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THE OPPORTUNITIES TO USE CONSORTIUM OF HIGHER AQUATIC PLANTS AND MICROALGAE IN THE TREATMENT OF POLLUTED AQUATIC ECOSYSTEMS

One of the priorities of modern environmental research is the development of theoretical and practical aspects of bioremediation of water bodies, based on the use of natural mechanisms of self-purification and self-healing of water bodies, the effect of which is associated with the activities of higher aquatic plants (HAP) and microorganisms belonging to different types of cyanobacteria and microalgae. The practical significance of these objects for bioremediation and post-treatment of water bodies is determined by the uniqueness of their metabolic abilities (photosynthesis, respiration, a variety of carbon sources, the ability to absorb atmospheric nitrogen, etc.), high cumulative and destructive ability against heavy metals and organic pollutants such as oil, petroleum products, phenols, etc. The aim of the research was to study the process of wastewater treatment from heavy metals by the consortium of HAP and microalgae in industrial wastewater. It is shown that the use of the consortium of HAP and microalgae in wastewater treatment is very effective compared to their use in purification processes in monoculture. It was found that the use of the consortium of HAP and microalgae improved water quality, and the effect of purification from pollutants on some indicators was more than 95-100 %.

Key words: higher aquatic plants, microalgae, consortium, bioremediation.

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Су экожүйелерін тазалауда микробалдырлар мен су өсімдіктері негізіндегі консорциумды қолдану мүмкіншіліктері

Заманауи экологиялық зерттеулердің басым бағыттарының бірі микробалдырлар мен цианобактериялардың әр түрлеріне жататын микроорганизмдердің және жоғары сатылы су өсімдіктері (ЖССӨ) әрекетімен байланысты су қоймаларының өзіндік қалпына келуі және өздігінен тазалану табиғи механизмдерін пайдаланудың негізінде су қоймалары биоремедиациясының теориялық және тәжірибелік аспектілерін өңдеу. Биоремедиация және су қоймаларын тазалау үшін осы объектілердің практикалық маңыздылығы олардың метаболитикалық қабілеттілігінің (фотосинтез, тыныс алу, көміртегі көзінің әртүрлілігі, атмосфералық азотты пайдалану қабілеттілігі және т.б.), ауыр металдарға қатысты және мұнай, мұнай өнімдері, фенол және т.б. органикалық ластағыштарға қатысты жоғары кумулятивті және деструкциялық қабілеттерінің ерекшелігімен анықталады. Зерттеудің мақсаты, өндірістік ағынды сулар жағдайында ЖССӨ және микробалдырлардың консорциумы көмегімен ағынды суларды ауыр металдардан тазалау үрдісін зерттеу болып табылады. ЖССӨ және микробалдырлар консорциумын ағынды суларды тазалау үрдісінде пайдалану, монодақылды пайдаланумен салыстырғанда тиімді екені көрсетілді. ЖССӨ және микробалдырлар консорциумын пайдалану судың сапасын жақсартатыны анықталды, ал ластағыштардан тазалану деңгейі кейбір көрсеткіштер бойынша 95-100 %-дан жоғары болды.

Түйін сөздер: жоғары сатылы су өсімдігі, микробалдырлар, консорциум, биоремедиация.

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Возможности использования консорциума высших водных растений и микроводорослей в очистке загрязненных водных экосистем

Одним из приоритетных направлений современных экологических исследований является разработка теоретических и практических аспектов биоремедиации водоемов, основанная на использовании природных механизмов самоочищения и самовосстановления водоемов, действие которых связано с деятельностью высших водных растений (ВВР) и микроорганизмов, принадлежащих к различным видам цианобактерий и микроводорослей. Практическая значимость этих объектов для биоремедиации и доочистки водоемов определяется уникальностью их метаболических способностей (фотосинтез, дыхание, разнообразие источников углерода, способность усваивать атмосферный азот и т.д.), высокой кумулятивной и деструктивной способностью в отношении тяжелых металлов и в отношении таких органических загрязнителей, как нефть, нефтепродукты, фенолы и т. п. Целью исследований являлось изучение процесса очистки сточных вод от тяжелых металлов с помощью консорциума ВВР и микроводорослей в условиях промышленных сточных вод. Показано, что использование консорциума ВВР и микроводорослей в очистке сточной воды весьма эффективно по сравнению с использованием их в процессах очистки в монокультуре. Установлено, что использование консорциума ВВР и микроводорослей улучшало показатели качества воды, а эффект очистки от загрязнителей по некоторым показателям составил более 95-100 %.

Ключевые слова: высшая водная растительность, микроводросли, консорциум, биоремедиация.

Introduction

In recent years, the problems of biodiversity conservation associated with increased anthropogenic impact on different ecosystems that have a great importance. Due to the difficult environmental conditions, in many regions of the world the geochemical circulation of heavy metals in the biosphere is carried out not only as a result of natural processes, but also due to anthropogenic impact. The problems of environmental pollution by various ecotoxicants are aggravated depending on the degree of urbanization and industrialization of the state [1; 2]. Since the majority of industrial, municipal and agricultural waste waters enter open water bodies without preliminary treatment, in some reservoirs with a high content of toxic elements of high concentration, ions of heavy metals and organic substances [3, 4]. Therefore, many water bodies are under threat to the environment and human health. The main pollutants were heavy metals, petroleum products, nitrites, nitrates, and various polycyclic aromatic hydrocarbons [5, 6]. In this regard, the study of pollution of the biosphere by these toxicants is one of the most important problems of modern ecology [7]. It is known that to enhance the effect of bioremediation used mixed cultures of microorganisms, not only the monocultures. To obtain them, it is necessary to take into account the peculiarities of intraspecific bonds of cyanobacteria and microalgae, photo – and heterotrophic microorganisms. In the literature there is very little information about the species ratio of microalgae and their effect on bacteria [8; 9].

The problems of environmental pollution with heavy metals at the present time are relevant. Because of the acute toxicity of metals and the possibility of accumulation in the environment which are dangerous for biota [10]. In recent years, environmentalists, along with the assessment of the level of environmental pollution, identify sources of pollution, take attention to the identification of "destiny" of lost substances in the natural environment and the study of their connection with living organisms [11]. For such studies, the most convenient object is highly effective aquatic plants, cyanobacteria and microalgae that can accumulate many elements in high concentrations and replace them in a non-toxic form, and they widely used for wastewater treatment for bioremediation purposes [12; 13].

In this regard, the aim of the work is to study the process of industrial wastewater treatment from heavy metals by microalgae consortium.

Materials and methods of research

Objects of research – highest aquatic plant *Pistia stratiotes* and collection strains of phototrophic microorganisms: *Scenedesmus quadricauda* B-1*, Ankistrodesmus sp*. [14].

The number of cells of phototrophic microorganisms in liquid cultures was determined by direct counting under a microscope in the Gorjaev's count chamber adopted in hydrobiological practice [15]. Higher aquatic plants were cultivated on the water with the addition of Steinberg medium (2 wt. %) under natural light and at room temperature [16].

COD was determined by photometric method, BOD by iodometric method, chlorides and sulfates – titrimetric method. The content of nitrates and nitrites was determined by photometric method [17-19]. Determination of phosphate ions and phosphorus-containing compounds was carried out by photocolorimetric method [20].

For the research used wastewater from treatment facilities of Almaty city after chemical treatment of polluted waters. The research in industrial conditions was performed in biopound on the basis of agrobiostation of al-Farabi KazNU.

Results and discussion

We conducted research on studying the wastewater treatment process from heavy metals with a help of selected consortium of HAP and microalgae in industrial wastewater conditions. Investigated in previous studies *Ankistrodesmus* sp., *Scenedesmus quadricauda* B-1 microalgae and *Pistia stratiotes* were found as consortium with high-voltage aquatic vegetation [21].

In wastewater with biochemical oxygen consumption (BOC) 62.2 mg/O2/l, ammonia content -13.7 mg/l, nitrites – 0.4 mg/l, nitrates – 0.8 mg/l and phosphates – 4.46 mg/l, cadmium -0.00036 mg/l, lead – 0.0088 mg/l, zinc – 0.043 mg/l, copper-0.0015 mg/l in the first variant introduced the biomass of *Ankistrodesmus sp*. microalga strain in 1.6.107 CL/ml amount and the higher aquatic plant *Pistia stratiotes* in the amount 45 specimen/m^2 (№1), in the second variant biomass of *Scenedesmus quadricauda* B-1 microalga strain in the amount $1.6 \cdot 10^7$ and higher aquatic plant *Pistia stratiotes* in the amount 45 specimen/m^2 (№2), after that incubated during 15 days for sorption of organo-mineral substances and accumulation of metal ions. Then the biomasses of microalgae, cyanobacteria and higher aquatic plants were separated from the medium by filtration.

The content of suspended particles in the experimental conditions throughout the study period tended to decrease, so in the first 2 days of the experiment the best results were observed in the version №1, the number of suspended particles decreased by 15%, in variant No.2, this index was reduced by 13% but already during the next sampling after 4 days of cultivation the indicators in both variants were almost equalized and amounted to 56-58% of their original content. At the time of experiment completion the total reduction of suspended particles was high and amounted to 87% in variant 1, slightly lower in variant 2 – 71%.

In addition to the organoleptic characteristics of the main group, we paid attention to the group of chemical-organoleptic characteristics and pH of water. During the consortium cultivation under the experiment conditions, the pH of water in all study periods was 7.0-7.9 which corresponds to the MAC values.

Nitrogen and phosphorus content has particular importance in biological wastewater treatment [23; 24]. According to the results of our studies, the nitrogen exchange rates have tended to significant fluctuations throughout the study period. Obviously, this is due to the high content of ammonia nitrogen (13.7 mg/l) at the beginning of the experiment and its transformation from ammonia form to nitrite, and later nitrate. This is evidenced by the dynamics of ammonia nitrogen in water. Its content in the cultivation of the consortium in the variant №1 was noticeably reduced. The most intensively ammonia nitrogen was oxidized in the first 2 days of the experiment, during this period about a third of its total content was destroyed, which was 48% in the variant №1, 28% – in the variant №2. The intensive oxidation of the ammonia form of nitrogen during purification by the consortium of HAP and microalgae is evidenced by the dynamics of nitrite-ions. In the first days of the experiment, the number of nitrate-ions varies slightly [25]. Starting from 4 days, the nitrate concentration decreases, that is, they are almost completely utilized by hydrobionts and by the end of the experiment no nitrates were found. The appearance of oxidized forms of nitrogen indicates a deep passage of the purification process, because their increase against the background of an overall decrease in BOD suggests that carbonaceous compounds are intensively oxidized [26; 27]. Thus, in terms of nitrate metabolism there is a positive trend when we used consortia based on HAP and microalgae for bio-purification. As the results of the studies showed the consumption of phosphates by consortium occurred at a fairly rapid pace in both cases. At the time of completion of the experiment phosphate extraction in both variants was approximately at the same level, 87.3% – in variant No.1, slightly lower in variant No.2 – 82.6 %.

Under experimental conditions the COD index during purification on the basis of the consortium of HAP and microalgae in all variants tended to decrease. Moreover, the most intense dichromatic oxidability decreased in the period from 6 to 8 days to 42%, then the intensity of the oxidation process was observed and in the end of experiment the dichromatic oxidability decreased to 87% in the variant № 1. This indicator decline slightly faster in the version using the consortium *Pistia stratiotes*+ *Scenedesmus quadricauda* B-1 in the period from 6 to 8 days was 45%, but the final index was lower compared with the variant 1 by 15-20%.

We also determined the indicator characterizing the degree of organic pollution of the reservoir and wastewater – biochemical oxygen consumption (BOD). During the entire period of the experiment a similar pattern was observed as in the case with the COD indicator. In particular, in the first 4 days of the experiment there was no significant variation of this indicator, however, from 6 to 8 days in both experimental variants of purification there was a sharp drop: from 4.6 mgO2/l to 1.26 mgO2/l which is 72% of its original value in variant No. 1 and from 4.5 mgO2/l to 1.5 mgO2 / l, which is 67% in variant No. 2. Despite the presence of difficult-to-oxidize organic compounds in water, the use of phyto-algo-cyanobacteric consortia in biological treatment reduced the values of these parameters in both variants by 95%.

A study of the sorption capacity of consortium of HAP and microalgae established that in the experimental variants with *Pistia stratiotes*+*Ankistrodesmus sp*. B-1 the concentration of heavy metal ions on 6th day of purification decreased by more than 90% of the initial concentration. In the case of *Pistia stratiotes*+*Scenedesmus quadricauda* B-1 consortium the concentration of all heavy metals decreased by more than 75% of the initial concentration (Table 1).

Table 1 – Dynamics of physical and chemical parameters of water during treatment with the help of HAP and microalgae consortiums

Water Quality Index	Option consortium	Incubation period, days	
		0	15
pH	№1	7,5±0,16	7,85±0,27
	№2	7,53±0,12	7,86±0,27
Suspended substances	№1	6,2±0,21	0,8±0,017
	№2	6,0±0,20	1,74±0,02
Ammonia	№1	0,87±0,01	0,018±0,001
	№2	0,79±0,02	0,17±0,02
Nitrite	№1	0,11±0,004	0,0059±0,001
	№2	0,11±0,004	0,0065±0,0012
Nitrates	№1	1,6±0,04	0,027±0,0001
	№2	1,8±0,06	0,052±0,0021

Phosphates	№1	2,76±0,06	0,35±0,017
	№2	2,76±0,05	0,48±0,014
COD	№1	38,52±1,2	4,9±0,001
	№2	36,8±1,12	5,7±0,002
BOD	№1	4,80±0,1	0,239±0,001
	№2	4,6±0,16	0,24±0,001
Cadmium	№1	0,068±0,001	0,0059±0,002
	№2	0,068±0,0014	0,0078±0,002
Zinc	№1	6,48±0,002	0,33±0,001
	№2	6,38±0,001	0,37±0,002
Copper	№1	0,108±0,001	0,017±0,002
	№2	0,11±0,01	0,02±0,0025
Lead	№1	0,07±0,001	0,0175±0,0001
	№2	0,067±0,003	0,0188±0,002

The absorption capacity of No.1 consortium in relation to heavy metal ions was significantly higher than No.2 consortium which makes it possible to recommend it for phytoremediation of wastewater.

Biological reserves of biocenoses including organisms with different biochemical possibilities should be used to accelerate the processes of purification and restoration disturbed by pollution of aquatic ecosystems. The use of cyanobacteria and microalgae resistant to polluted waters, introducing them into the consortium of higher aquatic plants allows creating a new biotechnology complex of purification and restoration of polluted water bodies.

For practical application of created HAP and microalgae consortium was used the wastewater from the state enterprise «Holding» Almaty Su» selected from the primary radial sedimentation tanks after mechanical cleaning and after the aeration tank.

In wastewater in the 1st variant introduced the biomass of *Ankistrodesmus* sp. microalga strain in the amount $1.6 \cdot 10^7$cl/ml and *Pistia stratiotes* aquatic plant in quantity 45 specimen/m^2 (№1), in the second variant increased the biomass concentration of consortium, so the biomass of *Ankistrodesmus* sp. microalga strain in the amount $2.5 \cdot 10^7$ cl/ml and *Pistia stratiotes* aquatic plant 65 specimen/m^2 (№2) were introduced. Introduced variants of consortium incubated during 20 days for sorption of organo-mineral substances and accumulation of metal ions. After that the biomasses of microalgae, cyanobacteria and higher aquatic plants were separated from the medium by filtration.

Should be noticed that during selection and before consortium introduction in the biopond the wastewater characterized by sharp smell with IV point of intensity. After 10 days of consortium cultivation in biopond the sharp smell in all studied variants decreased by two points. At the end of experiment the smell intensity was "light" and counted I point, consequently, we can argue that application of hydrophytic treatment had a positive tendency and decreased the level of smell sharpness.

As known, for maximum heavy metal ions extraction and purification from organo-mineral compounds it is necessary to introduce biomass of microalgae strains and higher aquatic plants in sufficiently amount to binding metal ions and organo-mineral impurities. Certainly, as shown in Table 2 the introducing of HAP and microalgae consortium into the wastewater with less volume of biomass (№1) doesn't provide full purification due to adsorbent deficiency. The concentration increasing of biomass consortium particularly the cells of microalgae in the variant №2 lead to significantly increasing the level of wastewater treatment because of optimal ratio between adsorbent quantity and pollutant (Table 2).

Therefore, to ensure a sufficiently high degree of purification from heavy metal ions and organo-mineral impurities using higher aquatic plants and microalgae, an optimal ratio between the number of cells and metal ions is necessary.

Table 2 – results of wastewater treatment with a help of HAP and microalgae consortium

Index		№ 1 option		№2 option	
	Initial concentration	Concentration after cleaning, mg/l	Power cleaning, %	Concentration after cleaning, mg/l	Power cleaning, %
$BOD_{5,}$ mg/O_2/l	62,2±0,01	7,6±0,002	93	4,6±0,002	97
Ammonia	13,7±0,01	-	100	-	100
Nitrite	0,4±0,002	-	100	-	100
Nitrates	0,8±0,0023	-	100	-	100
Phosphates	4,46±0,0034	0,6±0,003	98	-	100
Cadmium	200±0,01	29,4±0,002	85,3	14,8±0,01	92,6
Zinc	200±0,01	26±0,01	87	10±0,01	95
Copper	200±0,01	26,2±0,01	86,9	10,2 ±0,012	94,9
Lead	200±0,01	26,4±0,01	86,8	14,4±0,02	92,8

Thus, the use of the proposed method of purification of domestic and industrial wastewater from organo-mineral substances and heavy metal ions: Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2} can significantly simplify the process and improve the quality of treatment. Intensity of purification process due to the high rate of microalgae reproduction and large working area of vegetative organs of aquatic plants which allow to absorb more pollutants from the water. In addition, the roots of aquatic plants absorb a large number of other organic impurities, and their metabolites inhibit the processes of self-pollution of the aquatic environment.

Thus, it was found that the use of HAP and microalgae for water purification from various pollutants in biological ponds is the most effective purification system.

For wastewater treatment from heavy metals we have developed principles for the creation and practical application of structured biocenoses – multilevel purification consortia based on microalgae and HAP contributing to the maximum purification of effluents from heavy metals.

The scheme of wastewater treatment from heavy metals includes a sequentially located from the flow mechanical treatment of biopond and drainage with a consortium of HAP and microalgae in the volume of *Ankistrodesmus sp* microalgae 2.5*10^7 cl/ml and the high aquatic plant *Pistia stratiotes* in amount 65 specimen/m^2. The depth of the pond should be at least 1.5 – 2 m. Drainage and drain will be performed at the opposite wall of the biopond (Figure 1).

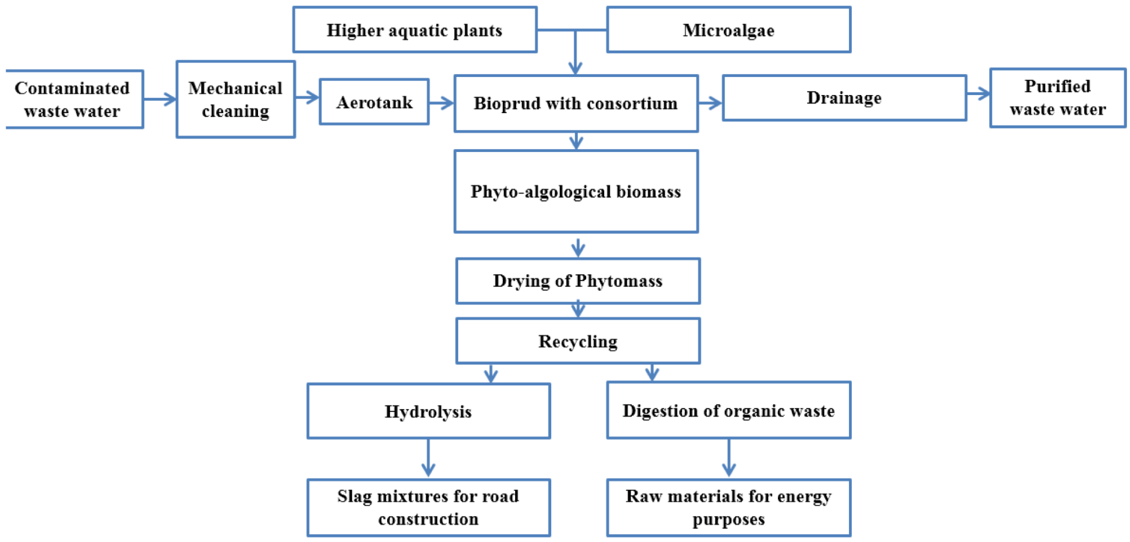

Figure 1 – Technological scheme of wastewater treatment from heavy metals based on HAP and microalgae consortium

The selected phyto-algological mass will be recycled according to the technological scheme. At the same time, secondary waste can be considered as a raw material for additional commercial products. Thus, ash residue (sludge) can be used in the production of building materials. This version of the technological scheme is suitable for the treatment of domestic and industrial wastewater contaminated with heavy metals.

In conclusion, it should be noted that by applying the technology of natural biological wastewater treatment using higher aquatic plants and microalgae on biological ponds, it is possible to solve the environmental problem of waste water disposal, reduce the concentration of nitrates, phosphates, heavy metals and other pollutants to the maximum permissible concentrations for their safe discharge into water bodies which can be the basis for the creation of environmentally friendly technologies. This technology of biological treatment is characterized by high efficiency of purification from pollution, ease of operation and efficiency and allows to dispose of waste water with minimal damage to the environment.

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