,458	140		
H	42,590	7	6,084.29	121.08*
C x H	12,237	21	582.71	11.59*
error b	5,631	112	50.28	
Total	72,604	159		

*$F_{.95}$=3.24 *$F_{.95}$=2.09 *$F_{.95}$=1.68

Table 8

Main effects for the percent transmittance of methanol extract of algal clumps at 650 mu (C), the harvest times (H), and the interaction between the two.

ANOV Summary Table

Source of Variation	SS	df	MS	F
Between conditions	4.14	19		
C	4.08	3	1.36	340*
error a	0.06	16	0.004	
Within conditions	8.14	160		
H	5.94	8	0.74	92.50*
C x H	1.16	24	0.05	6.25*
error b	1.04	128	0.008	
Total	12.28	179		

*$F_{.95}$=3.24 *$F_{.95}$=2.01 *$F_{.95}$=1.60

Table 9

Simple effects for harvests (H) and pH of culture fluids (C) for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

ANOV Summary Table

Source of Variation	SS	df	MS	F
H at C(Aw)	18,271.11	8	2,283.89	585,612.50*
H at C(ABw)	14,667.74	8	1,833.47	470,119.87*
H at C(Aw/o)	17,871.77	8	2,233.97	572,813.14*
H at C(ABw/o)	18,933.77	8	2,366.72	606,851.60*
H at C(Bw)	14,714.05	8	1,839.26	471,604.17*
Within conditions	0.063	160	0.004	

*$F_{.95} = 2.00$

Table 10

Simple effects for the dry weights of algal clumps (C), and the harvests (H).

ANOV Summary Table

Source of Variation	SS	df	MS	F
H at C(Aw)	378,419.65	7	54,059.95	50.07*
H at C(ABw)	41,833.55	7	5,976.22	5.54*
H at C(Aw/o)	188,155.02	7	26,879.29	24.90*
H at C(ABw/o)	234,128.75	7	33,446.96	30.98*
Within conditions	60,458.00	56	1,079.61	

*$F_{.95} = 2.17$

Table 11

Simple effects for harvests (H) and percent transmittance of methanol extract at 650 mu (C) for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

ANOV Summary Table

Source of Variation	SS	df	MS	F
H at C(Aw)	484.45	8	60.57	946.41*
H at C(ABw)	1,937.06	8	242.13	3,783.32*
H at C(Aw/o)	1,705.25	8	213.16	3,330.57*
H at C(ABw/o)	1,611.33	8	201.42	3,147.12*
Within conditions	8.14	128	0.064	

*$F_{.95}=2.02$

Table 12a

Newman-Keuls test of main effects of ordered mean differences of the arcsin transformations of percent transmittance of culture fluids at 550 mu for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Conditions = {n's

	Bw	ABw	ABw/o	Aw	Aw/o
	95.30	99.30	128.76	129.91	130.76
95.30		4.00*	33.46*	34.61*	35.46*
99.30			29.46*	30.61*	31.46*
128.76				1.15*	2.00*
129.91					.85*

* Significant values; see Table 12b

Table 12b

Newman-Keuls test of ordered mean differences of the arcsin transformations of the percent transmittance of culture fluids at 550 mu.

Harvests = ξn's

	1	2	3	4	9	5	7	6	8
	60.32	61.13	62.48	64.94	64.96	66.64	67.40	67.69	68.47
60.32		.81*	2.16*	4.62*	4.64*	6.32*	7.08*	7.37*	8.15*
61.13			1.35*	3.81*	3.83*	5.51*	6.27*	6.56*	7.34*
62.48				2.46*	2.48*	4.16*	4.12*	5.21*	5.99*
64.94					.02	1.70*	2.46*	2.75*	3.53*
64.96						1.68*	2.44*	2.73*	3.51*
66.64							.76*	1.05*	1.83*
67.40								.29	1.07*
67.69									.78*

* Significant values

$S_{CH} = MS_H$ Within conditions n=0.15

	r=2	r=3	r=4	r=5	r=6	r=7	r=8	r=9
q.95(r.160)	2.79	3.35	3.68	3.91	4.09	4.23	4.35	4.47
S_{CH} x q.95(r.160)	.42	.50	.55	.59	.61	.63	.65	.67

Table 13a

Newman-Keuls test of simple effects of ordered mean differences of pH of culture fluids (C) for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 1

C	Bw	ABw	Aw/o	Aw	ABw/o
	7.08	7.24	7.92	8.04	8.14
7.08		.16*	.84*	.96*	1.06*
7.24			.68*	.80*	.90*
7.92				.12*	.22*
8.04					.10*

Harvest 2

C	Bw	ABw	Aw/o	ABw/o	Aw
	7.10	7.20	8.08	8.34	8.55
7.10		.10*	.88*	1.14*	1.45*
7.20			.78*	1.04*	1.35*
8.08				.26*	.47*
8.34					.21*

Harvest 3

C	ABw	Bw	Aw/o	Aw	ABw/o
	7.58	8.26	9.00	9.16	9.62
7.58		.68*	1.42*	1.58*	2.04*
8.26			.74*	.90*	1.36*
9.00				.16*	.62*
9.16					.46*

* Significant values; see Table 13c.

Table 13b

Newman-Keuls test of simple effects of ordered mean differences of pH of culture fluids (C) continued for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 4

C	Bw	ABw	Aw/o	Aw	ABw/o
	8.12	8.18	9.18	9.22	9.58
8.12		.06	1.06*	1.10*	1.46*
8.18			1.00*	1.04*	1.40*
9.18				.04	.40*
9.22					.36*

Harvest 5

C	ABw	Bw	Aw	Aw/o	ABw/o
	8.12	8.28	9.28	9.56	9.74
8.12		.16*	1.16*	1.44*	1.62*
8.28			1.00*	1.28*	1.46*
9.28				.28*	.46*
9.56					.18*

Harvest 6

C	ABw	Bw	Aw/o	Aw	ABw/o
	8.46	8.48	9.20	9.38	9.56
8.46		.02	.74*	.92*	1.10*
8.48			.72*	.90*	1.08*
9.20				.18*	.36*
9.38					.18*

* Significant values; see Table 13c

Table 13c

Newman-Keuls test of simple effects of ordered mean differences of pH of culture fluids (C) continued for the following conditions: the alga alone in sucrose medium (Aw); the bacterium alone in sucrose medium (Bw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 7

C	ABw	Bw	Aw/o	Aw	ABw/o
	8.40	8.42	9.10	9.40	9.48
8.40		.02	.70*	1.00*	1.08*
8.42			.68*	.98*	1.06*
9.10				.30*	.38*
9.40					.08

Harvest 8

C	Bw	ABw	Aw/o	ABw/o	Aw
	8.36	8.70	9.12	9.20	9.36
8.36		.66*	.24*	.16*	1.00*
8.70			.44*	.50*	.66*
9.12				.08*	.24*
9.20					.16*

Harvest 9

C	Bw	Aw	ABw	ABw/o	Aw/o
	8.54	8.60	8.64	8.90	8.94
8.54		.06	.10*	.36*	.40*
8.60			.04	.30*	.34*
8.64				.26*	.30*
8.90					.04

* Significant values

$S_{CH} = MS_H$ Within conditions n=0.024

	r=2	r=3	r=4	r=5
q.95(r,160)	2.79	3.35	3.67	3.90
S_{CH} x q.95(r,160)	.07	.08	.09	.09

Table 14a

Newman-Keuls test of simple effects of ordered mean differences of dry weights of algal clumps (C) for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).*

Harvest 1

C	Aw	ABw	Aw/o	ABw/o
	22.80	23.00	25.00	25.60
22.80		.20	2.20	2.80
23.00			2.00	2.60
25.00				.60

Harvest 2

C	ABw/o	ABw	Aw/o	Aw
	3.20	4.60	6.80	8.20
3.20		1.40	1.60	5.00
4.60			2.20	3.60
6.80				1.40

Harvest 3

C	ABw	Aw/o	ABw/o	Aw
	3.80	4.20	5.00	15.80
3.80		.40	1.20	12.00
4.20			.80	11.60
5.00				10.80

*None of the above values were significant.

Table 14b

Newman-Keuls test of simple effects of ordered mean differences of dry weights of algal clumps (C) continued for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 4

C	ABw	ABw/o	Aw/o	Aw
	10.80	21.00	22.40	29.20
10.80		10.20	11.60	18.40
21.00			1.40	8.20
22.40				6.80

Harvest 5

C	ABw	ABw/o	Aw	Aw/o
	18.00	25.80	31.60	36.40
18.00		7.80	13.60	18.40
25.80			5.80	10.60
31.60				4.80

Harvest 6

C	ABw	Aw/o	ABw/o	Aw
	6.60	18.60	28.60	34.60
6.60		12.00	22.00	28.00*
18.60			10.00	16.00
28.60				6.00

* Significant values; see Table 14c.

Table 14c

Newman-Keuls test of simple effects of ordered mean differences of dry weights of algal clumps (C) continued for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 7

C	ABw	Aw/o	ABw/o	Aw
	9.80	21.60	34.00	64.00
9.80		11.80	24.20	54.20*
21.60			2.60	32.60
34.00				30.20

Harvest 8

C	ABw	Aw/o	ABw/o	Aw
	23.00	64.80	74.80	84.60
23.00		41.80*	51.80*	61.60*
64.80			10.00	19.80
74.80				9.80

* Significant values

$S_{CH} = MS_H$ Within conditions n=0.024

	r=2	r=3	r=4
q.95(r,112)	2.81	3.37	3.70
S_{CH} x q.95(r,112)	26.13	31.34	34.41

Table 15a

Newman-Keuls test of simple effects of ordered mean differences of the arcsin transformations of percent transmittance of methanol extract at 650 mu (C) for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).*

Harvest 1

C	Aw	ABw	Aw/o	ABw/o
	2.94	2.94	2.94	2.94

Harvest 2

C	ABw	Aw	Aw/o	ABw/o
	2.91	2.94	2.94	2.94
2.91		.03	.03	.03

Harvest 3

C	Aw	ABw/o	Aw/o	ABw
	2.74	2.74	2.84	2.94
2.74			.10	.20
2.74			.10	.20
2.84				.10

* None of the above values are significant.

Table 15b

Newman-Keuls test of simple effects of ordered mean differences of the arcsin transformations of percent transmittance of methanol extract at 650 mu (C) continued for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 4

C	Aw	ABw/o	Aw/o	ABw
	2.50	2.67	2.77	2.94
2.50		.17	.27	.44*
2.67			.10	.27
2.77				.17

Harvest 5

C	Aw	ABw/o	Aw/o	ABw
	2.56	2.71	2.86	2.94
2.56		.15	.30	.38*
2.71			.15	.23
2.86				.08

Harvest 6

C	Aw	ABw/o	Aw/o	ABw
	2.25	2.45	2.56	2.94
2.25		.20	.31	.69*
2.45			.11	.49*
2.56				.38*

* Significant values; see Table 15c.

Table 15c

Newman-Keuls test of simple effects of ordered mean differences of the arcsin transformations of percent transmittance of methanol extract at 650 mu (C) continued for the following conditions: the alga alone in sucrose medium (Aw); the alga and bacterium together in sucrose medium (ABw); the alga and bacterium together in the sucrose-lacking medium (ABw/o); the alga alone in sucrose-lacking medium (Aw/o).

Harvest 7

C	Aw	ABw/o	Aw/o	ABw
	2.20	2.39	2.64	2.94
2.20		.19	.44*	.74*
2.39			.25	.55*
2.64				.30

Harvest 8

C	Aw	ABw/o	Aw/o	ABw
	2.04	2.39	2.54	2.94
2.04		.35*	.50*	.90*
2.39			.15	.55*
2.54				.40*

Harvest 9

C	Aw/o	Aw	ABw/o	ABw
	2.67	2.80	2.81	2.94
2.67		.13	.14	.27
2.80			.01	.14
2.81				.13

* Significant values

$S_{CH} = MS_H$ Within conditions n=0.024

	r=2	r=3	r=4
q.95(r,128)	2.80	3.36	3.69
S_{CH} x q.95(r,128)	.28	.34	.37

Table 16

Modified medium of Hughes et al. (Allen 1968). Amounts per liter of distilled water.

macroelements	grams
$NaNO_3$	1.5
K_2HPO_4	0.039
$MgSO_4 \cdot 7H_2O$	0.075
Na_2CO_3	0.02
$CaCl_2$	0.027
$Na_2SiO_3 \cdot 9H_2O$	0.058
EDTA	0.001
$H_3C_6H_5O_7 \cdot H_2O$	0.006
$FeC_6H_5O_7 \cdot xH_2O$	0.006

microelements	milligrams
H_3BO_4	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.222
$Na_2MoO_4 \cdot 2H_2O$	0.391
$CuSO_4 \cdot 5H_2O$	0.079
$Co(NO_3)_2 \cdot 6H_2O$	0.0494

VITA

James Rexford Brownell was born on April 14, 1946 in Leesburg, Virginia. He was married in May, 1970 to the former Miss Martha Jean Saunders. They have one child, Samuel.

He attended Loudoun Valley High School from which he graduated in June 1964. The following September he began study at Virginia Polytechnic Institute and State University in Blacksburg, Va. from which he received a Bachelor of Science Degree in Biology in June 1969. He began graduate study in Biology at the University of Richmond in September 1969. His requirements for the Master of Science Degree in Biology were completed in August 1972.