Prospective Strains of Microalgae for Cleaning of the Industrial Drains Polluted by Ions of Heavy Metals

B. K. Zayadan, A. K. Sadvakasova, M. M. Hassan, and A. Zh. Beisenova

Abstract—It is studied absorption properties of wild and mutant strains of Chlamydomonas reinhardtii and Chlorella sp. in regard to ions of copper, zinc and cadmium.

It is established that high accumulation ability are characterized for cells resistant to cadmium of mutant strains of Chlamydomonas reinhardtii CC-124 Res-1, CC-124 Res-2 and for cells of a wild strain of Chlorella sp.3 allocated from water polluted various organic substances. Opportunities are studied and there are shown prospects of the investigation strains in the sewage treatment, polluted by organic substances and ions of various heavy metals.

Keywords—Bioremediation, consortium, heavy metals, microalgae.

I. Introduction

IN the conditions of anthropogenous activity pollution of natural fresh waters by heavy metals has become especially acute problem. Relevance of this problem doesn't raise doubts. It is enough to tell that there are no reliable mechanisms of self-cleaning for heavy metals. Being in environment they aren't exposed to chemical biodegradation as it is peculiar to organic compounds, and only redistributed between abiotic and biotic components and interact with them, everywhere leaving visible undesirable consequences of this interaction.

Nowadays the main source of pollution of natural waters by heavy metals is industrial pollution. Heavy metals get to natural waters with used industrial waters containing chemical compounds and traces of elements, with the rain water which is filtering through dumps, and also at accidents of various chemical installations and storages [1]. As a result of all this self-clearance ability of natural waters decreases so that there is a risk of emergence of an emergency ecological situation.

One of the most perspective methods of biological cleaning of the water environment from ions of heavy metals is introduction in the polluted ecosystem of active strains of the microorganisms possessing high cumulative ability [2]. The success of use of this method is provided with the correct selection of the optimum microorganisms bioaccumulators, being characterized high metal-accumulation ability.

It is known that active bioaccumulators of ions of heavy metals are microalgae. So, it is shown that such heavy metals as Cu, Pb, Cd, are collected by green and diatomaceous algae at insignificant their small contents in a reservoir. Natural association of a sea phytoplankton green algae of *Scenedesmus* sp. *Selenastrum* sp. *Chlorella* sp. at the equimol contents in the environment accumulate metals in sequence of Ag>Cu>Cd>Zn [3].

Sea microalgae *Tetraselmus* sp. *Dunaliella tertiolecta*, and *Phaeodactium tricornutum* are capable to grow directly on sewage and completely to delete from them Cd, Cu, Fe, Mn, Zn in concentration to 10^{-6} M [4].

The work purpose is studying of accumulation of ions of zinc, copper and cadmium by cells of mutant and wild strains of green microalgae, for the purpose of identification of their opportunities in cleaning of the polluted water ecosystems.

II. Materials and Research Methods

Objects of research are cultures of mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1 and *Chlamydomonas reinhardtii* CC-124 Res-2 steady to cadmium, zinc and copper, received by a method of induced mutagenesis. *Chlorella* sp.3 strain is allocated from water polluted by various organic substances. Mutant strains of microalgae are deposited with RGCE "Republican Collection of Microorganisms" at numbers A-RKM 0175, A-RKM 0176.

Microalgae were cultivated in conic flasks of 1000 ml when lighting by fluorescent lamps (4000 lux), at the temperature 25-28^{0}C to density (1,6-2,5).10^7 C/ml on Tamiya nutrient medium and L2-min. Number of cells were determined by calculation method in Goryaev's camera. Cadmium was brought in the form of CdC_{l2}*$7H_2O$ salt, zinc was in the form of $ZnSO_4$*$7H_2O$, copper was in the form of $CuSO_4$*$5H_2O$ in concentration of 100 mg/l counting on metal ions.

In order to define accumulation of ions of heavy metals, a biomass of microalgae was deleted from cultural liquid by centrifugation. Concentration of heavy metals in over deposit liquid was determined by method of atomic-absorption Spectrophotometer (AAS of MGA-915, with mercury-hydric prefix) [5]. Quantitative indices of sorbed metals were determined by a difference of the brought concentration and concentration of ions of heavy metals in a supernatant.

Bolatkhan Zayadan is with the Department of Biotechnology, Al-Farabi Kazakh National University, Kazakhstan (corresponding author to provide phone: +7(727)377 33 28 fax:;+7(727)3773255: e-mail: zbolatkhan@mail.ru).

Asem Sadvakasova is now with the Department of Biotechnology, Al-Farabi Kazakh National University, Kazakhstan (e-mail: sadvakasova1979@mail.ru).

Aizhan Beisenova is with the Department of Biotechnology, Al-Farabi Kazakh National University, Kazakhstan (e-mail: aizhan.beisenova@mail.ru).

Marwa Hassan is with the Department of Biotechnology, Al-Farabi Kazakh National University, Kazakhstan (e-mail: marwasaleh2000@yahoo.com).

Processing of the obtained data was carried out by the standard techniques by use of Student's *t*-cryteria.

III. Results and Discussion

Zinc, copper, cadmium and their compound can be carried to number of priority pollutants of the water environment as they meet in sewage of the different industrial enterprises. These metals are dangerous, first of all, as the algaecide agents breaking functioning of algae - the first link of a trophic chain of water ecosystems. It is established that zinc in concentration of 0,1-0,5 mg/ml has toxic effect on the lowest water organisms, concentration of 1,0 mg/ml are reduced number of the major types of algae [6].

Studying of mechanisms of toxic influence of heavy metals on cells of microalgae causes great theoretical and practical interest. The first step of such researches is clarification of a question, whether these metals are occluded by cells.

In this regard, we investigated ability of cells of various strains of microalgae to accumulate ions of copper (Cu ++), cadmium (Cd ++) and zinc (Zn ++).

Studying of accumulation of ions of copper (Cu ++) by cells of various strains of green microalgae of *Chlorella* sp.3, *Chlamydomonas reinhardtii* CC-124Res-1, CC-124 Res-2 and CC-124 with their growth in the environment containing 100 mg/l of ions of copper were carried out within 24 hours after the beginning of cultivation. About accumulation of copper in cells were judged on change of concentration of this element in the cultural medium.

It is revealed that concentration of copper in a supernatant within 24 hours after the beginning of cultivation were: for *Chlamydomonas reinhardtii* CC-124 strain - 90 mg/l, for *Chlorella* sp.3 – 10 mg/l, for mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1 and CC-124 Res-2 5 - 18 mg/ml respectively (Fig. 1).

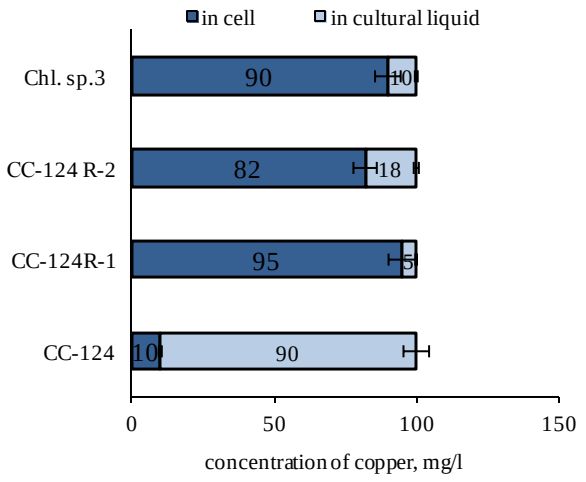

Fig. 1 Bioaccumulation of copper ions by cells of various strains of microalgae at 24 hours of cultivation

Received results give evidence of high accumulation of ions of copper by cells steady to cadmium, mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1 and CC-124 Res-2, and cells of the wild strain allocated from polluted water *Chlorella* sp.3. Thus growth of cells of mutant strains CC-124 Res-1, CC-124 Res-2 and Ch. sp.3. were 100% whereas the survival of cells of the wild type *Chlamydomonas reinhardtii* CC-124 was considerably low and were 28%. The wild strain of *Chlamydomonas reinhardtii* CC-124 accumulated copper ions to 10 mg/l.

The similar situation was when studying accumulation of ions of zinc by cells of these strains of green microalgae. Initial concentration of zinc in the medium was also 100 mg/l counting on zinc ions. The maximum absorbtion of metal at 24 hours of cultivation is established for mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1 and CC-124 Res-2-96, 2-98,4 mg/l respectively (Fig. 2).

Cells of the wild strain allocated from polluted places of *Chlorella* sp.3 accumulated zinc ions to 93,5 mg/l. For a wild strain of *Chlamydomonas reinhardtii* CC-124 concentration of zinc in supernatant liquid within 24 hours after the beginning of cultivation was 91,6 mg/l that gives evidence of considerable low extent of accumulation of ions of this heavy metal - to 8,4 mg/l.

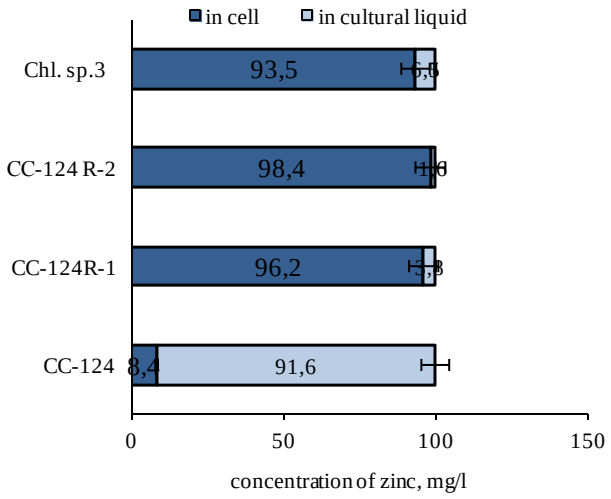

Fig. 2 Bioaccumulation of zinc ions by cells of various strains of microalgae at 24 hours of cultivation

Nature of growth of cells of studied cultures in the presence of ions of zinc is comparable to ones for the cultures developing in the presence of ions of copper.

As it is known the metabolic processes proceeding in a cell, have influence on absorption of metals [6]. Both zinc and copper are heavy metals, however, as it is known they are necessary in a small concentration and even are vital for a metabolism of cells.

Active accumulation of ions of heavy metals by cells of microalgae is observed from the first hours of experiment. Probably, the main impact of heavy metals on cells of

microalgae consists in destruction of a diffusive barrier which promotes free receipt into cells of various substances, including, toxic metals. This phenomenon can begin with external adsorption of ions of heavy metals on a cellular wall with their subsequent penetration into protoplasm of cells.

Unlike zinc and copper, cadmium is a heavy metal, toxic for living organisms even in small concentration as possesses very high cumulative ability because its ions are capable to be adsorbed on firm particles and to be transferred to long distances. It is known that cadmium is capable to have toxic effect on the most different bodies and systems owing to what, its hit into cells of plants and animals is extremely undesirable [7].

The analysis of literary data showed that sensitivity of microalgae to ions of cadmium is various and is caused by a functional condition of cells, their genotype and features of a metabolism of each species.

Studying of accumulation of ions of cadmium in cells of microalgae at introduction of chloride of cadmium into medium in concentration of 100 mg/l counting on ions of metal showed very considerable accumulation of them by mutant cultures steady to cadmium *Chlamydomonas reinhardtii* CC-124 Res-1 and CC-124 Res-2. Thus the ions of metal occluded by cells CC-124 Res-1 account for 91,0 mg/l, cells of strain SS-124Res-2 this indicator is 83,2 mg/l (Fig. 3). Ions of cadmium sorbed by cells of *Chlorella* sp.3 account to 76,3 mg/l. Sorption of ions of cadmium by cells of a wild strain of *Chlamydomonas reinhardtii* CC-124 isn't observed. Thus it should be noted low survival of this culture under toxic influence of metal.

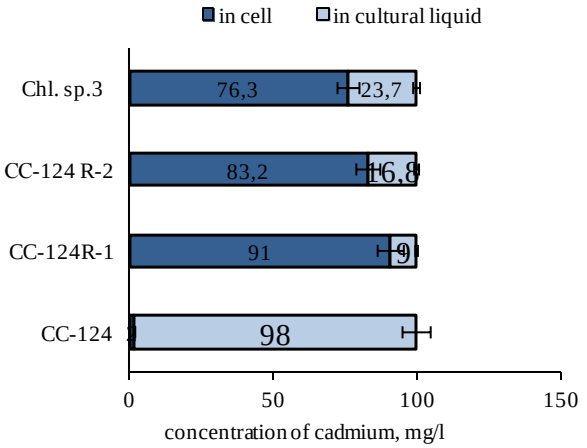

Fig. 3 Bioaccumulation of cadmium ions by cells of various strains of microalgae at 24 hours of cultivation

In the previous researches we showed that cells of a mutant strain of *Chlamydomonas reinhardtii* CC-124 Res-1 differed with resistance to considerable concentration of cadmium. As well as it was supposed, these cells accumulated cadmium ions in high quantity in comparison with cells of wild type. The analysis of the received results allows to conclude that resistance to cadmium of a mutant strain of *Chlamydomonas reinhardtii* CC-124 Res-1 correlates with absorption of ions of metal into a cell.

Thus, the analysis of the obtained experimental data gives evidence of essential distinction in ability to accumulation of heavy metals by cells of various strains of green microalgae.

Apparently there are species and strains dependence of representatives of green algae concerning heat-sink ability of the studied heavy metals, it is explained by many factors, including, features of a metabolism of each species, its genotype, a functional condition of cells, etc.

The analysis of the received results testifies that dynamics of accumulation of zinc and copper by cells of the studied strains is generally much higher in comparison with cadmium accumulation ability. Thus cells of resistant to cadmium of mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1, CC-124 Res-2 have high accumulation. Only zinc and copper ions are capable to accumulate in very low concentration cells of a wild strain of *Chlamydomonas reinhardtii* CC-124. Toxic influence of metals on microalgae is obvious.

The received results allow to recommend the mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1, CC-124 Res-2, resistant for cadmium and the wild strain of *Chlorella* sp.3 allocated from water polluted by various organic substances, received by us in laboratory condition, to use in processes of cleaning of the water environment, polluted by various heavy metals, and also for extraction of their ions from the diluted solutions.

Studying of dynamics of accumulation of ions of copper, zinc and cadmium by cells of wild and mutant strains of microalgae in time, showed that sorption activity of cell of microalgae is connected with species and genotypic features of a strain.

In the following series of experiences we made observation over process of clarification of the polluted sewage at cultivation on them of the mixed culture of mutant strains of *Chlamydomonas reinhardtii* CC-124 Res-1 and *Chlamydomonas reinhardtii CC*-124 Res-2 steady to cadmium, received by a method of induced muthagenesis and strain of *Chlorella* sp.3.

In model experiments we used sewage with addition of salts of cadmium, copper and zinc in various concentration. The content of ions of copper was 100,0 mg/l, cadmium – 50,0 mg/l, zinc – 100,0 mg/l. Physical properties and extent of purification of these waters were defined at cultivation of microalgae on them.

The initial density of cells were 1,5 x 10^6 Cell/ml, in 3 days – 14,6 x 10^6 Cell/ml. Suspensions of cells of microalgae were brought in tests of the sewage enriched by ions of metals, and we watched change of its physical and chemical structure.

According to the received results, pH in the sewage II of a settler used by us is about 6,8 to 7,8, a smell - 5 points, color – the dark-brown, dissolved oxygen is absent, concentration of the weighed substances is 18 mg/l, biochemical consumption of oxygen (BCO_5) is 62,2 mg/O_2/l, oxidability is 16,6 mg/O_2/l, concentration of ammonia is 13,7 mg/l, nitrites are 0,4 mg/l,

nitrates are 0,8 mg/l and phosphates are 4,46 mg/l. For detection of ability of microalgae to grow in the polluted water strains *Chlamydomonas reinhardtii* CC-124 Res-1, CC-124 Res-2 and *Chlorella* sp.3 were cultivated in laboratory conditions in this water.

It is established that in 4 days of growth of microalgae on experimental sewage (real sewage with addition of heavy metals) a putrefactive smell of sewage disappeared, the content of oxygen increased to 10,4 mg/l, biochemical consumption of oxygen decreased to 5,6 mg of O_2/l, oxidability to 3,1 mg of O_2/l, ammonia, nitrites and nitrates were acquired by algae completely, ions of copper, zinc and cadmium weren't found (see Table I).

TABLE I
PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF SEWAGE BEFORE CULTIVATION OF THE MIXED CULTURE OF MICROALGAE

Indicator	Physical-chemical structure of sewage		Treatment %
	Before cultivation of microalgae	After cultivation of microalgae	
temperature ^{0}C	23-28	24-28	-
smell, point	5	00	100%
pH	7,0-7.8	8-9	-
BCO_5, mgO_2/l	62,2	5.6	91%
oxidability, mgO_2/l	16,6	3.1	81,3%
Weighted substances, mg/l	18	4.7	73,8%
ammonia, mg/l	13,7	-	100%
nitrites, mg/l	0,4	-	100%
nitrate, mg/l	0,8	-	100%
phosphate, mg/l	4,46	-	100%
copper, mg/l	100	-	100%
cadmium, mg/l	50	-	100%
zink, mg/l	100	-	100%

Thus, according to the received results, cultivation of consortium of microalgae on model sewage renders a noticeable positive effect on their physical properties and promotes sewage treatment from ions of heavy metals.

These data can be interpreted as follows: microalgae consortium created by us, which use for purification of model sewage in laboratory experiments showed its high efficiency not only concerning cleaning of heavy metals, but also concerning a number of bacteriological and physical and chemical indicators, it is possible to recommend it as a basis for receiving a biological product with remediation effect.

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