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IMPACT OF SILICATES AND DETERGENTS ON PRIMARY PRODUCTION AND TOTAL BIOMASS OF ALGAE IN A SHORT-TERM LABORATORY EXPERIMENT

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WPŁYW KRZEMIANÓW I DETERGENTÓW NA PRODUKCJE PIERWOTNĄ I BIOMASĘ GLONÓW W KRÓTKOTERMINOWYM EKSPERYMENCIE LABORATORYJNYM

Krzem, jeden z ważniejszych pierwiastków dla okrzemek, jest często poza głównym nurtem zainteresowań w czasie badań wód powierzchniowych. W ostatnim dziesięcioleciu wiele związków krzemu z detergentów trafiło do wód powierzchniowych wraz ze ściekami komunalnymi. W niniejszej pracy opisano wpływ krzemianów oraz detergentów krzemianowych na wielkość produkcji pierwotnej oraz ilość wytworzonej biomasy. Testy wykazały, że detergenty zawierające krzemiany zwiększały produkcję biomasy oraz miały wpływ na produkcję pierwotną.

Summary

Silicon, an important element for diatoms, is often beyond the main object of freshwater researches. In the last decade, many detergents containing silicon compounds have been released by municipal sewage to surface water ecosystems. In this paper, an influence of silicates and washing agents which contain silicates on algal primary production and biomass growth has been shown. The experimental analysis revealed that detergents with the silicate addition increased the rate of the biomass growth and had a direct impact on algal primary production.

INTRODUCTION

Silicon is one of the most important biogenic elements for water phytoplankton. It is a life-essential element particularly for diatoms (*Baccillariophyceae*) whose cell walls are incrustated by silicates [22]. Earlier studies confirm that this element is not cumulated by organisms and, therefore, it could be a growth-limiting factor for algae [4].

The minimal concentration of silicates necessary for diatom life is still questionable. Results of many studies on diatoms revealed that the silicate concentration below 0.1 mg SiO_2 dm^{-3} led to algal growth inhibition [14, 18]. However, sometimes diatoms did not grow, in spite of

good thermal and light conditions, even in water containing 2.0 mg SiO_2 dm^{-3} [2] or 9.6 mg SiO_2 dm^{-3} [3]. In these cases, there were probably some other not detected factors, like synergism of chemical compounds or presence of specific toxic compounds, which had a direct impact on algal growth. Algal blooms caused by *Asterionella formosa* have been stated at 0.4–0.5 mg SiO_2 dm^{-3}, on the other hand, in laboratory experiments this species was able to reduce the silicate concentration to the level of 0.1 μg dm^{-3} and survive [2]. This wide range of growth-limiting concentrations of silicates results from different experimental or environmental conditions. Many parameters, such as light, temperature, pH-value, salinity, presence of other species and toxic compounds influence silicate uptake of various species of diatoms [22, 23].

In Europe, silicates appeared in households in the late 70-ties. At that time many research works, focusing on sodium polyphosphate $Na_2P_3O_{10}$ substitution, were done. This chemical compound has been used as filling material of good washing properties in all popular washing powders since the 50-ties. It fitted for water softening, impurities removal and for supporting the alkaline reaction of the washing process. Sodium polyphosphate, being a non-toxic compound, has been successfully used in household chemicals [15]. However, phosphates have been transported through domestic sewages to the surface water, leading to its eutrophication [13, 25, 27, 33].

This process is connected with intensive algal blooms and water quality deterioration and, consequently, a biodiversity reduction in a freshwater ecosystem [11]. Moreover, a harmful effect of water blooms on aquatic biota has been intensified by algal toxins, which are secreted, first of all, by blue-green algae *Cyanophyta* [11, 27, 29]. Aerobic decomposition of organic matter released into the environment after blooming algae mortification, often results in complete oxygen depletion in the benthic zone and simultaneously sulfur hydrogen production [25, 27]. Bacterial decay of aquatic biota remains and precipitated calcium triphosphate $Ca_3(PO_4)_2$ leads to soluble compound formation and repeated nutrient enrichment of water [19, 26].

A relation between carbon, nitrogen and phosphorus (C:N:P) in the photosynthesis equals 106:16:1 [21]. Thus, phosphorus is the main element limiting this process. Therefore, zeolites (calcium alumino-silicates) have been used in the washing chemical production in order to substitute sodium polyphosphate. Zeolites as synthetic ion exchangers reveal water softening properties, among others [16]. In some countries, such as Norway, Japan and Italy, the use of phosphates in the washing agent production has been forbidden by law [7]. In the 90-ties big blooms of diatoms were observed in the North Sea. Additionally, awful foam on the shelf bottom of the Adriatic has been observed since 1989. Investigations performed in these regions revealed that zeolites made an adequate medium for bacteria blooms [15]. Unfortunately, our knowledge of the silicate content in surface water ecosystems, particularly freshwater ones, and their impact on aquatic biota is still limited.

In this paper, an impact of silicates and washing agents, which contain silicates, on the primary production of algae and their biomass growth has been presented.

MATERIALS AND METHODS

Plankton samples for laboratory experiments were collected from a natural pond without filtering or other separation methods. Then, an insecticide Karate 25 EC was applied in order to remove eggs and insect larvae. In the laboratory, beakers of 250 cm^3 volume or

crystallizers of 300 cm^3 volume were used as microcosm containers. As a light source imitating natural solar radiation a photostat with ten fluorescent lamps of 40 W was used [30]. Full light streams obtained by means of this photostat equaled 10 klx [28]. The daily light cycle, set up as 10-hour night and 14-hour day, was controlled by an electronic time-recorder [32].

The following media for algal culture were used: Knopp and two modified Knopp's media, enriched by μ-nutrients medium (Tab. 1).

Table1. Components of mediums applied in the study [mg dm^{-3}]

Medium	$Ca(NO_3)_2$ $4H_2O$	$MgSO_4$ $7H_2O$	K_2SO_4	KCl	KH_2PO_4	$FeCl_3$ 1% solution
Knopp (i)	360	120	–	30	60	1 drop
Knopp (ii) PO_4^{-3} lack	360	120	38	30	–	1 drop
Knopp (iii) 1 mg dm^{-3} PO_4^{-3}	360	120	38	30	1,5	1 drop
μ-nutrients medium		H_3BO_3 114 mg	Na_2EDTA 100 mg	$ZnCl_2$ 3.1 mg	$MnSO_4$ $5H_2O$ 17.1 mg	Water ad 100 cm^3

Detergent solutions (labeled as P1–P10) were made from common washing agents in the ratio of 1 g of dry detergent (1 cm^3 of liquid one P8) to 1 dm^3 of distilled water. The selected detergents represent: six modern washing powders (P1–P4 and advertised as „phosphate free" P9–P10), an old one from 1985 year (P7), water softening agent used only as an addition to washing powders (P5), chemical compound Na_2SiO_3 (P6) and water-glass liquid available in chemical shops (P8) (Tab. 2).

Table 2. Properties of agents and detergents used in laboratory experiments

Washing agent	pH	EC [mS cm^{-1}]	PO_4^{-3} [mg dm^{-3}]	SiO_2 [mg dm^{-3}]	Si/P
P1	10.5	1.11	9.33	20.5	3.15
P2	10.5	1.13	9.05	22.8	3.61
P3	10.5	1.03	7.83	35.3	6.46
P4	10.1	1.14	12.0	45.0	5.37
P5	10.8	1.31	5.10	0.00	0.0
P6	11.1	0.66	0.23	110	685
P7	10.4	1.03	3.60	0.00	0.0
P8	10.6	0.50	0.16	350	3134
P9	10.0	0.86	0.70	85.3	174
P10	9.8	1.46	1.18	17.6	21.37

At the beginning of laboratory investigations, the 21-day pre-test was done with different detergents. Particular washing agent solutions of 2 cm^3 volume were added to microcosm containers filled up with 250 cm^3 of Knopp medium (iii) and 10 cm^3 of algae suspension.

Five experiments were performed during the laboratory studies. Four experiments focused on an algal biomass production, using various Knopp's media (Tab. 1) and selected washing chemicals, which differ in the silicon and phosphorus content (Tab. 2). They were performed according to the following scheme:

Experiment I – medium: Knopp (i); time of exposure – 24 days:

Exp. 1	PO_4 [mg dm^{-3}]	SiO_2 [µg dm^{-3}]
Control	41.3	–
A (P1)	41.3	4.1
B (P1)	41.3	20.5
C (P1)	41.3	41

Experiment II – medium: Knopp (iii); time of exposure – 24 days:

Exp. 2	PO_4 [mg dm^{-3}]	SiO_2 [mg dm^{-3}]
Control	1.23	0.02
A (P8)	1.22	3.52
B (P8)	1.2	7.00
C (P8)	1.25	13.53

Experiment III – medium: Knopp (ii); time of exposure – 10 days:

Exp. 3	PO_4 [mg dm^{-3}]	SiO_2 [mg dm^{-3}]
Control	0.01	0.01
A (P4)	0.09	0.10
B (P5)	0.02	0.01
C (P8)	0.01	0.7

Experiment IV – medium: Knopp (iii); time of exposure – 31 days:

Exp. 4	PO_4 [mg dm^{-3}]	SiO_2 [mg dm^{-3}]
K (control)	0.91	0.01
E (P8)	0.91	3.62

A control container (K) filled with appropriate Knopp medium and algal suspension was set during each experiment. Additionally, pH of the medium as well as the phosphate and silicate content were analyzed.

The fifth experiment focused on the algal primary production, which was measured by means of the method of light-dark bottles.

Exp. 5	PO_4 [mg dm^{-3}]	SiO_2 [mg dm^{-3}]
K Light/Dark	41.3	0.02
E (P8) Light/Dark	41.3	6.24

Prior to the experiment, two plastic vessels with Knopp medium (i) and algal suspension were exposed to natural sun rays in order to obtain phytoplankton blooms. The control vessel (K) was left without any additions, whereas the experimental one (E) contained also P8 agent. Consequently, the silicate concentration was 6.24 mg SiO_2 dm^{-3}. When algal blooms appeared, thirty small bottles were filled up with media from K and E vessels, respectively, and left for further incubation. The dissolved oxygen concentration was analyzed in both light and dark bottles after 12, 24 and 36 hours of incubation. In Knopp's growth medium an ethylenediaminetetraacetic acid (Na_2EDTA) as a source of sodium was used.

RESULTS AND DISCUSSION

Pre-test

Algae incubated in particular vessels were characterized by a diverse growth rate in the time of three weeks. After seven days, media in vessels P1, P3 and P9 remained transparent without any visible algal colonies. Whereas, clear blooms were observed in vessels P2, P6 and P7. Further incubation led to the progressive algal growth in all media. However, at the end of the pre-test the most intensive blooms were observed in the vessel P7, where algae formed not only green suspension in the whole volume, but also dark green blankets on the medium surface and bottom. The old detergent P7 addition resulted in the highest biomass increase, i.e. to the amount of 436.3 mg dm^{-3}, which was almost two times higher than in the control group (220.1 mg dm^{-3}). On the contrary, the lowest biomass increase was obtained in vessels P1 and P3 (67.1 and 82.4 mg dm^{-3}, respectively), where only some small agglomerations of green algae were observed on the bottom of containers (Fig. 2).

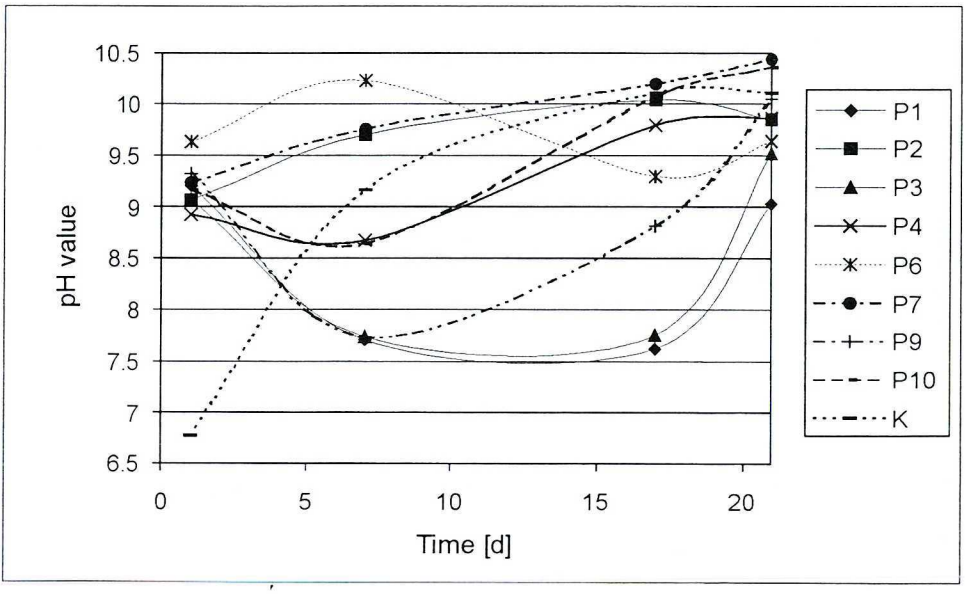

Fig. 1. pH values of media with different washing agents in pre-test (n = 3)

Algae development affected pH of the medium (Fig. 1). Within the first week of the incubation, increase of pH values of about 0.5 unit was stated in vessels with significant algal blooms (P2, P6 and P7); while pH values decreased over one unit in vessels revealing the shortage of algal growth (P1, P3 and P9). The highest pH growth (over two units) was stated in the control group. This change resulted from the biological activity of algae and the lowest initial pH value of the medium due to the lack of alkaline compounds from washing chemicals. Results of the chemical analysis revealed that there were significant differences among pH values of particular testing vessels (Tab. 3) The highest values of correlation coefficients have been obtained between P1 and P3 (0.958), P2 and P7 (0.901), P4 and P10 (0.990).

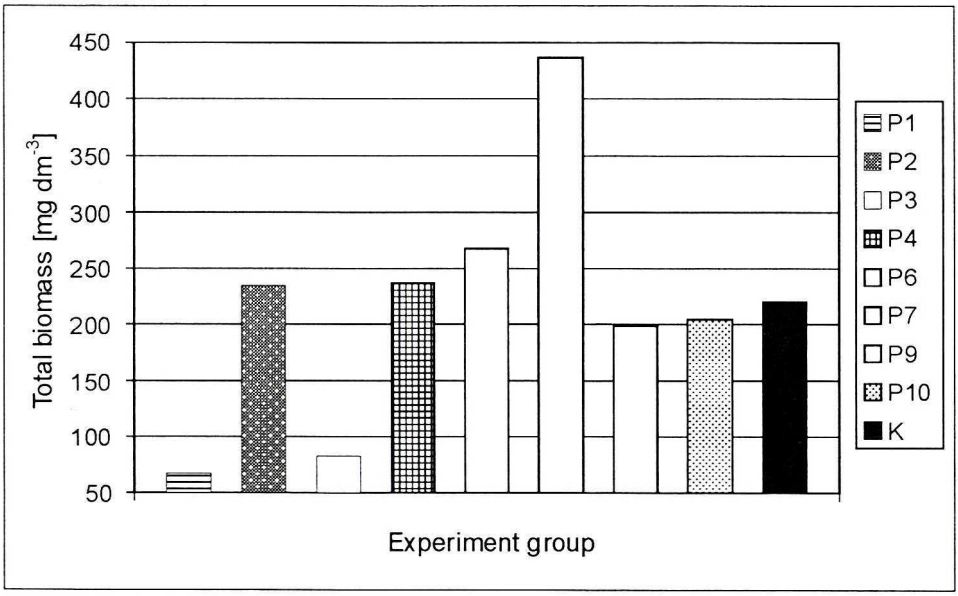

Fig. 2. Total biomass of algae in 21 days pre-test with different washing agents (n = 3)

Table 3. Statistical differences of biomass (*) and pH-value (#) among experimental groups (n = 3) after 21 days of incubation

	P1	P2	P3	P4	P6	P7	P9	P10	K
P1	–	##	##	##	##	##	##	##	##
P2	**	–	#			##		##	
P3		**	–	#		##	##	##	##
P4	**		**	–		##		##	
P6	**	*	**	*	–	##	#	##	##
P7			**	**	**	–	#		#
P9	**	*	**	*	**	**	–	#	
P10	**	*	**	*	**	**		–	
K	**		**		**	**			–

* – significant difference; ** – very significant difference; no mark – non-significant
# – significant difference; ## – very significant difference; no mark – non-significant

Experiment I

P1 detergent was chosen for the experiment purpose due to the poor algal growth in the Knopp medium (iii) fertilized by this agent in the pre-test. Results of the pre-test suggested that P1 detergent might have contained some other toxic to algae compounds. However, after the complete Knopp medium (i) application, the regular algal growth was observed in testing vessels A, B and C. The 24-day exposure period has shown to be sufficient for algal development, which was diverse due to different amount of phosphates in particular vessels. In general, the P1 detergent addition caused the algal biomass increase

by 15.3%, 19.4% and 80.0%, respectively in vessels A, B and C, in comparison with the control one.

Experiment II

The objective of this experiment was the assessment of the silicate-rich washing agent impact on algae. Testing vessels A, B and C were filled up with the Knopp medium (iii) with a various amount of P8 (Si/P = 327) additive. Consequently, silicate concentrations at the beginning of the experiment were as follows: 0.02 mg SiO_2 dm^{-3} (K-control), 3.52 (A), 7.00 (B) and 13.53 mg SiO_2 dm^{-3} (C).

Both, the algal growth and development and the reduction of the phosphate concentration were simultaneously recorded (Fig. 3 and 4). The highest rate of changes of the algal biomass and the phosphate content occurred between the 4th and the 9th day of the experiment. Generally, the biomass increase and the reduction of the phosphate content were lower in the control vessel (K) in comparison with the others. A comparable algal biomass rate has been obtained within 8 days of incubation also after agricultural fertilizers application [31].

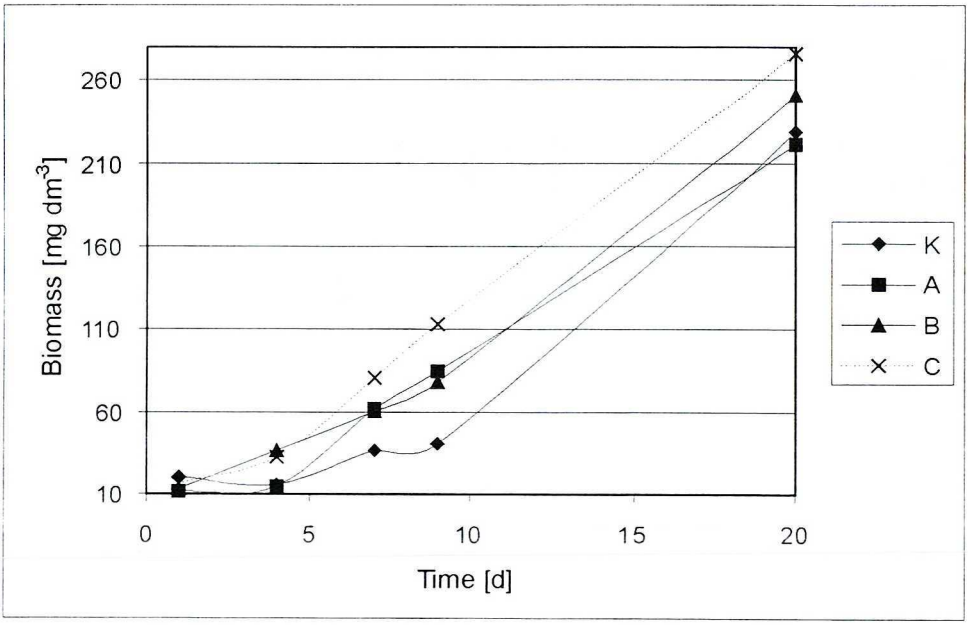

Fig. 3. Total algal biomass in 20-day experiment with silicates addition

Silicon concentrations, unlike phosphate ones, varied significantly during the exposure time (Fig. 5). A stable increase in concentration was recorded only in the control group. In the testing group with the minimal P8 agent addition (A), the silicate content fluctuated considerably, revealing a small growth at the beginning, then a drastic downfall (between the 4th and the 7th day) and finally a slow growth at the end of the incubation. Another course of silicate content changes was observed in vessels B and C. The sequence of changes was as follows: a stable content during a few days – a small increase of the silicate amount – a slow decrease in the silicate concentration.

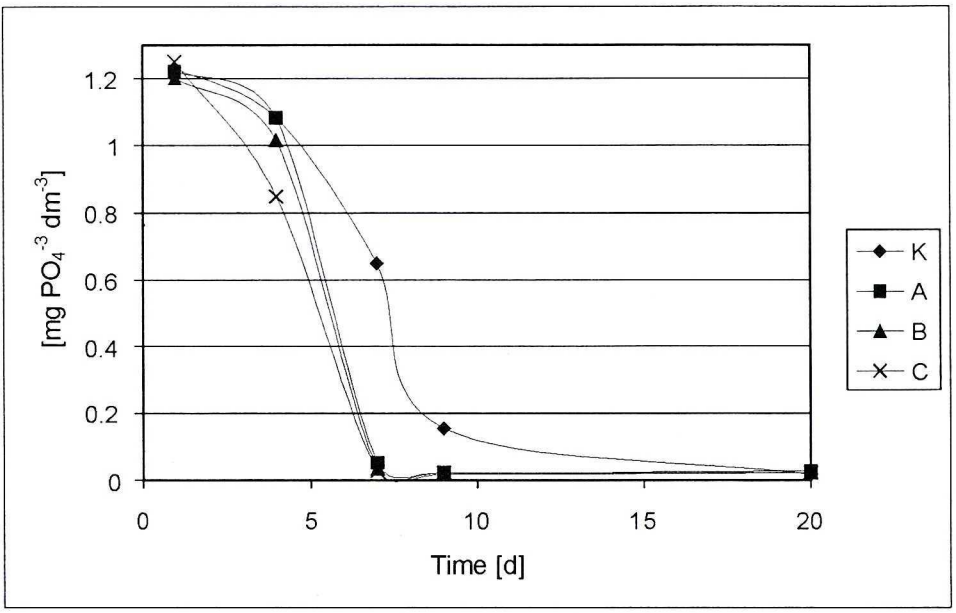

Fig. 4. Phosphates depletion during 20-day experiment with silicates addition

Notwithstanding silicate variations, a gradual algae development was stated. In comparison with the control vessel (K), the algal biomass increased by 15.4%, 31.5% and 51.9%, respectively in vessels A, B and C. Similar phenomenon has been described by Wu [32].

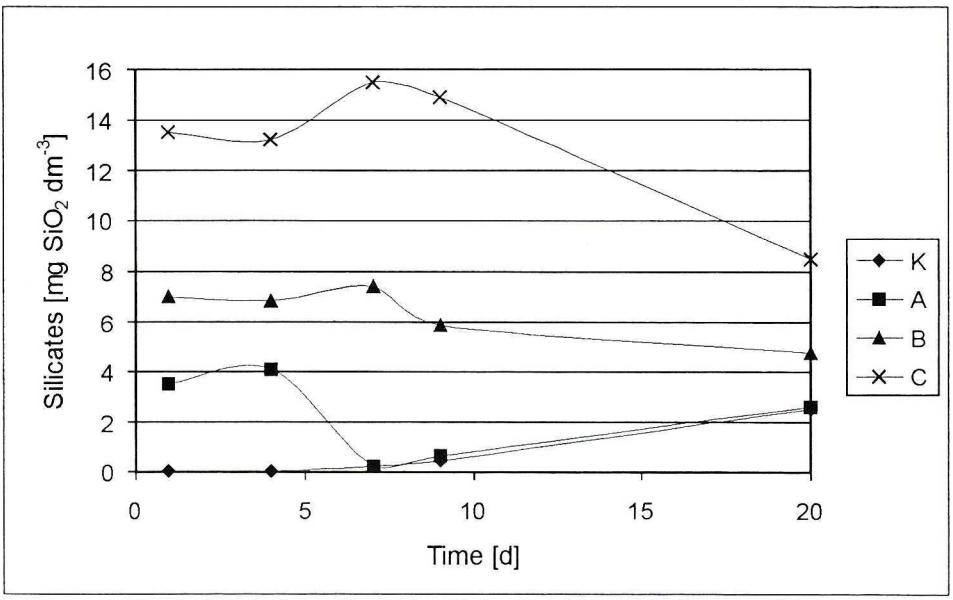

Fig. 5. Silicate fluctuation during 20-day experiment with silicates addition

Experiment III

The experiment focused on observations of the algal growth rate in the phosphate-poor Knopp medium (ii) supplemented by three detergents of various Si:P ratio, i.e. P4 (Si/P = 5.38), P5 (Si/P = 0) and P8 (Si/P = 327).

Although there was a low concentration of phosphates in the control vessel, 10 days of incubation were sufficient for the algal growth to the level of 47.9 mg dm^{-3} (Fig. 6). The algal biomass was lower only in the case of the medium with the P8 agent addition. Whereas, in the other testing vessels evident algal blooms were observed, with higher biomass recorded for P5 agent (4.5 times higher than in the control group). It occurred that in media characterized by extremely low phosphate concentrations (0.054 and 0.067 mg PO_4 dm^{-3}, respectively in vessels K and P8), silicates did not induce the algal development. The biomass reached by algae after 10 days of the incubation in media with P4 and P5 detergent additions was comparable to that obtained in the experiment II, where the phosphate-poor medium with P8 detergent addition was applied. This suggests that the phosphate uptake by phytoplankton occurs even in the case of much diluted solutions.

Experiment IV

Knopp medium (iii) containing 0.907 mg PO_4 dm^{-3} and P8 detergent were used to assess the algal growth during the 31-day exposure period. The initial phosphate and silicate concentrations in the testing vessel E were 0.893 mg PO_4 dm^{-3} and 3.62 mg SiO_2 dm^{-3}, respectively. The addition of P8 detergent caused also changes of pH values from 5.44 (mean value in K) to 7.41 (mean value in E).

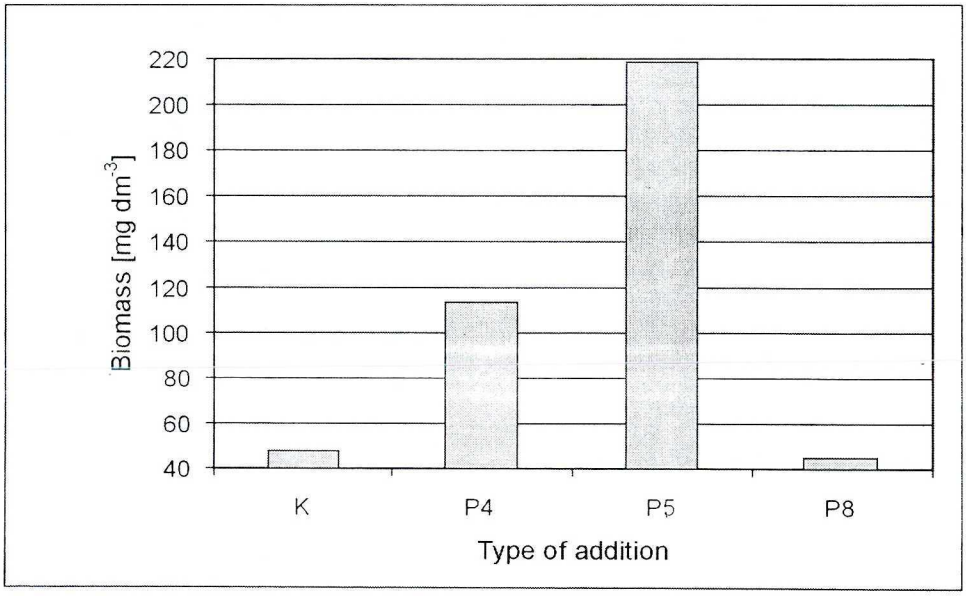

Fig. 6. Algal biomass in 10-day experiment on (ii) medium with different agent addition

Algal blooms were observed in both groups of vessels (K and E). The amount of the algal biomass fluctuated over time, particularly at the beginning of the exposure. This fluctuation was more strongly marked in the control group (Fig. 7). Final rebuilding of the algal biomass was much higher in E group than in K group. At the same time, phosphate concentrations decreased during the incubation to the level of 0.027 and 0.017 mg PO_4 dm^{-3} in K and E group, respectively. On the contrary, silicate concentrations increased by 3.11 and 2.99 mg SiO_2 dm^{-3}. This phenomenon, observed also in other experiments, probably results from the degradation process of allochtonic silicon dioxide from cell walls of diatoms. Silicate leaching from the vessel walls is very small and in experimental conditions does not exceed 0.17 mg SiO_2 dm^{-3} at pH 7.0–9.6 and 0.025 mg SiO_2 dm^{-3} at pH 4.7–5.1 [5, 9, 12].

Experiment V

Algal blooms were observed in both groups of vessels (control K and experimental E). However, a primary production (PP) and oxygen consumption (OC) were evidently higher in the second one (Fig. 8). In comparison with the control group, the primary production in the testing group E was higher by 102%, 97% and 90%, respectively, after 12, 24 and 36 hours of the incubation.

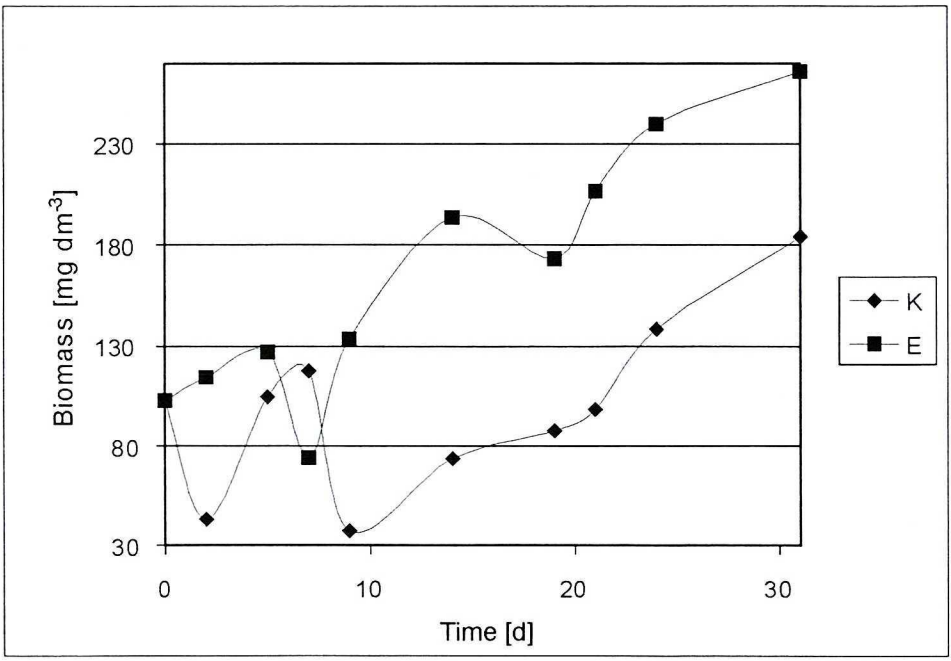

Fig. 7. Biomass in 31-day experiment with P8 addition

A glucose production, calculated on the basis of PP and OC values, reached the level of 3.27 and 5.34 mg $C_6H_{12}O_6$ dm^{-3} in groups K and E, respectively, after 12 hours of incubation. Finally, after 36 hours of incubation, glucose production increased to the level of 4.99 (K) and 7.27 mg $C_6H_{12}O_6$ dm^{-3} (E). The results of the investigation revealed significant statistical differences among majority of „light" and „dark" bottles.

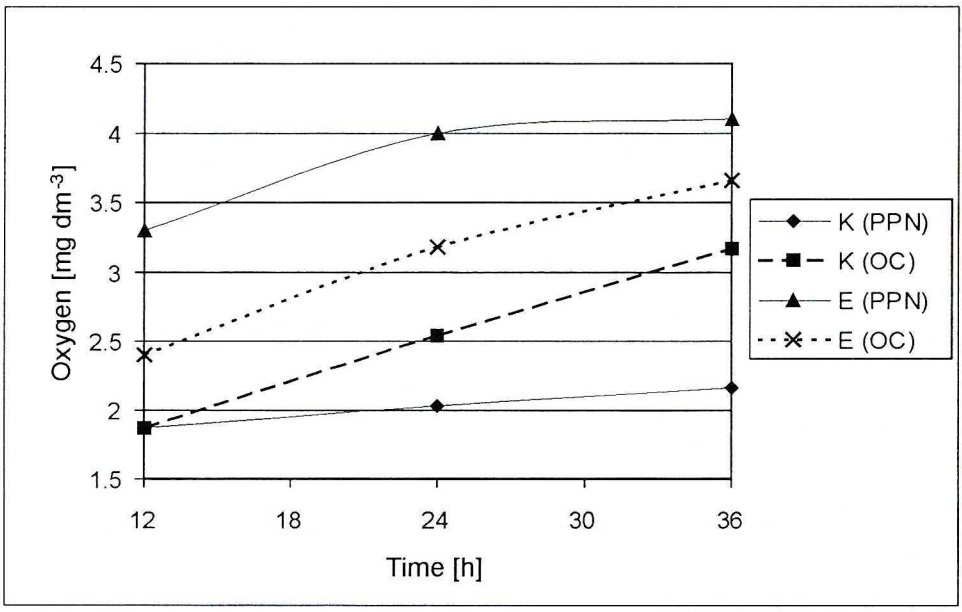

Fig. 8. Net primary production (PPN) and oxygen consumption (respiration) (OC) in the experiment with P8 addition

Generally, the primary production obtained in this experiment is comparable with the level which has been stated in natural conditions during algal blooms [6, 8, 17]. The algal biomass in natural ecosystems, like lakes, is usually smaller than the biomass quantity that was recorded during the experiment [1, 10, 12, 20], of course except for highly eutrophic ecosystems [24].

CONCLUSION

Impact of silicates on algae might be different, depending on the exposure time. A short-term contact could lead to algal biomass decline due to violent changes of pH values of the medium. However, longer period of the silicate action (over 10–20 days) resulted in algal blooms. Influence of silicates on the algal primary production and biomass growth is very significant, though the intensity of changes is much higher in the case of phosphates impact. There is, however, possibility that municipal sewages, containing a large amount of soluble silicates, will affect freshwater ecosystems in the same way as in the described laboratory experiments, leading to water quality deterioration.

NOTE

All researches were made during doctoral studies in the Department of Limnology and Fishery of Agricultural University in Wroclaw.

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