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THE EFFECT OF SOME ORGANIC SOLVENTS ON THE GROWTH OF CHLORELLA ALGEA, STRAIN 366

1. INTRODUCTION

Studies on the effect of organic compounds on phytoplankton are related not only to the organic matter commonly to be found in aquatic environments [2], but also to the compounds which penetrate water reservoirs as pollutants. These include, in particular, such compounds as hydrocarbons, mainly the alcanes, cycloalcanes or aromatic hydrocarbons which are components of crude oil or those obtained from coal tar.

The influence of hydrocarbons on higher plants is well known [7]. In the case of phytoplankton, it was found that irrespective of the toxic effect on many pollutants, stimulation of the growth of the algae was noted. Such an effect, under controlled laboratory conditions was found by Ukeles and Rose [8]. These authors observed hetero- or mixotrophic growth of the unicellular marine algae studied, in the presence of some organic compounds. Heterotrophic growth of algae was observed for three strains of Chlorophyta grown in the dark, in the presence of ethanol, and sodium acetate, as the carbon sources.

The above ability of Chlorophyta to utilize the organic carbon, which indicates considerable ease of adaptation to the changing conditions of development, made these algae a suitable model for studies of their growth in the presence of organic solvents. For our investigation we chose liquid hydrocarbons, namely; hexane, cyclohexane and benzene and for comparative interpretation of the results — ethanol. In investigations on the effect of ethanol on the growth of algae, data from the paper by Ukeles and Rose [8] were used as a starting point.

2. MATERIAL AND METHODS

The objects of the investigations were Chlorella algae, strain 366 [3], from the collection of the Algological Laboratory, Institute of Animal Husbandry at Zator, Polish Academy of Sciences. The algae were cultivated in Erlenmayer flasks, in 500 ml liquid sterilized medium L_{5m}, after Lefévre [5], with the addition of microelements according to [9], as modified by Jankowski [3, p. 31]. Ethanol (rectified, 96%) and the hydrocarbons were added directly to the medium, one day after the inoculation of the media with the algae. The cultures with ethanol were maintained at room temperature at constant illumination and in the dark, when the initial concentration of ethanol in the medium was equal to 0.01 M/dm^3 or 0.03 M/dm^3. The cultures with hydrocarbons were kept at constant illumination after adding 1 ml of each hydrocarbon to 500 ml of the medium. The growth of the cultures was checked by counting the algae cells in a Bürker chamber. In cultures with ethanol, the decrease of the ethanol concentration in the medium was determined during the algal growth, by applying Widmark's [4] modified method. The experiments with cultures in the presence of the solvents studied, including 4 versions of the culture with ethanol, were repeated 3 or 4 times. Each culture was maintained in identical conditions as the parallel control culture, with the exception of the cultures with 0.01 and 0.03 M ethanol, where only one served as the control for each pair.

A mercury lamp was used (5000 lx) for the illumination. The cultures were incubated at a temperature of 25°C, with continuous mixing using a magnetic stirrer.

3. RESULTS

The observed growth of Chlorella algae, strain 366, in the investigated and control cultures was compared after calculation of the algal growth factor N/N_o; where N and N_o are the numbers of algae cells on a given day and on the first day, respectively. The results of the effect of ethanol and given hydrocarbons on algal growth, are presented in Tables 1 and 2 and in Figs. 2 and 3. The typical progress of the tested and control cultures in the experiments with ethanol is illustrated in one example in Fig. 1, since the results presented in Table 1 do not give the complete image. In Fig. 1, the method of determining the N/N_o factor for the control cultures kept in the dark, in the form of the mean value of the factor, covering the whole period of cultivation, is also illustrated.

The N/N_o values presented in Table 1 are the means of the final values calculated for the phase of stationary growth of the culture. The values in Table 1 represent the percentage increment of the growth

Table 1. Comparison of the quantitative indicators of the growth of Chlorella algae, strain 366, in cultures with ethanol and in the control culture, in the light and in the dark

Tab. 1. Porównanie wskaźników wzrostu glonów Chlorella, szczep 366, w hodowlach z etanolem i w hodowlach kontrolnych, w świetle i w ciemności

No. Lp.	Cultures in the light / Kultury hodowane w świetle					Cultures in the dark / Kultury hodowane w ciemności				
	control kontrola	0.01M ethanol 0.01M etanol		0.03M ethanol 0.03M etanol		control kontrola	0.01M ethanol 0.01M etanol		0.03M ethanol 0.03M etanol	
1	11.4	14.2	125%	16.0	140%	2.5	5.0	200%	8.3	332%
2	18.0	27.0	150%	28.8	160%	3.5	7.0	200%	9.8	280%
3	19.0	21.8	115%	23.5	124%	2.8	5.0	178%	5.6	200%
4	10.0	13.5	135%	16.4	164%	4.0	6.4	160%	7.1	177%

factor in the tested as compared with the control culture (the value of N/N_o for the control culture is equal to 100%).

This means that the results in Table 1 enable not only comparison of the tested and control cultures, but also between the tested cultures.

It results from the data in Table 1 that the absolute values of N/N_o

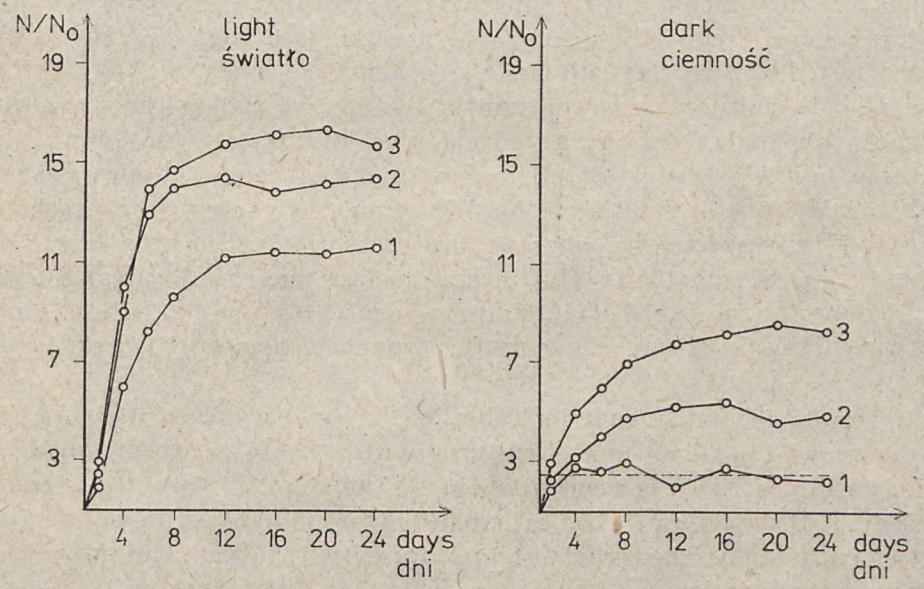

Fig. 1. The growth of Chlorella algae, strain 366, in cultures with ethanol, as compared with the control; on the left — cultures in the light, on the right — in the dark

1 — the control culture

2 and *3* — the cultures with 0.01M and 0.03M ethanol, respectively

Rys. 1. Wzrost glonów Chlorella, szczep 366, w hodowlach z etanolem, w świetle i w ciemności, w porównaniu z hodowlami kontrolnymi; strona lewa — hodowle w świetle, strona prawa — w ciemności

1 — hodowle kontrolne

2 — hodowle z 0.01M etanolem

3 — hodowle z 0.03M etanolem

Table 2. The percentage decrease of ethanol in the media with Chlorella, strain 366, cultivated in the presence of ethanol, in the light and in the dark

Tab. 2. Ubytek procentowy etanolu w pożywkach glonów Chlorella, szczep 366, hodowanych w obecności etanolu, w świetle i w ciemności

Day of cultivation / Dzień hodowli	Cultures in the light / Kultury hodowane w świetle								Cultures in the dark / Kultury hodowane w ciemności							
	0.01M ethanol / 0.01M etanol				0.03M ethanol / 0.03M etanol				0.01M ethanol / 0.01M etanol				0.03M ethanol / 0.03M etanol			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
2[a]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
3	78	74	80	69	85	80	78	79	70	79	75	80	80	85	78	72
4	49	55	48	45	55	49	45	40	45	46	50	52	59	60	55	50
5	25	31	21	26	40	35	30	25	25	21	25	30	38	40	35	30
6	00	04	00	00	18	12	08	05	00	00	00	02	15	12	12	08
7	00	00	00	00	05	00	00	00	00	00	00	00	00	00	00	00

[a] First day of the culture with addition of ethanol to the medium. — Pierwszy dzień hodowli z dodanym do pożywki etanolem.

in the cultures cultivated in the dark are lower in each case, than the values of this factor in the illuminated cultures. The dark control cultures show practically no growth of algae (see Fig. 1). Comparison of the tested and control cultures shows that the values of N/N_o, in the case of both cultures kept constantly illuminated and in the dark, are in each case higher for the tested cultures. In the cases of illuminated cultures in the presence of ethanol with initial concentrations of 0.01 and 0.03 M/dm^3, the values of the N/N_o factor increased on average by 31 and 47%, respectively, whereas in the case of the dark cultures this increase was 86 and 167%. This means that the increase in algal growth is greater at a higher (0.03 M) alcohol concentration and that alcohol has a particularly strong effect on the growth of algae cultivated in the dark.

It results from the data in Table 2, that in each case the increase of the growth of algae in the cultures with ethanol is accompanied by a decrease of ethanol concentration in the medium. Finally, the ethanol present in the medium becomes exhausted around the sixth or seventh day of cultivation. This is fastest in cultures with lower (0.01 M) initial ethanol concentration.

The cultures with hexane and cyclohexane presented in Figs. 2 and 3 are in each case compared with three parallel control cultures, averaged for technical reasons. In the experiments with the hydrocarbons tested and among them with benzene, marked differences were found in their influence on algae. The retardation of growth of the algae, visible in Figs. 2 and 3, is observed after 1 - 2 and 11 - 13 days in the case of hexane and cyclohexane, respectively, but in the case of benzene it maintained throughout the whole period of cultivation. The development

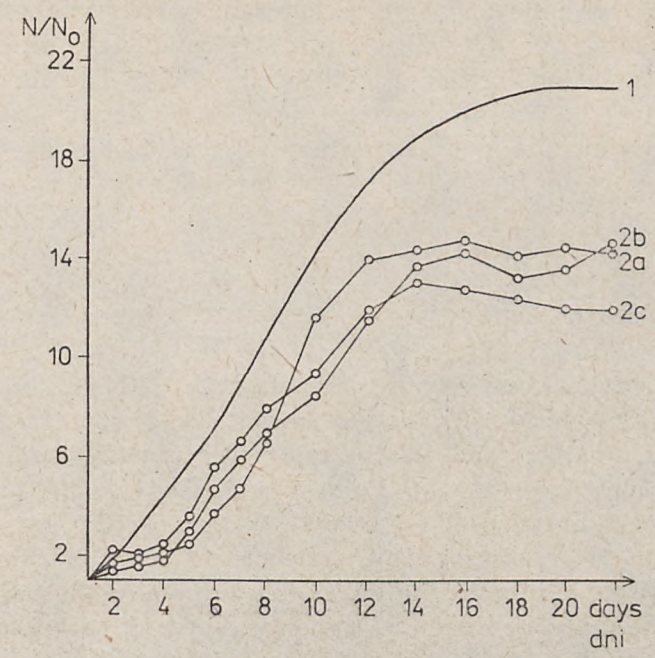

Fig. 2. The growth of Chlorella algae, strain 366, in cultures with hexane, as compared with the averaged results for the control cultures
1 — averaged for three control cultures
2a, b, c — three cultures with hexane

Rys. 2. Wzrost glonów Chlorella, szczep 366, w hodowlach z heksanem, w porównaniu z uśrednionymi wynikami hodowli kontrolnych
1 — uśrednione wyniki przebiegu hodowli kontrolnych
2a, b, c — poszczególne hodowle z heksanem

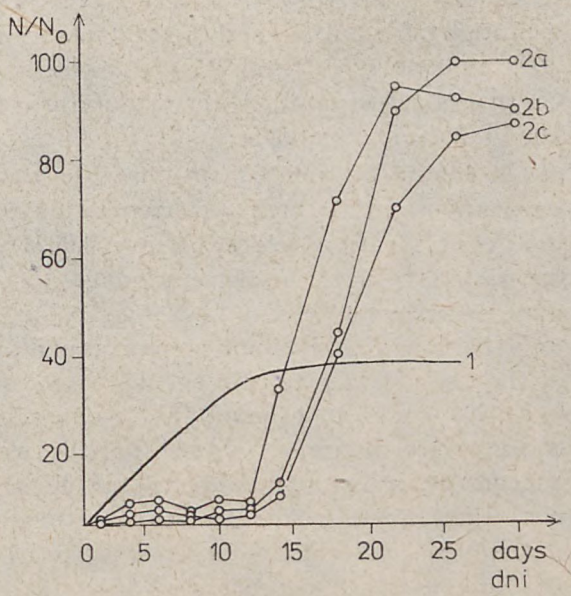

Fig. 3. The growth of Chlorella algae, strain 366, in cultures with cyclohexane, as compared with the averaged results for the control cultures
— legend as in Fig. 2

Rys. 3. Wzrost glonów Chlorella, szczep 366, w hodowlach z cykloheksanem, w porównaniu z uśrednionymi wynikami przebiegu hodowli kontrolnych
— legenda jak na rys. 2

of the culture with hexane and cyclohexane which follows the retardation effect observed at the beginning has completely different quantitative effects during further growth of the algae. The final values of the N/N_0 factor in the cultures with hexane are about 30 - 40% lower as compared with the average values of this factor in the control cultures, whereas in the cultures with cyclohexane they are around 130 - 170% higher.

4. DISCUSSION

The results obtained, concerning the effect of ethanol on the algae studied show the ability of Chlorella, strain 366 [3, p. 31], to adapt to the conditions of mixo- and also heterotrophic growth, similar to other chlorophytes [8]. According to Palmer and Togasaki [6], mixotrophy consists in the stimulation of the growth in light by the given substrate (being a source of organic carbon), which is reflected in enhacement of the photoautotrophic growth. Heterotrophic growth in the dark proceeds by utilizing the given substrate as the only source of carbon (completely replacing the assimilation of CO_2 by photosynthesis). In our studies, the stimulation of the growth of our algae by ethanol, both in the dark and in the illuminated culture was established quantitatively by direct counting of the cells. It was also found that mixo- and heterotrophism are accompanied by disappearance of the ethanol in the medium, obviously due to its assimilation by the algae. The results obtained therefore supply information on the utilization of ethanol in the cultures of Chlorella (strain 366). It was found that the algae studied have better conditions for mixo- and heterotrophic growth at the higher (0.03 M) starting concentration of ethanol, exceeding many times the concentration of this alcohol applied for Chlorella autotrophica, species and clone U-710, by Ukeles and Rose [8].

It should be pointed out that the algal growth would also probably be possible with a higher concentration of ethanol in the medium, as at both concentrations used (0.01 and 0.03M) the ethanol in the cultures was exhausted before the algae reached the stationary growth phase. The data presented here can therefore be useful from the point of view of detoxication of the aquatic environment, also from the practical aspect of the use of algae to prevent water pollution.

As opposed to ethanol the hydrocarbons studied — hexane, cyclohexane and benzene — are hardly water soluble [1]. Their initial concentrations in the media were therefore much lower as compared with that of ethanol. Furthermore, they may undergo further decrease during the cultivation, as it was noted that continuous diffusion of vapours of the hydrocarbons tested takes place through the microbio-

logical corks used to close the culture flasks. The facts described are strictly related to the possibility of interpreting the results of the influence of hydrocarbons on the growth of the algae studied. These facts primarily reflect the toxic effect leading to temporary or complete retardation of the growth of algae. In the estimation of this efect, it would be interesting to determine the "threshold" concentrations of hexane and cyclohexane below which the algal growth starts, and compare the values of these concentrations with the lowest concentration of benzene continually causing retardation of the growth of algae.

Efforts are at present being made to gain the technical facilities to carry out proper, highly sensitive analytical determinations for such low concentrations of hydrocarbons in our laboratory.

In the light of the very low concentration of hexane and cyclohexane which is easy to predict in the phase of logarythmic growth of algae following the retardation period, the enhancement of algal growth (as compared with the control) in the cultures with cyclohexane is particularly interesting. This fact may suggest that the participation of cyclohexane in the metabolism of Chlorella (strain 366) probably differs from the effect of both hexane and benzene. It seems also that, in contrast to ethanol, the cyclohexane cannot be treated as a substrate for mixotrophic growth of the algae studied, as the assumption of such a hypothesis could be difficult in light of the very low concentration of this substrate in the medium. It is also known that hexane could be more susceptible for utilization in the mixotrophic growth of algae than cyclohexane as, of the groups of hydrocarbons tested (alcanes, cycloalcanes and aromatic hydrocarbons), the alcanes [7] have the highest assimilability.

It was thus found that as compared with the control cultures, the reduction in the algal growth in those with hexane, constitutes additional justification for avoiding, in the interpretation of the results, the possibility of mixotrophic assimilation of cyclohexane by the algae studied.

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WPŁYW WYBRANYCH ROZPUSZCZALNIKÓW ORGANICZNYCH NA WZROST GLONÓW CHLORELLA, SZCZEP 366

Streszczenie

Wykazano, że glony Chlorella, szczep 366, zdolne są do utylizacji etanolu, na drodze wzrostu miksо- oraz heterotroficznego. Powodowanej przez etanol, ocenionej ilościowo stymulacji wzrostu badanych glonów towarzyszył spadek wyjściowego stężenia etanolu i ostatecznie nastąpiło wyczerpanie się etanolu w pożywce, jeszcze przed osiągnięciem przez glony fazy stacjonarnego wzrostu hodowli. Badane węglowodory: heksan, cykloheksan i benzen, obecne w pożywkach w nieporównywalnie mniejszych stężeniach od etanolu, wykazywały rosnące toksyczne oddziaływanie, przejawiające się występowaniem przejściowego (heksan, cykloheksan) lub całkowitego (benzen) zahamowania wzrostu glonów. Cykloheksan, w odróżnieniu od heksanu, powodował jednak stymulację glonów, przejawiającą się intensywniejszym w porównaniu z hodowlą kontrolną wzrostem glonów, następującym po okresie zahamowania wzrostu.

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