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Detoxication of Water Media, Containing Thiazine Dye with Use of Microalgae

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Abstract: The process of purifying wastewater which contains methylene blue by using green microalgae *Chlorella* and *Chlamidomonas* sp. has been researched. It has been found out that in the process of cultivating both Chlorella and Chlamydomonas in such water, the concentration of dye is reduced and the purification process with Chlamydomonas is more efficient. It has been demonstrated that with increasing the concentration of methylene blue to 10 μM L^{-1}, the obvious increase of degradation degree is observed at using these algae which indicates the microalgae's ability to sorbate the dye. The degree of purification from the xenobiotic in the variants with Chlamydomonas amounts to 70% and with Chlorella amounts to 40%. Using microalgae for wastewater treatment wouldn't require rearrangement or capital construction of new wastewater treatment facilities. Besides there is an opportunity of using microalgae after the wastewater purification as green manure for melioration agriculture, soils remediation or waste composting. The application of algae strains endued with principally new opportunities of wastewater treatment will allow amending the environmental situation and creating a secure system of environmental enhancement.

Key words: Detoxication, methylene blue, microalgae, *Chlorella* sp., *Chlamidomonas* sp.

INTRODUCTION

The global technogenic burden causes the significant environmental pollution which results from the imperfection of the used technologies. Among the ways of water ecosystems restoration and the prevention of their pollution the microbiological methods attract the most interest as biomembranes have the high adsorption capacity (Munoz and Guieysse, 2006; Ogbonna *et al*., 2000) and microorganisms' enzyme systems are characterized by high activity and selectability, particularly to xenobiotics. It's essential to create a microbiocenosis in which one of the members partially decomposes the hazardous substances; others mineralize them the third provide the optimal ecological factors for their existence. It should be peculiar symbiotic biocenoses, adapted to a certain wastewater composition and to specific xenobiotics. Such, systems can be used and already find application not only for remediation of environmental disasters at water bodies but for the industrial waste neutralization, purification of municipal sewerage wastewater, neutralization of gas emissions, decontamination of soils from polluting agents (Ogbonna *et al*., 2000; Goncharova *et al*., 2009; Tebbutt, 1998). Besides, nowadays a number of ways of using algae are known, e.g. for producing valuable products and biologically active substances from algae biomass (Perales-Vela *et al*., 2006). This allows suggesting the further integrated application of purification technologies and algae processing which may result in creating the waste-free technological systems.

A large number of scientific papers have been devoted to the decomposition and/or adsorption of various xenobiotics with bacteria or mold fungi. The processes of purifying wastewater from heavy metals:

Chromium, cadmium, zinc, copper, lead, molybdenum, by means of green algae has been well researched (Chisti, 2007; Wilde and Benemann, 1993; Filip *et al*., 1979; Nakajima *et al*., 1981; Ting *et al*., 1989). At the same time this question remains underexplored for the waters, containing organic impurities and for the role of microalgae in the detoxication process. Introducing the algae to the artificial symbiosis should provide a number

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of advantages for the biosystem. In particular, microalgae create the optimal conditions for aerobic microorganisms as they oxygenate the system which contributes to the more intensive acidification of xenobiotics. The role of algae is not always positive though: their cumulation can result in water enrichment in the active colonization of buildings' and constructions' surfaces by the algae which causes the significant environmental damage (Goncharova *et al*., 2014; Goncharova and Vasilenko, 2013). Among the various functions of algae in the environment, the most attractive is their ability to adsorb considerable amounts of polluting substances with their cell walls (alginates, contained in them) and to occlude carbon-dioxide gas intensively, purifying the environment from greenhouse gases.

The purpose of this research work was to test the possibility of detoxication of water media, containing methylene blue dye by using unicellular green algae.

The Methylene Blue dye (MB) is widely applied in various branches of industry: in light industry as a pigment for coloured pencils production in printing industry (basic varnishes) for paper coloration as well as in veterinary medicine, agriculture, analytical chemistry, healthcare, etc. The MB thiazine dye in high concentrations induces the transformation of haemoglobin to methaemoglobin, i.e., it is a hemolytic poison. Besides, methylene blue is a widely know model substance which is used to detect Mg, Ca, Cd, Co, Ni, Zn and other metals and as an oxidation-reduction indicator it is toxic for many microorganisms, besides as a result of photo transformation in the environment, it becomes even more toxic. The limiting nuisance value of MB's influence on the organoleptic properties of water is the change of color; the threshold concentration amounts to 0.012 mg L^{-1}.

Due to the multifold application of this chemical in various branches of industry and the toxicity of its large concentrations for living organisms, the purification of wastewater from this dye is a crucial task.

MATERIALS AND METHODS

The possibility of purifying wastewater from dye was researched by using two species of algae *Chlorella* and *Chlamidomonas* sp. The various conditions of experiments on purifying a model medium, containing methylene blue as a primary pollutant are presented in Table 1.

The test media and media containing microalgae were exposed to the light of 36 W white luminous lamp («cool light»), located at 15 cm from the surface of the media, during 5 days.

To determine the MB content in different variants of experimental suspensions 5 mL of liquid was taken from each glass and centrifuged within 5 min. The MB concentration was evaluated by photocolorimetric method with a PE-6100 UF double-beam spectrometer using the Baly tubes with optical thickness 1 cm. As a comparison solution the distilled water was used. In Fig. 1, the spectra of model water solutions with various concentrations of MB and a calibration chart are presented.

The algae were cultivated in Tamia liquid nutrient medium. The amount of microalgae cells was determined with an Axio Scope A1 (Carl Zeiss Jena) microscope by counting up.

The amount of heterotrophic microorganisms in the media after their purification was determined by inoculation on solid media.

Table 1: Conditions of experiments to research the process of purifying wastewater from methylene blue in the presence of microalgae

Microalga	Initial concentration of MB (μM L^{-1})	Preparation	Notes
Chlorella	0	50 mL of medium+1 mL	To evaluate the growth inhibition
Chlamydomonas	0	of algae suspension	under the action of MB
No algae	1	50 mL of medium with MB+1 mL	To prevent decomposition of MB
–«–	4	of distilled water	under exposure to light
–«–	7		
–«–	10		
Chlorella	1	50 mL of medium with MB + 1 mL	
–«–	4	of algae suspension	
–«–	7		
–«–	10		
Chlamydomonas	1		
–«–	4		
–«–	7		
–«–	10		

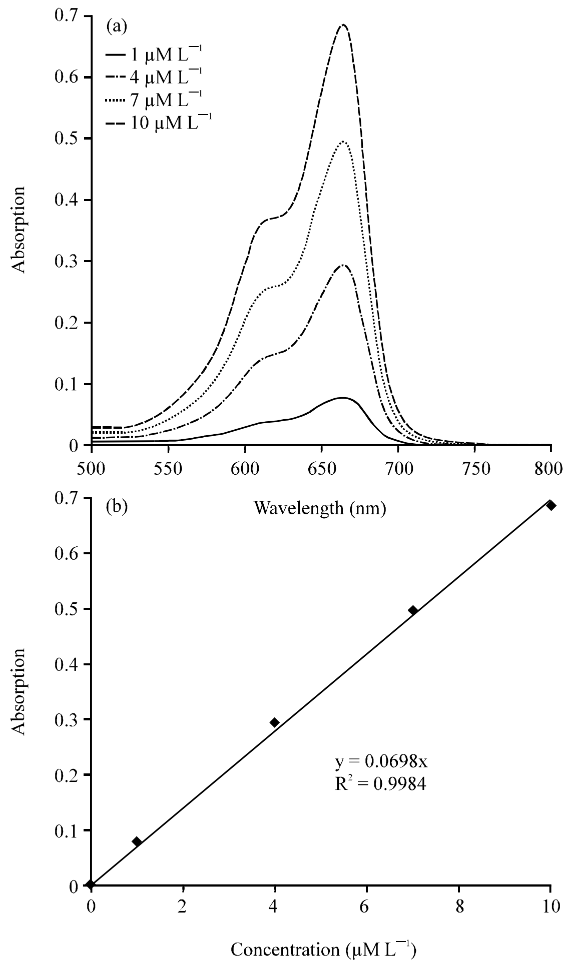

Fig. 1: a) Absorption spectra of MB and b) a calibration chart for determining the dye concentration in the solution

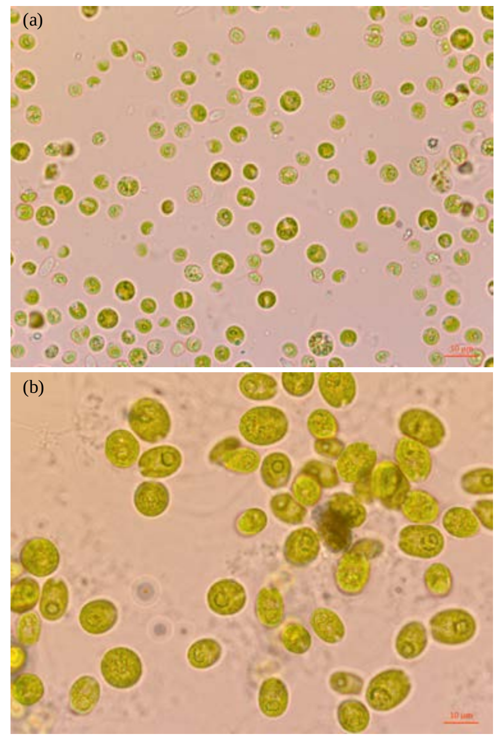

Fig. 2: a) Chlorella and b) Chlamydomonas in Tamia liquid nutrient medium

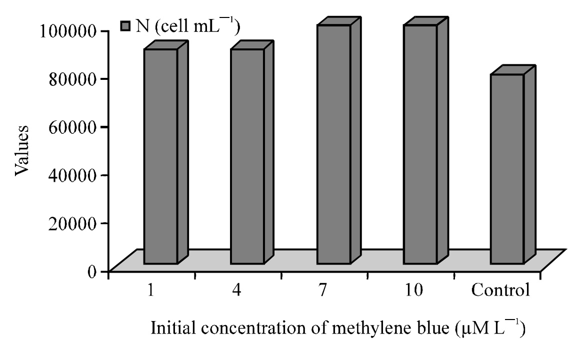

Fig. 3: Alteration of the Chlorella cells amount depending on the concentration of a thiazine dye-methylene blue

RESULTS AND DISCUSSION

The visual appearance of green algae *Chlorella* and *Chlamidomonas* sp. is presented in Fig. 2. In the process of purification no morphological changes of algae cells were detected.

The initial concentration of Chlorella in the model medium amounted to 2×10^4 cell mL^{-1}. The experimental data of dependence of the chlorella cell amount on the concentration of dye at the end of the experiment (after 5 days) at the presence of the dye and without it are given in Fig. 3. In the course of experiment, the amount of Chlorella increased by 3-5 times.

As we can see in Fig. 3, the amount of algae in all the researched space of the pollutant (1-10 μM L^{-1}) is more or equal to the amount of algae cells in the test experiment, i.e., the xenobiotic didn't inhibit the algae growth in the given range of concentrations alteration.

The experiments were carried out with other green algae as well including Chlamydomonas. It is a unicellular alga, covered with a relatively hard pectin membrane, pear-shaped, sometimes round or oval it can move actively by means of flagella. The visual appearance of Chlamydomonas is presented in Fig. 2b.

The initial concentration of Chlamydomonas in the model medium amounted to 3.6×10^3 cell mL^{-1}. The data of alga cells calculation for various initial concentrations of dye, determined by the Counting-up Method in a

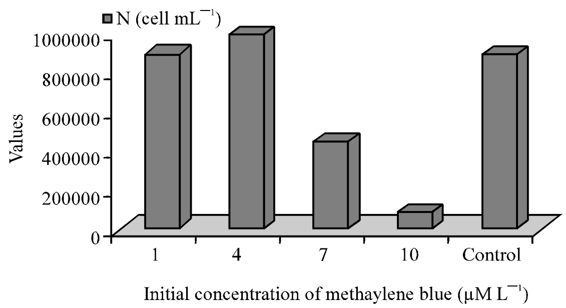

Fig. 4: Dependence of the amount of Chlamydomonas cells on the methylene blue concentration

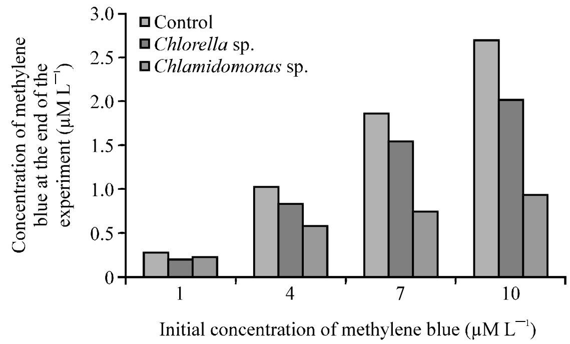

Fig. 5: The dependence of residual concentration of the dye in the microalgae growth medium on its initial concentration

Goryaev-Thoma chamber by the end of the experiment (after 5 days) are presented in Fig. 4. The xenobiotic concentration of 1-4 μM L^{-1} didn't influence the algae growth the growth rate remained the same 250-280 as in the test medium, not containing the dye. It should be noted that Chlamydomonas was growing faster then Chlorella.

As we can see in Fig. 4 at the concentration 7-10 μM L^{-1} the methylene blue inhibited the growth of algae, the culture density of the algae reduced to 0.9×10^5-4.5×10^5. The cells generation time in the presence of the dye with concentration 1-4 μM L^{-1} and in the test experiment was more or less equal and amounted to about 15 h, so, the xenobiotic concentration in the range from 1-4 μM L^{-1} didn't inhibit the algae growth.

Probably, the detected process of Chlamydomonas growth inhibition is conditioned by its reaction to the xenobiotic introduction which results in micoalgae cells issuing the reactive oxygen intermediates to the medium. These oxygen intermediates not only protect the micoalgae cells against the adverse effect of xenobiotic by decomposing it but also inhibit the growth of microorganisms.

The comparative analysis of the variants of purifying wastewater from the dye by using *Chlorella* and *Chlamidomonas* sp. in conditions of various concentrations of xenobiotic is presented in Fig. 5.

It was determined that in the process of cultivating algae Chlorella as well as Chlamydomonas in the wastewater with the dye, the concentration of toxic agent in the medium is reduced, more effectively in the variant with Chlamydomonas. The degree of xenobiotic degradation in the variant with Chlamydomonas amounted to 70% (Fig. 5).

The obvious increase of the degradation degree of the dye correlates with the increase of its initial concentration. This indicates the ability of these microalgae to capture (adsorb on cell walls and/or destroy) the thiazine dye at the initial concentration up to 10 μM L^{-1} without a considerable inhibition of their growth. At the concentration over 10 μM L^{-1} the inhibition of Chlamydomonas growth was observed but the degree of xenobiotic degradation was not reduced. This can indicate the participation of other aerobic microorganisms, symbiotic with the algae in the reaction of xenobiotic destruction.

In spite of the fact that at cultivating Chlorella within the above-mentioned range of concentration the inhibition of its growth wasn't detected, the highest degree of purification was lower than in the experiments with Chlamydomonas the maximum degree of xenobiotic degradation amounted to 40%.

So, we can arrive at the conclusion that it's efficient to treat wastewater containing methylene blue up to 10 μM L^{-1} by using the Chlamydomonas alga. The high concentrations of the pollutant obviously inhibited the algae growth and the purification was not so effective. In this case to continue the purification, it's necessary to dilute wastewater before treatment to maintain the initial concentration of the dye within the desired range or to create an artificial symbiotic biocenosis which could increase the purification efficiency.

The calculation of heterotrophic microorganisms' content in nutrient media with algae after the experiment was over has shown that the amount of bacteria in the medium with Chlamydomonas was 3-4 times higher than in the medium with chlorella. The high efficiency of purification from methylene blue in this case could be conditioned by the presence of symbiotic microbial flora in the medium. Chlorella appears to exude the antibacterial metabolites to the medium which provides a bactericidal effect.

CONCLUSION

The process of purifying wastewater from methylene blue is more efficient in the suggested conditions by using Chlamydomonas. But to improve the efficiency of purification it's necessary to provide conditions for the controlled cultivation of Chlorella and for creating optimal parameters for its growth. The use of alga looks attractive for the fact that its presence provides the purification not only from the dye but from bacteria as well.

Applying algae for the detoxication of water media looks promising because after the wastewater purification they can be used as green manure for melioration agriculture, soils remediation, bioactive agents' production or waste composting. Besides, the algae can be used for purifying wastewater which has bactericidal properties as in the example given and for this reason the conventional techniques of biological purification can't be applied to it.

Using microalgae for wastewater treatment doesn't require rearrangement or capital construction of new wastewater treatment facilities. The application of active algae strains will allow creating a new integrated biotechnology for the purification and remediation of disturbed ecosystems. The application of microalgae endued with principally new opportunities of wastewater treatment will allow amending the environmental situation and creating a secure system of environmental enhancement.

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