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EFFECTS OF PHYTOPLANKTON ON THE GROWTH OF BACTERIA UNDER EXPERIMENTAL CONDITIONS

UTJECAJ FITOPLANKTONA NA RAST BAKTERIJA U EKSPERIMENTALNIM UVJETIMA

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The paper deals with the effects of phytoplankton species (*Tetraselmis suecica, Dunaliella tertiolecta, Phaeodactylum tricornutum*) on the growth of bacterial strains (*Vibrio, Flavobacterium, Moraxella, Pseudomonas, Alcaligenes, Aeromonas, Acinetobacter*) in mixed cultures at varying ecological enviromental factors (temperature, salinity, light intensity, enrichment of media with yeast extract, glucose and vitamis B_1 and B_{12}).

Bacterial growth was also observed in live and dead cultures and in the algal culture filtrate, as well as the effects of phytoplankton on bacterial growth in dependence of growth stage of the algal population.

The algae *T. suecica* and *D. tertiolecta* inhibited the growth of all bacteria. The alga *P. tricornutum* stimulated the growth of bacterium *Flavobacterium sp.*, did not affect *Acinetobacter sp.* and inhibited that of all other bacteria.

INTRODUCTION

Phytoplankton organisms as primary producers and heterotrophic bacteria as decomposers of organic matter and regenerators of primary nutrients, are closely connected with the complex processes of the cycling of matter in the sea.

In addition to their relationship within food chains, the interactions of these organisms through their excretes are also of importance. It is well known that excretion products of phytoplankton and bacterial organisms may have both stimulative and inhibitive effects on the organisms in their environment (Lucas, 1955).

An intensive growth of bacteria in the presence of some algae several authors attributed to the dying of algal cells which enrich thus the environment with easily accessible organic matter providing a favourable substrate

for bacterial growth (Waksman and Hotchkiss, 1937, Steeman-Nielsen, 1955; Fukmani *et al.*, 1981). It was also found that the metabolic products of phytoplankton may stimulate bacterial growth as potential nutrients (Hellebust, 1965; Brock, 1966; Fogg, 1966; Vela and Guerra, 1966; Berland *et al.*, 1970. Alexander, 1971; Niewolak, 1971; Samuel *et al.*, 1971; Thomas, 1971; Whittaker and Feeny, 1971; Belly *et al.*, 1973; Allen and Garrett, 1977). Many authors reported the number of bacteria and their activity to increase in the vicinty of the zone of phytoplankton bloom (Wood, 1963; Vaccaro *et al.*, 1968). In the report by Bell and Mitchell (1972) a term »phicosphere« was used to denote the zone in the vicinity of phytoplankton bloom stimulative for bacterial growth.

On the other hand, many authors established that phytoplankton organisms produced antibacterial substances having inhibitory or even lethal effects on individual bacteria (Waksman *et al.*, 1938., Jorgensen, 1956; Sieburth, 1959, 1964a, b; Burkholder *et al.*, 1960, Droop and Elson, 1966; Duff *et al.*, 1966; Berland and Maestrini, 1969).

In the present paper we studied the effects of phytoplankton species *Tetraselmis suecica, Dunaliella tertiolecta* and *Phaeodactylum tricornutum* on the growth of several bacterial strains.

MATERIAL AND METHODS

Seven bacterial strains of genera *Vibrio, Flavobacterium, Moraxella, Pseudomonas, Alcaligenes, Aeromonas* and *Acinetobacter* and axenic monocultures of three phytoplankton species *Tetraselmis suecica (Kylin) Butch* (Prasinophyceae), *Dunaliela tertiolecta Butcher* (Chlorophyceae) ond *Phaeodactylum tricornutum Bohlin* (Bacillariophyceae) were used in the experiment.

Bacterial strains were isolated from the Kaštela Bay (the Adriatic Sea) and identified by several identification schemes (Shewan *et al.*, 1960a, 1960b; Hendrie *et al.*, 1964; Gibson *et. al.*, 1977).

Algal cultures were grown in Walne's medium (Walne, 1956) with 10 mg/l Na_2SiO_3 added to the medium in which the alga *P. tricornutum* was grown. The medium was pre-autoclaved at 121°C for 15 min for sterilization. When algae reached necessary concentrations of 3×10^4 to 1×10^5 cells /l — *T. suecia,* 7×10^5 to 1×10^6 cells/l — *D. tertiolecta* and 8×10^4 to 1×10^5 cells/l — *P. tricornutum,* 100 ml of each culture was placed in 300 ml E. flasks to which bacterial cells were inoculated. Flasks where bacterial and algal cultures were grown separately under the same conditions were used as controls. Flasks with cultures were placed on the shaker throughout the experiment to prevent sedimentation of cells to flask walls.

Phytoplankton and bacterial growth was observed every 24 h. Algal cells were counted on light microscope with the use of hemocytometer. Bacterial growth was observed by inoculation to ZoBell's 2216 medium (Zo Bell, 1946).

Effects of individual phytoplankton species on the growth of isolated bacterial strains was studied under varying experimental conditions (Table 1).

Table 1. Experiment

Parameter		Alga used in the experiment
I Environmental factors		
1. Temperature	a) 17°C b) 21°C c) 25°C	*T. suecica, D. tertiolecta* *P. tricornutum*
2. Salinity	a) 15×10^{-3} b) 25×10^{-3} c) 35×10^{-3}	*T. suecica, D. tertiolecta* *P. tricornutum*
3. Light intensity	a) 800 Lx b) 2000 Lx c) 4000 Lx	*T. suecica, D. tertiolecta* *P. tricornutum*
II Organic matter		
1. Yeast extract	a) O mgl^{-1} b) 100 mgl^{-1} c) 500 mgl^{-1}	*P. tricornutum*
2. Glucose	a) O mgl^{-1} b) 50 mgl^{-1} c) 500 mgl^{-1}	*D. tertiolecta*
3. Vitamins ($B_1 + B_{12}$)	a) $10^{-1} + 10^{-3}$ μg ml^{-1} »low« b) $1 + 10^{-2}$ μg ml-1 »medium« c) $10 + 10^{-1}$ μg ml^{-1} »high«	
III Algal culture		
1. State of culture	a) live b) dead c) culture filtrate	*D. tertiolecta* *P. tricornutum*
2. Growth stage	a) Lag stage (Â day old culture) b) Exponential stage (4 day old culture) c) Stationary stage (7 day old culture)	

The experiment was carried out under 24-h light regime (flourescent lamps of 40W, 5200 K-daily light).

Filtrate of the *P. tricornutum* culture was obtained by filtering the culture in the exponential growth stage through a 0.45 μm Millipore HA filter.

RESULTS

Three types of algal effects on the growth of studied bacterial strains were established (Table 2).

Table 2. M/C ratio (growth rate of bacteria in mixed culture to growth rate of bacteria in controls, during the exponential growth stage)

	T. suecica	*D. tertiolecta*	*P. tricornutum*
Vibrio	0.64	0.69	0.93
Flavobacterium	0.79	0.96	1.20
Moraxella	0.60	0.85	0.87
Pseudomonas	0.75	0.75	0.91
Alcaligenes	0.64	0.86	0.90
Aeromonas	0.72	0.89	0.91
Acinetobacter	0.91	0.86	0.99

Algae *T. suecica* and *D. tertiolecta* inhibited the growth of all bacterial strains. Alga *P. tricornutum*, however, stimulated the growth of *Flavobacterium* sp., not affecting at all the growth of the *Acinetobacter* sp. and inhibiting the growth of all the other bacteria.

Three types of algal effects on bacterial growth are shown in Figs. 1, 2 and 3. It may be observed that the algal effects were most intensive between the third and fifth day, that is in the period of algal exponential growth, irrespective of the fact whether it stimulated or inhibited bacterial growth. In the initial growth stage (lag stage) and at the end of the experiment, when algae reached stationary stage, their, effects on bacterial growth were considerably reduced.

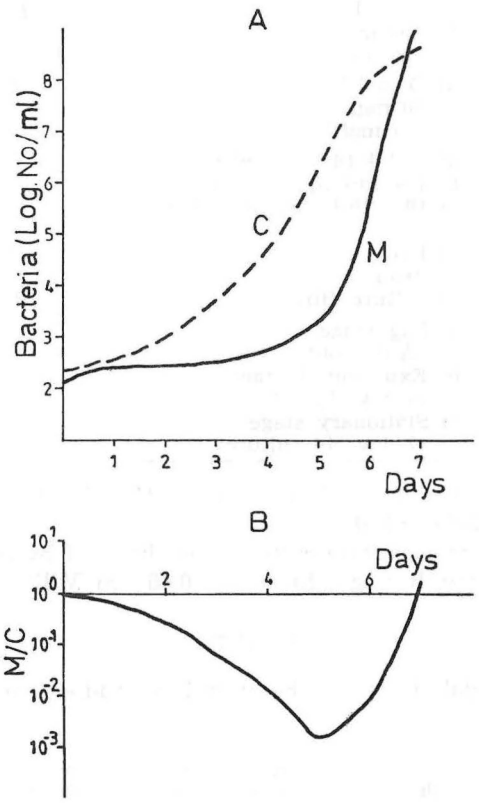

Fig. 1. A) Growth of ***Alcaligenes*** in mixed culture with *T. suecica* (M) and in the controls (C)
B) Relationship between the growth of *Alcaligenes* in the mixed culture and controls (M/C ratio)

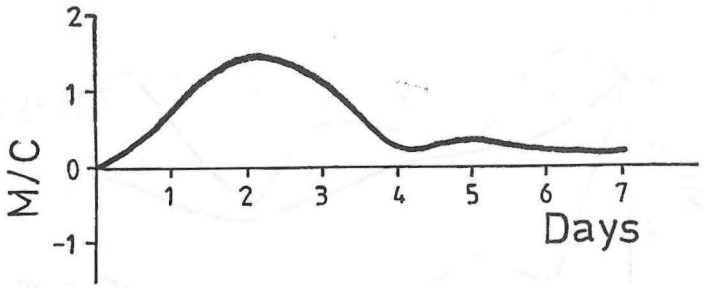

Fig. 2. M/C ratio for *Flavobacterium* in the culture with *P. tricornutum*

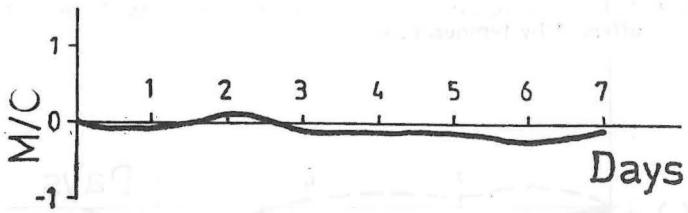

Fig. 3. M/C ratio for *Acinetobacter* in the culture with *P. tricornutum*

Temperature

Temperature increase intensified the influence of algae on bacterial growth (Table 3).

Table 3. M/C ratio as affected by temperature

	T. suecica			*D. tertiolecta*			*P. tricornutum*		
	25°C	21°C	17°C	25°C	21°C	17°C	25°C	21°C	17°C
Vibrio	0.62	0.69	0.86	0.41	0.55	1.06	0.93	0.84	1.04
Flavobacterium	0.58	0.65	1.09	1.06	0.85	1.42	1.26	1.17	1.16
Moraxella	0.41	0.64	0.69	0.48	0.83	1.21	0.80	0.86	0.94
Pseudomonas	0.78	0.69	0.65	0.66	0.71	0.90	0.90	0.93	0.95
Alcaligenes	0.58	0.76	0.87	0.69	0.86	1.03	0.90	0.90	0.99
Aeromonas	0.60	0.63	0.67	0.76	0.86	1.02	0.89	0.93	0.94
Acinetobacter	0.90	0.90	0.90	0.81	0.89	1.07	0.84	0.98	1.08

So the inhibitory influence of *T. suecica* and *D. tertiolecta* on bacterial growth was most pronouced at 25°C temperature, and much less pronounced at lower temperatures (21 and 17°C) (Figs. 4, 5).

At the same time stimulative influence of *P. tricornutum* on *Flavobacterium* sp. growth was intensified by temperature increase (Fig. 6).

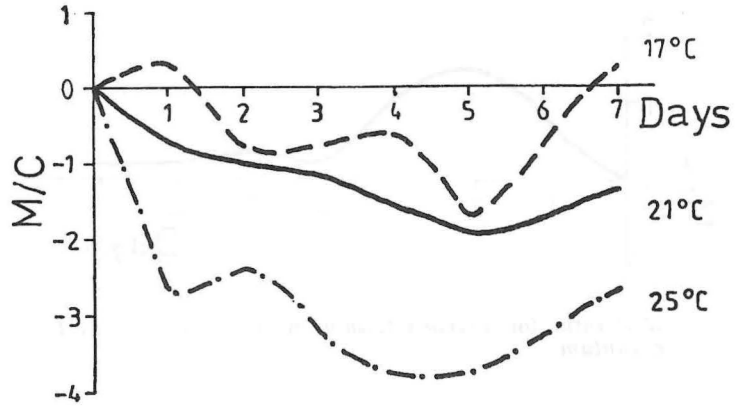

Fig. 4. M/C ratio for *Alcaligenes* in the culture with *T. suecica* as affected by temperature

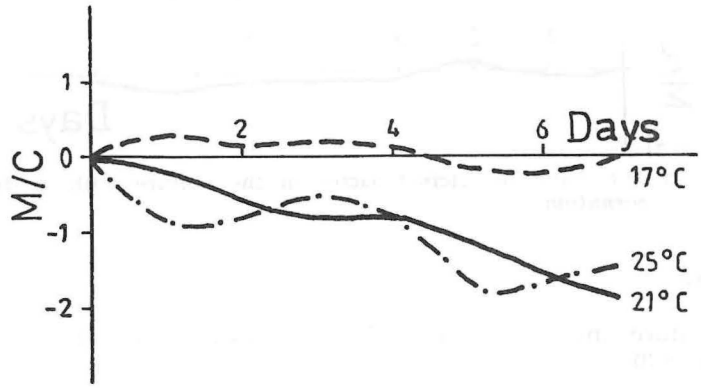

Fig. 5. M/C ratio for *Vibrio* in the culture with *D. tertiolecta* as affected by temperature

Salinity

At different salinities individual algae affected differently bacterial growth (Table 4).

Table 4. M/C ratio as affected by salinity

	T. suecica			*D. tertiolecta*			*P. tricornutum*		
	35‰	25‰	15‰	35‰	25‰	15‰	35‰	25‰	15‰
Vibrio	0.69	0.30	1.08	0.41	0.97	0.71	0.96	0.98	0.97
Flavobacterium	0.65	0.72	1.18	1.06	0.86	0.92	1.29	1.26	1.02
Moraxella	0.64	0.53	0.70	0.48	1.37	0.89	0.80	0.85	0.94
Pseudomonas	0.69	0.64	1.20	0.66	0.93	0.76	0.90	0.86	0.88
Alcaligenes	0.76	0.32	0.91	0.69	0.93	0.99	0.90	0.87	0.88
Aeromonas	0.72	0.69	1.02	0.76	1.20	0.91	0.89	0.90	1.06
Acinetobacter	0.90	0.92	0.92	0.81	0.96	0.92	0.84	1.05	1.00

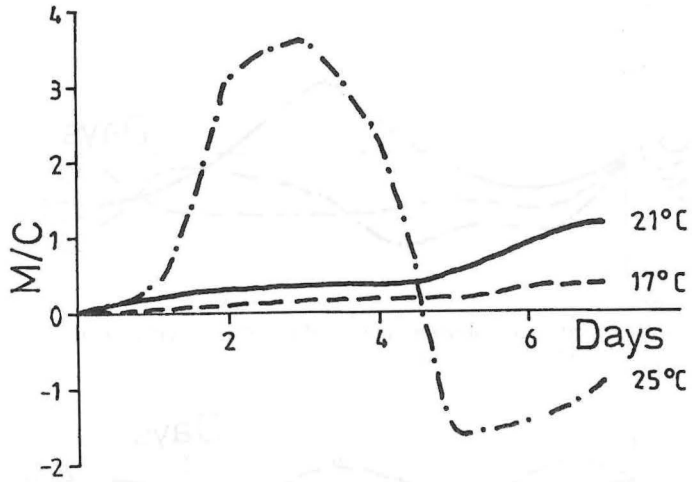

Fig. 6. M/C ratio for *Flavobacterium* in the culture with *P. tricornutum* as affected by temperature

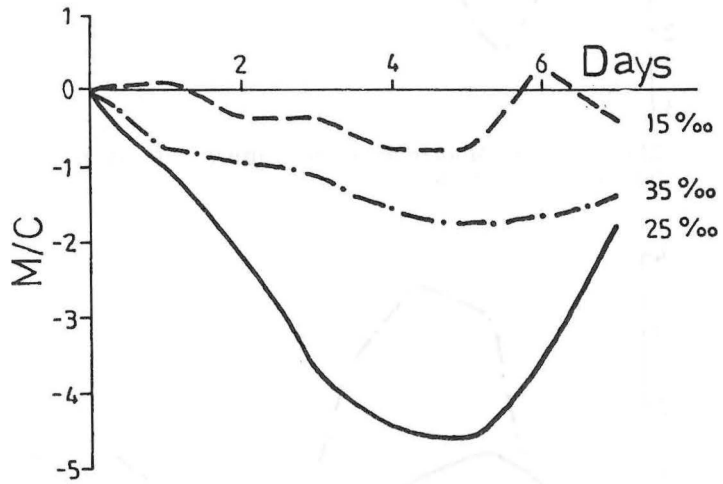

Fig. 7. M/C ratio for *Alcaligenes* in the culture with *T. suecica* as affected by salinity

It was found that *T. suecica* inhibited most the bacterial growth at 25×10^{-3} salinity. At higher salinity of 35×10^{-3} inhibitory influence was reduced, while at 15×10^{-3} salinity no inhibitory influence was recorded (Fig. 7).

As to the algae *D. tertiolecta* and *P. tricornutum* salinity variations were less important for the intensity of their influence on bacteria. However, they inhibited bacterial growth most frequently at 35×10^{-3} salinity. (Figs. 8, 9).

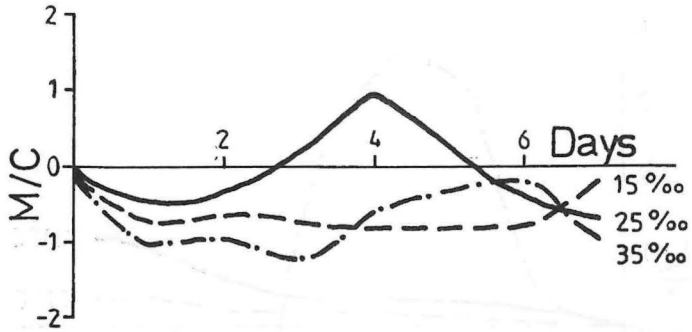

Fig. 8. M/C ratio for *Aeromonas* in the culture with *D. tertiolecta* as affected by salinity

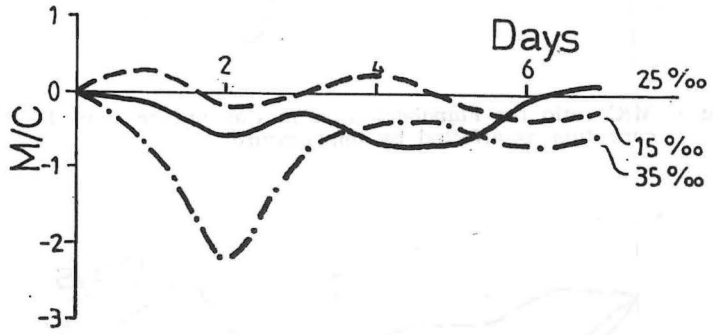

Fig. 9. M/C ratio for *Moraxella* in the culture with *P. tricornutum* as affected by salinity

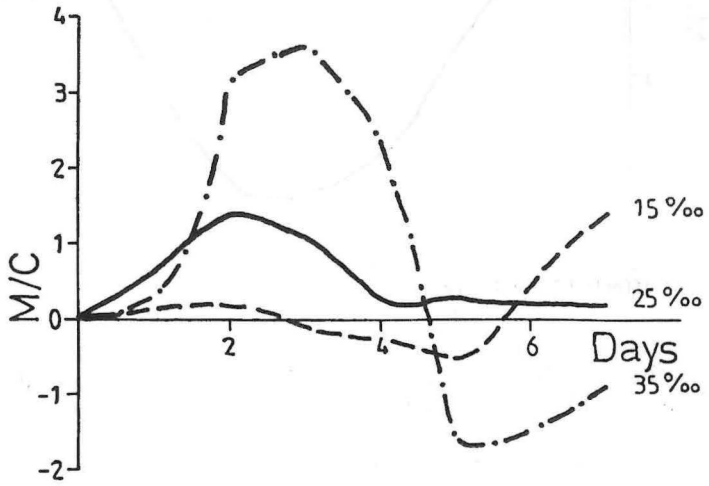

Fig. 10. M/C ratio for *Flavobacterium* in the culture with *P. tricornutum* as affected by salinity

Intensity of stimulative influence of *P. tricornutum* on the growth of *Flavobacterium sp.* was best pronounced at 35×10^{-3} decreasing at lower salinities (Fig. 10).

Light intensity

Increased light intensity resulted in intensified influence of algae on bacteria (Table 5).

Table 5. M/C ratio as affected by light intensity

	T. suecica			*D. tertiolecta*			*P. tricornutum*		
	4000Lx	2000Lx	800Lx	4000Lx	2000Lx	800Lx	4000Lx	2000Lx	800Lx
Vibrio	0.59	0.30	0.64	0.71	0.55	0.82	0.81	0.86	0.98
Flavobacterium	0.93	0.72	0.58	0.70	0.85	0.92	1.25	1.17	1.18
Moraxella	0.32	0.53	0.92	0.72	0.83	0.87	0.85	0.86	0.93
Pseudomonas	0.68	0.63	0.81	0.55	0.71	0.90	0.83	0.93	0.98
Alcaligenes	0.45	0.32	0.78	0.78	0.86	0.91	0.88	0.90	0.84
Aeromonas	0.59	0.69	0.91	0.74	0.86	0.88	0.87	0.93	0.82
Acinetobacter	0.87	0.94	0.95	0.52	0.89	0.93	1.12	0.98	0.95

The most intensive inhibitory (Figs. 11, 12) and stimulative (Fig. 13) influence of algae on bacterial growth was recorded at 4000 Lx. The intensity of influence decreased with light intensity reduction.

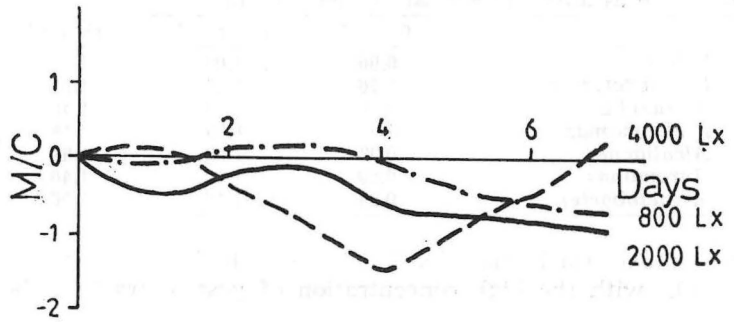

Fig. 11. M/C ratio for *Pseudomonas* in the culture with *D. tertiolecta* as affected by light intensity

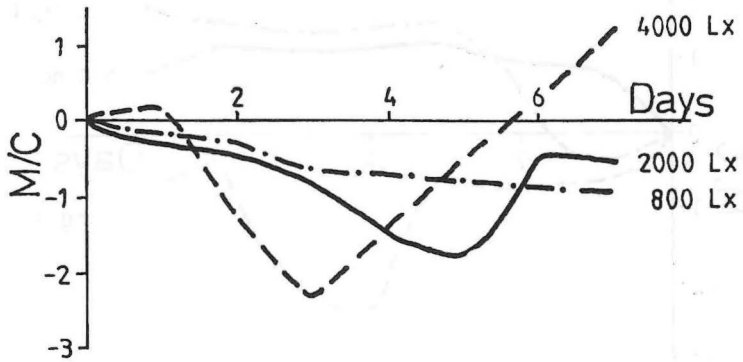

Fig. 12. M/C ratio for *Moraxella* in the culture with *T. suecica* as affected by light intensity

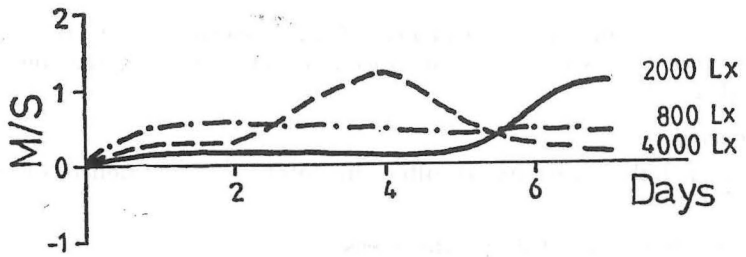

Fig. 13. M/C ratio for *Flavobacterium* in the culture with *P. tricornutum* as affected by light intensity

Adding of organic substances to the medium

Yeast extract

Yeast extract added to the medium affected better bacterial growth in the mixed culture with the alga *P. tricornutum* than in the controls as distinct from the medium without yeast extract, in which *P. tricornutum* inhibited the growth of all studied bacterial strains except that of *Flavobacterium sp.* (Table 6).

Table 6. M/C ratio as affected by yeast extract concentration

	0 mg/l	100 mg/l	500 mg/l
Vibrio	0.96	1.08	1.11
Flavobacterium	1.26	1.23	1.13
Moraxella	0.80	1.53	1.48
Pseudomonas	0.90	1.19	1.26
Alcaligenes	0.93	1.03	1.61
Aeromonas	0.89	1.42	1.40
Acinetobacter	0.84	1.12	1.26

Growth of bacteria in the mixed culture with the algae was better than in the controls with the high concentration of yest extract in the medium (Fig. 14)

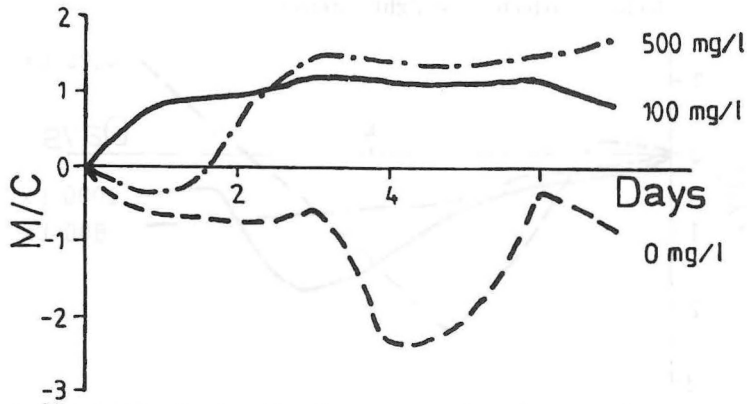

Fig. 14. M/C ratio for *Pseudomonas* in the culture with *P. tricornutum* as affected by the yeast extract concentration

On the other hand, added yeast extract caused poorer stimulative effects of *P. tricornutum* on the growth of *Flavobacterium sp.* (Fig. 15).

Glucose

Glucose added to the medium considerably increased inhibitory influence of *D. tertiolecta* on bacterial growth (Table 7).

Table 7. M/C ratio as affected by glucose concentrations

	0 mg/l	50 mg/l	500 mg/l
Vibrio	0.75	0.62	0.41
Flavobacterium	0.80	0.72	0.54
Moraxella	0.78	0.71	0.52
Pseudomonas	0.81	0.73	0.61
Alcaligenes	0.73	0.64	0.38
Aeromonas	0.83	0.72	0.52
Acinetobacter	0.92	0.88	0.78

Inhibition of bacterial growth was more pronounced with the higher glucose concentration (Fig. 16).

Vitamins

Adding of vitamins B_1 and B_{12} also intensified inhibitory influence of alga *D. tertiolecta* on bacterial growth (Table 8).

Table 8. M/C ratio as affected by vitamins B_1 and B_{12} concentrations

	High	Medium	Low
Vibrio	0.43	0.43	0.55
Flavobacterium	0.78	0.79	0.85
Moraxella	0.53	0.54	0.83
Pseudomonas	0.47	0.65	0.71
Alcaligenes	0.67	0.83	0.86
Aeromonas	0.69	0.79	0.86
Acinetobacter	0.68	0.74	0.89

Inhibitory effects were better pronounced with higher vitamin concentrations (Fig. 17).

Growth of bacteria in the live and dead (autoclaved) algal culture and in the filtrate of algal culture

Filtrate of culture of alga *D. tertiolecta* also inhibited the growth of studied bacteria, although to a lesser extent than live algal culture. As shown by Table 9, only the growth of he strains of *Flavobacterium sp.* and *Acinetobacter sp.* was not inhibited in algal culture filtrate.

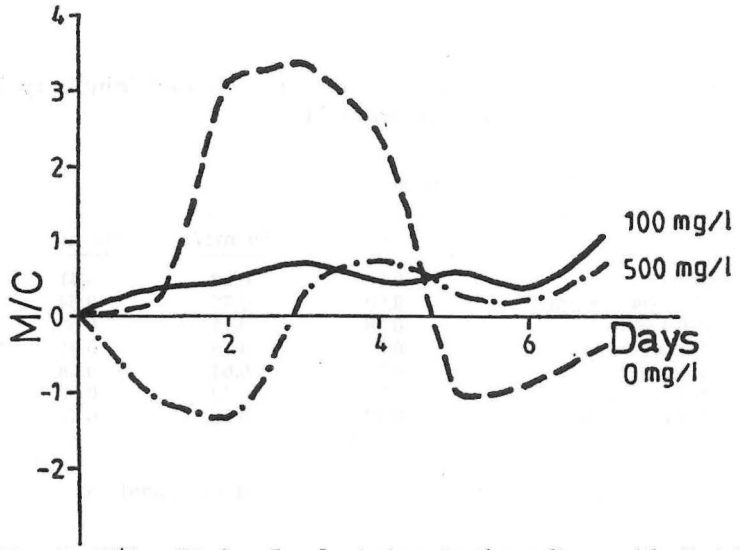

Fig. 15. M/C ratio for *flavobacterium* in the culture with *P. tricornutum* as affected by yeast extract concentration

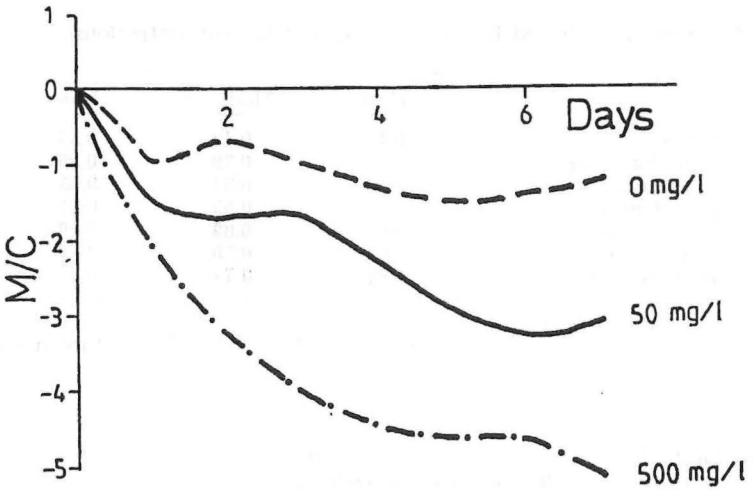

Fig. 16. M/C ratio for *Vibrio* in the culture with *D. tertiolecta* as affected by glucose concentration

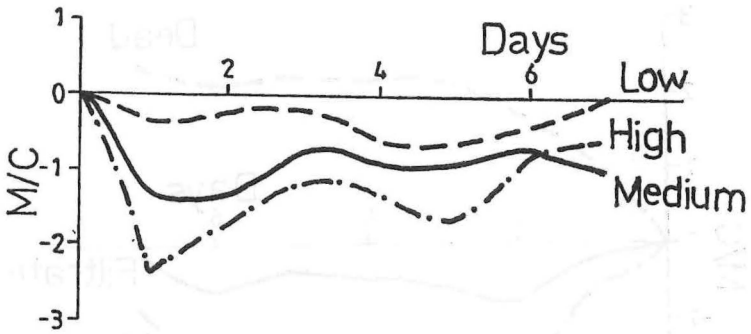

Fig. 17. M/C ratio for *Aeromonas* in the culture with *D. tertiolecta* as affected by vitamins concentration

Table 9. M/C ratio in the live and dead culture as well as in the filtrate of the culture of alga *D. tertiolecta*

	Live	Filtrate	Dead
Vibrio	0.33	0.68	1.23
Flavobacterium	0.84	1.02	1.39
Moraxella	0.82	0.81	1.47
Pseudomonas	0.86	0.95	1.44
Alcaligenes	0.63	0.84	1.45
Aeromonas	0.63	0.79	1.50
Acinetobacter	0.75	1.01	1.34

On the other hand, dead algal cells enriched the medium with organic matter liable to bacterial degradation which stimulated the growth of studied bacteria (Fig. 18).

Growth of bacteria in mixed culture with alga P. tricornutum *with respect to the growth stage of alga*

The alga *P. tricornutum* affected bacterial growth much more actively during the exponential (log) growth stage than during the initial (lag) stage (Table 10).

Table 10. M/C ratio as affected by the growth stages of the agla *P. tricornutum*

	Lag	Exp.	Stat.
Vibrio	0.97	0.72	1.28
Flavobacterium	1.27	2.35	1.93
Moraxella	0.86	0.83	1.80
Pseudomonas	0.94	0.91	1.38
Alcaligenes	0.92	0.78	1.63
Aeromonas	0.94	0.70	1.31
Acinetobacter	1.02	1.00	2.12

Accordingly, both stimulative effects on the growth of the strain of *Flavobacterium sp.* (Fig. 19) and inhibitory effects on other studied bacteria were best pronounced during exponential stage of *P. tricornutum* growth. (Fig. 20).

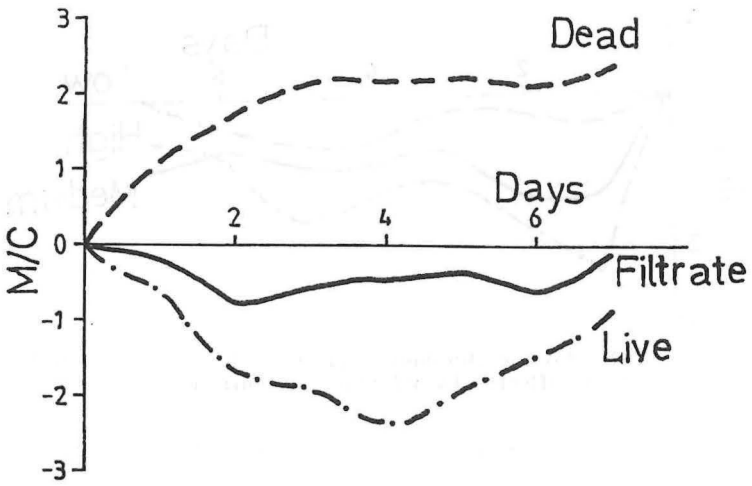

Fig. 18. M/C ratio for *Pseudomonas* in live and dead culture of *D. tertiolecta*

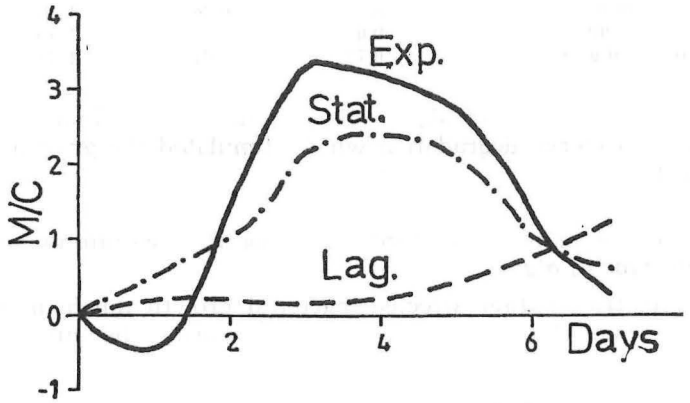

Fig. 19. M/C ratio for *Flavobacterium* in the culture with *P. tricornutum* as affected by the growth stage of alga

However, growth of all studied bacteria in the culture with *P. tricornutum* was stimulated when this alga was in the stationary stage. This stimulation, however, is very likely only a passive reflection of the climax of the culture and intensified dying of algal cells.

These results point to the conclusion that the intensity of influence of algae on bacteria, irrespective of the fact whether this influence is stimulative or inhibitive, is directly dependent on the growt intensity of the algal population itself (Figs. 21, 22, 23).

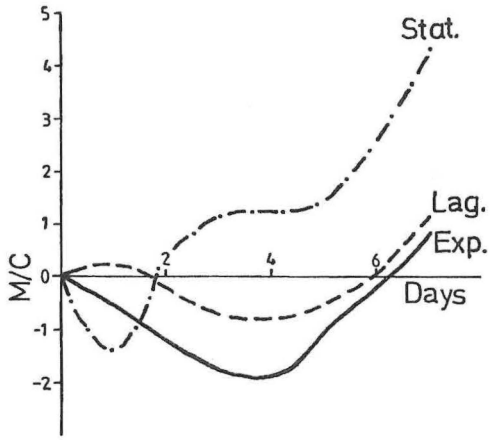

Fig. 20. M/C ratio for *Aeromonas* in the culture with *P. tricornutum* as affected by the growth stage of alga

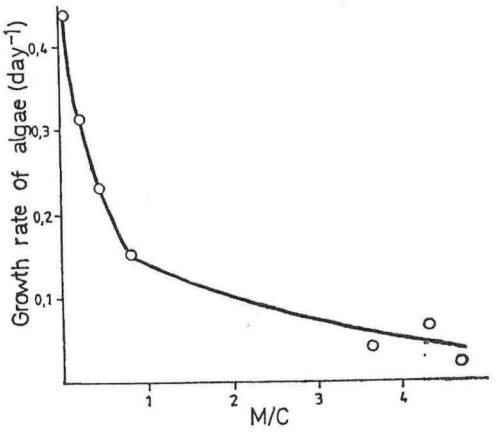

Fig. 21. Dependence of the degree of inhibition of *Pseudomonas* (expressed as M/C ratio) on the growth intensity of *T. suecica* (expressed as growth rate)

DISCUSSION

Results pointed to the selective inhibition and stimulation of bacterial growth by phytoplankton. The algae *T. suecica* and *D. tertiolecta* inhibited the growth of all studied bacteria, while *P. tricornutum* stimulated the growth of *Flavobacterium sp,* did not affect *Acinetobacter sp.,* and inhibited the growth of remaining bacteria.

Similar results were reported by some other authors. Niewolak (1971) established inhibitory effects of phytoplankton species *Chlorella vulgaris* and

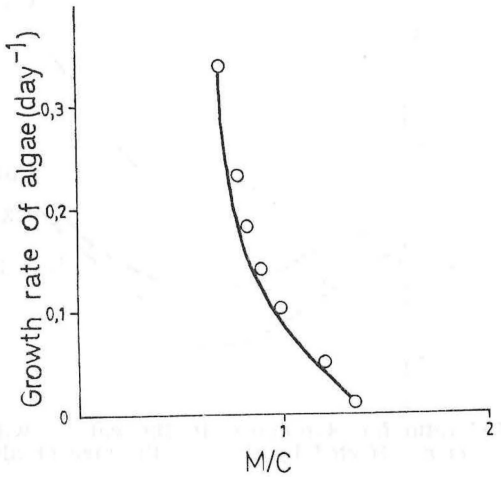

Fig. 22. Dependence of the degree of inhibition of *Aeromonas* (expressed as M/C ratio) on the growth intensity of *D. tertiolecta* (expressed as growth rate)

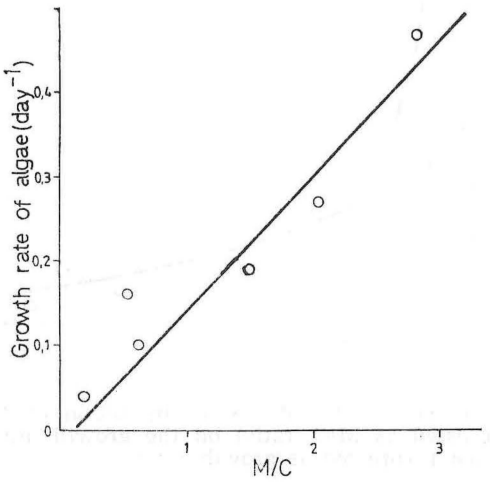

Fig. 23. Dependence of the degree of stimulation of *Flavobacterium* (expressed as M/C ratio) on the growth intensity of *P. tricornutum* (expressed as growth rate)

Scenedesmus obliquus on the bacterial strains *Azotobacter sp.*, *Pseudomonas sp.* and *Aeromonas sp.* Selective inhibition of the genus *Vibrio* by phytoplankton was discussed by Sieburth (1968) and Simidu *et al.*, (1977).

Results obtained by Kogure *et al.* (1979) are almost identical to the ours. They reported stimulative effects of the daiatom *Skeletonem costatum* on bacteria of the genera *Pseudomonas* and *Vibrio* and stimulation of the genus *Flavobacterium*, finding no essential difference in the growth between

mixed culture and controls as far as the genus *Acinetobacter* was concerned. The case of stimulation of the genus *Flavobacterium* by the alga *S. costatum*, which like *P. tricornutum*, used in our experiment, belongs to diatoms may be of interest. This phenomenon of stimulative effects of diatoms on the genus *Flavobacterium* was reported by a number of other authors. Sieburth (1968) found that the variations in the population of bacterial genus *Flavobacterium* corresponded to the variations in the diatom population. Bell *et al.* (1974) reported *S. costatum* to stimulate selectively individual bacterial strains. Kogure *et al.* (1979) reported that microscopic examinations showed the tendency of *Flavobacterium* strain to attach to the surface of algae what points to the specific interactions between these organisms. Jolley and Jones (1977) mentioned a stimulative interactions between the *Flavobacterium* genus and another diatom, *Navicula muralis*. Some authors suggested the symbiosis between the genus *Flavobacterium* and some diatoms (Sieburth, 1918; Shiba, 1978).

Results showed that the intensity of either inhibitory or stimulative influence of algae was, in the first place, dependent on the intensity of their population growth. Namely, more intensive the growth of algal population their overall cell activity was more intensive and therefore, very likely, the excretion of some substances which either inhibited or stimulated bacterial growth. The excretion of these substances to the outer environment was confirmed by the experiment with the algal culture filtrate which affected bacteria in the same manner as live algal culture. Similar results were obtained by Kogure *et al.*, (1979) who reported that the growth of the genera *Vibrio* and *Pseudomonas* was inhibited in the filtrate of the culture of *S. costatum*. Intensity of inhibitory influence of the filtrate was, like in our experiment, somewhat lower than that of the live culture. These authors suggested that this was due to the fact that bactericide excretes were present in the filtrate, but that the inhibition was less pronounced owing to the lability of these substances.

Temperature, salinity and light intensity, as well as some organic substances added (vitamins, glucose, yeast extract) affected primarily the growth intensity of algal population, and indirectly the intensity of their either inhibitory or simulative influnce on the bacterial growth.

Temperature increase considerably intensified algal activity, as reported by many authors (Jorgensen, 1968; Eppley, 1972; Golman and Carpenter, 1974; Goldman, 1977a, b).

Similar may be said for the light intensity, the increase of which stimulated algal growth and consequently the intensity of both their inhibitory and stimulative effects on baterial growth. Stimulative effects of light intensity on algal growth was reported by a number of authors (Steeman-Nielsen, 1955; Harris and Lott, 1973; Falkowski and Owens, 1978).

Salinity variations showed insignificant influence on the growth and activity of algae. This may be explained by the fact that all studied algae were euryhaline (Ben-Amotz and Avron, 1978; Laing and Utting, 1980; Fabergas *et al.*, 1984; 1985).

Adding of vitamins B_1 and B_{12} also caused intensified influence of algae on bacterial growth. This is in agreement with the resluts given by Droop

(1957, 1958), Provasoli (1958) and Braarud (1961) who found better growth of algae in the media enriched with vitamins. We established the same when added glucose. Yeast extract neutralized inhibitory influence of the alga *P. tricornutum* on bacetria. An identical phenomenon was reported by Kogure *et al.* (1979) for the alga *S. costatum.*

Inoculation of bacteria to diferent stages of growth of the *P. tricornutum* growth since dead cells were a good substrate for the development of bacteria. Similar results with the dead algal cells were reported by Niewolak (1971).

Inoculation of bacteria to different stages of growth of the *P. tricornutum* population showed its most intensive influence on bacteria during the exponential (log) stage of growth. During this stage *P. tricornutum* stimulated most the growth of *Flavobacterium sp.* and at the same time inhibited most the growth of other studied bateria. More intensive antibacterial activity during the exponential stage was also reported by Bell and Mitchell (1972). Influence of algae was considerably reduced during the lag stage, while bacterial growth was stimulated in the culture with the alga in the stationary growth stage, probably due to the climax of the culture and progressive dying of cells. This agrees with the results of Niewolak (1971) who established the inhibited growth of bactarial strains *Azotobacter* sp., *Pseudomonas* sp. and *Aeromonas* sp. during the exponential stage of growth of algae *Chlorella vulgaris* and *Scenedesmus obliquus* and sudden development of bacterial populations after the initiation of stationary stage. Similar results with other algal species were reported by Bell *et al.*, (1974), Kogure *et al.* (1979) and Fukami *et al.* (1981).

CONCLUSIONS

The results obtained by the present study show the following:

1. The algae *Tetraselmis suecica* and *Dunalliella tertiolecta* inhibited the growth of all studied bacteria. The alga *Phaeodactylum tricornutum* stimulated the growth of *Flavobacterium* sp., did not affect *Acinetobacter* sp. inhibiting the growth of all other studied bacteria.
2. Temperature increase caused intensified inhibition of bacterial growth. So the strongest inhibition of bacterial growth was recorded for all three studied algae at 25°C; it was slightly lower at 21°C and lowest at 17°C.
3. Increased light intensity also caused an increase of inhibitory effects on bacterial growth by all three algae. So the degree of inhibition was highest at 4000 Lx light intensity, slightly lower at 2000 Lx and lowest at 800 Lx.
4. The strongest inhibition by the alga *T. suecica* was found at 25‰ Sal. while the inhibition was less intensive at 15‰ and 35‰ Sal. The algae *D. tertiolecta* and *P. tricornutum* inhibited most intensively the bacterial growth at 35‰ Sal, while the intensity of inhibition decreased at lower salinities (25 and 15‰).
5. Adding yeast extract to the medium caused better growth of bacteria in mixed cultures with phytoplankton than in the controls.
6. Adding glucose to the medium as well as adding vitamins B_1 and B_{12} caused increased inhibition of bacterial growth by algae. So the degree

of inhibition was higher at glucose concentration of 500 mg/l than at 50 mg/l. In both cases inhibition was more intensive than when there was no glucose in the medium.

The highest degree of inhibition of bacterial growth was recorded at the concentration of 10 μg/ml — of vitamin B_1 and 0.1 μg/ml of vitamin B_{12}, than at two other concentrations which were lower for two and one order of magnitude respectively.

7. Algal culture filtrate less inhibited bacterial growth than live culture while dead algal cells stimulated bacterial growth.
8. Inhibitory or stimulative effects of algae on bacterial growth were most intesnive during exponential stage of algal growth, being considerably lower during the lag stage. During its stationary stage algal culture stimulated bacetrial growth.

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UTJECAJ FITOPLANKTONA NA RAST BAKTERIJA U EKSPERIMENTALNIM UVJETIMA

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KRATKI SADRŽAJ

U radu je ispitivan utjecaj fitoplanktonskih vrsta *(Tetraselmis suecica, Dunaliella tertiolecta, Phaeodactylum tricornutum)* na rast bakterijskih sojeva *(Vibrio, Flavobacterium, Moraxella, Pseudomonas, Alcaligenes, Aeromonas, Acinetobacter)* u mješovitim kulturama, pri promjeni različitih ekoloških faktora okoline (temperatura, salinitet, intenzitet svjetlosti, obogaćivanje medija kvaščevim ekstraktom, glukozom i vitaminima B_1 i B_{12}).

Također je ispitivan rast bakterija u živoj i mrtvoj kulturi, te filtratu kulture alga, kao i utjecaj fitoplanktona na rast bakterija u ovisnosti o fazi rasta u kojoj se nalazila populacija alga.

Alge *T. suecica* i *D. teritolecta* inhibirale su rast svih ispitivanih bakterija. Alga *P. tricornutum* je stimulirala rast bakterija *Flavobacterium sp.* a inhibirala sve ostale bakterije.

Povećanje temperature imalo je za posljedicu i povećanje stupnja inhibicije rasta bakterija. Tako je kod sve tri ispitivane alge zapažena najveća inhibicija bakterijskog rasta pri temperaturi od 25°C, da bi kod 21°C inhibicija bila nešto manja, a kod 17°C najmanja.

Povećani intenzitet svjetlosti također je uvjetovao povećanje stupnja inhibicije bakterijskog rasta od strane svih triju ispitivanih alga. Tako je stupanj inhibicije bio najveći kod intenziteta svjetlosti od 4000 Lx, nešto manji kod 2000 Lx, a najmanji kod 800 Lx.

Najveći stupanj inhibicije alga. *T. suecica* pokazala je kod saliniteta od 25‰, dok je kod saliniteta od 15‰ i 35‰ inhibicija bakterijskog rasta bila slabija. Alge *D. tertiolecta* i *P. tricornutum* su najjače inhibirale rast bakterija kod saliniteta od 35‰, dok pri nižim salinitetima (25‰ i 15‰) stupanj inhibicije opada.

Dodatak kvaščevog ekstrakta u medij imao je za posljedicu bolji rast bakterija u mješovitim kulturama s fitoplanktonom nego u kontroli.

Dodavanjem glukoze, te vitamina B1 i B12 u medij, stupanj inhibicije bakterijskog rasta od strane alga se povećao. Tako je stupanj ihibicije bio veći kod koncentracije glukoze od 500 mg/l, a u oba slučaja inhibicija je bila jače izražena nego kada u mediju nije bilo glukoze. Također je najveći stupanj inhibicije bakterijskog rasta ustanovljen kod koncentracija od 10 μg/ml vitamina B1 i 0,1 μg/ml vitamina B12, nego kod dviju drugih koncentracija koje su bile za jedan odnosno dva reda veličine niže.

Filtrat kulture alge manje je inhibirao rast bakterija od žive kulture, dok ubijene stanice alga djeluju stimulativno na rast bakterija. Inhibicijski odnosno stimulativni utjecaj alge na bakterijski rast bio je najjači kroz eksponencijalnu fazu rasta alge, dok je kroz lag fazu bio znatno manji. U stacioniranoj fazi kultura alge stimulativno je djelovala na bakterijski rast.