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PROSPECTS FOR THE USE OF MICROORGANISMS FOR THE BIOLOGICAL TREATMENT OF SURFACE WATER BODIES FROM BIOGENIC ELEMENTS

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ABSTRACT

The threat of water scarcity and inefficient management of water resources can become a major obstacle to sustainable economic growth and social development in Kazakhstan. Pollutants enter surface water bodies mainly as a result of human activities. Among the various substances present in wastewater, nitrogen and phosphorus compounds deserve the most attention. Getting into water bodies, cause eutrophication, which can be dangerous for the ecosystem as a whole and humans. Among the various methods of wastewater treatment, biological methods are considered the most promising and effective. In this regard, the search for active microorganisms and microalgae for the purification of water bodies from biogenic elements is especially relevant. The object of the study was Lake Bolshoi Taldykol, recreated as a result of the reclamation of the Taldykol wastewater evaporator and being one of the important water bodies of the city of Nur-Sultan. It has been revealed that in recent years the main substances polluting the water bodies of the capital are total phosphorus, calcium, magnesium, chlorides, sulfates, total iron, mineralization and chemical oxygen demand (COD). Chemical analysis of water samples from Lake Bolshoi Taldykol showed excesses in chlorides, sulfates, COD, total iron, suspended solids, and dry residue. The prospects for the use of microorganisms that are destructors of biogenic and organo-mineral pollutants are considered. Cultures of bacteria and green microalgae were isolated from the water of Lake Bolshoi Taldykol, and their cultural and morphological properties were studied. The biological activity of isolated microorganisms has been studied, as a result, thenumber of biogenic elements may decrease, thereby reducing their potential for eutrophication.

Key words: reservoir, pollution, microorganisms, cultivation, microalgae, bio-purification, biogenic elements, destruction.

1.INTRODUCTION

In recent years, the process of cleaning reservoirs has been of great ecological importance. This issue has been widely discussed in the scientific literature. The functioning of enterprises, especially those located in cities, causes irreparable damage to the environment. Increasing requirements for the standard indicators of surface water bodies makes it necessary to look for more efficient and environmentally friendly ways to remove pollution [1,2].

There are several thousand lakes in Kazakhstan. Most of them are concentrated in the north, the largest (Balkhash, Zaisan, Alakol) are located in the eastern and south-eastern regions. The increased mineralization of water in many lakes prevents their economic use. In the country as a whole, over the past 5 years, the volume of annual water consumption in all sectors of the economy averaged 22.1 km^3 and 95% of it was due to surface water [3].

The threat of water scarcity and inefficient management of water resources can become a major obstacle to sustainable economic growth and social development in Kazakhstan. According to Kazhydromet data on the state of the environment of the Republic of Kazakhstan, in 2019, 661 cases of high pollution (HP) and 19 cases of extremely high pollution (EHP) were detected in the Republic at 61 water bodies; in 2020, 248 cases of HP and 6 cases of EHP were detected in 33 water bodies; in 2021, 7 cases of HP and 144 cases of EHP were detected in 21 water bodies [4].

Pollution of water bodies can be caused by a number of reasons: erosional processes of the area, emissions of plant and livestock resources, the supply of biogenic elements with municipal wastewater, with target points, as well as with precipitation. Biogenic elements are difficult to biodegrade, and passing through treatment facilities, they are found in the past, are often found in water bodies, and have a significant negative impact not only on aquatic ecosystems, but also on human health, even in small quantities [5]. An increase in the content of biogenic elements (carbon, potassium, nitrogen, phosphorus) can lead to an increase in the composition of domestic wastewater [6].

When treating wastewater from biogenic elements, the main attention is paid to the removal of nitrogen and phosphorus [5]. An increase in the concentration of biogenic elements causes the process of eutrophication of reservoirs, leading to an increase in the level of primary production of reservoirs, which creates the basis for the development of a richer food base for fish and other hydrobionts, contributes to an increase in their number, and in connection with this, a deterioration in water quality due to its «blooming», reducing transparency and oxygen content in it. Anthropogenic eutrophication is caused by the discharge of biogenic substances with wastewater, including surface water (rain and snowmelt), and differs from the natural high rate of the process [7].

Among the various methods for removing biogenic elements, biological methods are the most preferable from an economic point of view [6]. Biological treatment is based on the ability of microorganisms to use many organic and inorganic compounds contained in polluted waters as nutrients [8].

Unlike many representatives of the microflora of water bodies, algae can completely utilize phosphorus. Using them, it is possible to carry out a deep removal of biogenic elements [9]. Green algae are the most favorable, as they also constitute a food base for hydrobionts [10].

In this regard, the search for active microorganisms and microalgae for the purification of water bodies from biogenic elements is especially relevant.

The aim of the work was to search for promising destructor microorganisms for the removal of biogenic elements in polluted surface reservoirs of the city of Nur-Sultan.

2. MATERIALS AND METHODS

The object of the study was Lake Bolshoi Taldykol, which is located in the southwestern part of Nur-Sultan and has a water area of 292 hectares. This lake was recreated as a result of the reclamation of the wastewater storage tank Taldykol. In order to minimize the inflow of flood waters, the water intake area (1004 ha) of the lake is cut off by an artificially constructed watershed. The water of the lake is prone to eutrophication in the warm season as a result of an excess of phosphorus and ammonium nitrogen.

Chemical analysis of water was carried out on the basis of the analytical laboratory of the State Communal Enterprise «Astana Su Arnasy» in accordance with the current methods within the Republic of Kazakhstan (ST RK ISO 10523-2013, SR RK 5815-2-2010, PND F 14.1:2:4.190-2003, ST RK 2015-2010, ST RK 2016-2010, ST RK ISO 6332-2008, ST RK 1983-2010 , ST RK ISO 5814-2014, PND F 14.1:2:4.128-98, GOST 26449.1-85, GOST 26449.1-85, GOST 3304-2014).

Isolation of cultures of microalgae and bacterial isolates was carried out by the method of limiting dilutions followed by inoculation on pre-prepared Petri dishes with differentiated media of meat-peptone agar, dry nutrient agar, Chapek, Saburo, Endo for bacteria and plates with Tamiya, 04, Lmin media for microalgae. The plates were incubated at 30°C and 37°C for 1-3 days. Pure cultures were obtained after re-plating isolated colonies on plates with the appropriate medium for each type of microorganism [11, 12].

The morphological features and purity of the isolated isolates were checked by staining with a set of dyes for differential Gram staining of microorganisms using a Micros MC 300X laboratory microscope (Austria) with immersion oil and a hundredfold magnification.

Antagonistic activity was determined by diffusion in agar by the width of the zone of absence of growth of opportunistic pathogenic cultures [12] available in the collection of the Republican Collection of Microorganisms: *Escherichia coli* ATCC 25922 B-RKM 0447, *Staphylococcus aureus* ATCC-6538 B-RKM 0470, *Salmonella enteriditis* B- RKM 0680, *Klebsiella pneumoniae* B-RKM 0444, *Enterococcus faecium*, *Pseudomonas taiwanensis* CB2R-1B-RKM 0726, *Pseudomonas aeruginosa* G13 B-RKM 0427, *Aeromonas punctata* G30 B-RKM 0287.

Determination of the phosphate mobilizing activity of bacteria was detected on Muromtsev's medium by the Gerretson precipitation method [13]. Proteolytic activity, lipolytic activity, nitrifying activity, amylolytic activity, carbohydrate fermentation were carried out according to standard microbiological methods. [12, 14].

The isolated bacterial pure cultures were identified by mass spectrometry on a MALDI-TOF analyzer (Bruker) [15].

3. RESULTS AND DISCUSSION

In recent years, surface water quality in the Republic has deteriorated significantly and has become more critical. 67.8% of reservoirs are polluted to varying degrees. For the 1st half of 2021, 27 cases of HP and 1 case of EHP were detected in the city of Nur-Sultan and Akmola region. So, in the water bodies of the city of Nur-Sultan, the following cases of air pollution were found: the Yesil River - 2 cases of high pollution, the Sarybulak River - 1 case of EHP and 21 cases of HP, the Akbulak River - 3 cases of HP (Table 1) [16].

According to the above data in Table 1, it can be seen that

Table 1. Water quality of reservoirs in Nur-Sultan according to the Unified Water Quality Classification

Number	Reservoir	Water quality class		Pollutants
		2020	2021	
1	Yesil river	4 cells	>4 cells (not standardized)	Phosphorus total
2	Akbulak river	>5 cells (not standardized)	>5 cells (not standardized)	Calcium, Magnesium, Chlorides, Mineralization
3	Sarybulak river	>5 cells (not standardized)	>5 cells (not standardized)	Magnesium Sulfates Mineralization
4	Nura-Yesil Channel	>5 cells (not standardized)	4 cells	Magnesium
5	Vyacheslav reservoir	3 cells	2 cells	Phosphates Phosphorus total COD

relative to the 1st half of 2020, the surface waters of the Akbulak and Sarybulak rivers has not changed to a significant extent, the water quality of the Nura-Yesil channel has moved from above class 5 to class 4, the Yesil river has moved from class 4 to above class 4, in the Vyacheslav reservoir, the water quality has improved and moved from the 3rd to the 2nd class. It has been revealed that in recent years the main substances polluting the water bodies of the capital are total phosphorus, calcium, magnesium, chlorides, sulfates, total iron, mineralization and chemical oxygen demand (COD).

Lake Bolshoy Taldykol is located in the southwestern part of Nur-Sultan and has a water area of 292 hectares. This lake was recreated as a result of the reclamation of the wastewater storage tank Taldykol. In order to minimize the inflow of flood waters, the water intake area (1004 ha) of the lake is cut off by an artificially constructed watershed. The water of the lake is prone to eutrophication in the warm season as a result of an excess of phosphorus and ammonium nitrogen.

In the summer of 2021, water samples were taken from Bolshoy Taldykol Lake to study the chemical analysis of water samples (Table 2) and to isolate microorganisms of various taxonomic groups.

tion in the volume of water in reservoirs. Recently, the lake has significantly decreased in size. The increase in the amount of sulfates is mainly due to the death of organisms and the oxidation of substances of plant and animal origin, as well as wastewater from utilities and agricultural production. The increase in iron in reservoirsis associated with wastewater from mines, metallurgical, machine-building and chemical enterprises. The increase in COD is mainly due to household wastewater, underground and surface runoff, and atmospheric precipitation. The table shows that during the study period, the concentration of nutrients and pollutants gradually increases and reaches a maximum by autumn.

One culture of green microalgae and 8 bacterial isolates were isolated from water samples of Bolshoy Taldykol Lake (Figure 1). The cultural-morphological and physiological features of isolated microorganisms were studied. It was established that 4 isolates are represented by gram-positive (BT 1, BT 2, BT 3, BT 5/1) and 4 isolates by gram-negative bacteria (BT 4, BT 5/2, BT 6, BT 7).

Bacterial isolates were identified using MALDI Biotyper mass spectrometric analysis (Table 3).

As a result of mass spectrometric analysis, bacterial iso-

Table 2. Chemical analysis of water samples of Lake Bolshoy Taldykol for the summer period of 2021

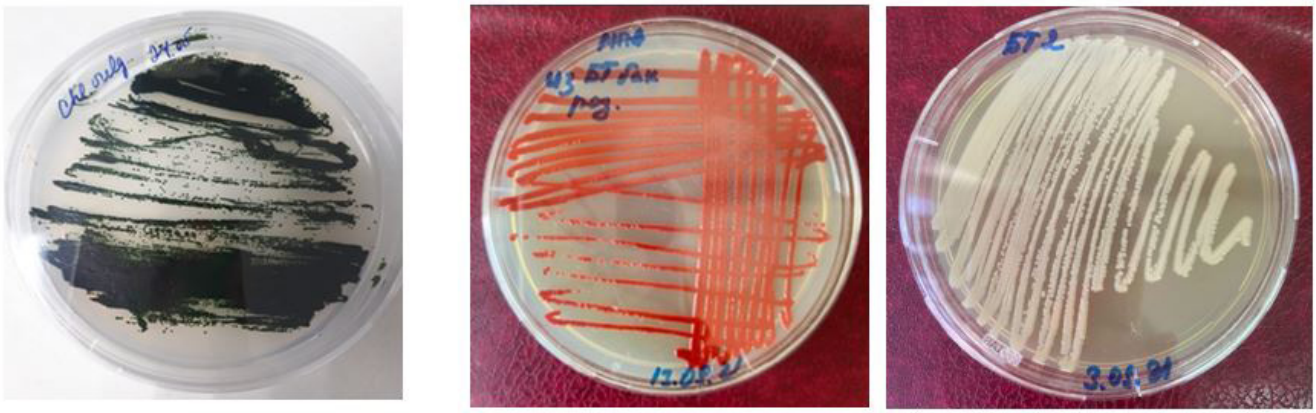
№	Defined indicator, mg/dm^3	Month			MPC,mg/dm^3
		May (mg/dm^3)	July (mg/dm^3)	September (mg/dm^3)	
1	pH	7.90	8.19	8.02	6.5 - 8.5
2	Dissolved oxygen	6.06	6.85	8.69	not less than 4 mg/l
3	BOD_5	3.0	5.0	2.0	3
4	COD	24.4	101	168	30
5	Suspended substances	30.6	281.7	58.0	25
6	Chlorides	983	1249	1702	350
7	Sulphates	1255	1650	2153	500
8	Phosphates	0.58	0.35	0.32	1.2
9	SAAs	0.18	0.20	1.24	0.5
10	Nitrates	0.30	1.53	12.25	45.0
11	Nitrites	0.198	0.018	0.321	3.3
12	Ammonium nitrogen	1.65	1.09	1.87	2.6
13	Iron	0.72	3.79	0.93	0.3
14	Petroleum products	>0.005	0.024	0.028	0.3
15	Dry residue	3682	5024	2009	1000

As a result of the chemical analysis of water samples, excesses of MPC (maximum permissible concentrations) for fishery reservoirs [4] were revealed for the following indicators: chlorides - from 2.8 to 4.8 MPC, sulfates - from 2.5 to 4.3 MPC, total iron - from 2.4 to 12.6 MPC, COD - 3.3 to 5.6 MPC, suspended solids - from 1.2 to 11.2 MPC, dry residue - from 3.6 to 5.0 MPC.

The increased concentration of chlorides is subject to noticeable seasonal fluctuations, and is also caused by a reduc-

lates were identified with a high degree of reliability (score close to 2 or higher). The isolated isolates belong to the following genera: *Bacillus* - 1 isolate, *Arthrobacter* - 2 isolates, *Rhodococcus* - 1 isolate, *Serratia* - 2 isolates, *Pseudomonas* - 1 isolate, *Enterobacter* - 1 isolate.

The widest world of microbial diversity provides an opportunity to reveal the biochemical potential of microorganisms to obtain biotechnologically significant products. One of the important factors in the screening of active cultures is



A B C

Figure 1 - Pure cultures of microorganisms isolated from Lake Bolshoy Taldykol:
A) microalgae culture SV-5; B) bacterial culture isolate BT 4;
C) bacterial culture isolate BT2

Table 3. Identification results by MALDI TOF Biotyper

№	Working name	Identification results	Score value	NCBI ID	Gram staining
1	BT 1	*Bacillus subtilis*	2.2	1423	gram+
2	BT 2	*Arthrobacter nicotinovorans*	1.976	29320	gram+
3	BT 3	*Rhodococcus erythropolis*	2.12	1833	gram+
4	BT 4	*Serratia marcescens*	2.277	615	gram-
5	BT 5/1	*Arthrobacter nicotinovorans*	2.35	29320	gram+
6	BT 5/2	*Pseudomonas fluorescens*	2.09	294	gram-
7	BT 6	*Serratia marcescens*	2.3	615	gram-
8	BT 7	*Enterobacter cloacae*	2.32	550	gram-

antagonism to various pathogenic and opportunistic microorganisms, as well as sensitivity to antibiotics, because. in the process of biopurification, the strains should exhibit antimicrobial properties. For this, the biological activity of the isolated strains was studied in order to screen the most promising strains that have destructive properties with respect to biogenic elements in the aquatic environment. The results are presented in table 4.

Table 4. Biological activity of the most active isolates

Strains	AnA	PMA	PA	LA	AmA	NA	SA
Bacillus subtilis BT 1	+	-	+++	+	++	+++	I
Arthrobacter nicotinovorans BT 2	++	++	+++	+	-	+++	I
Rhodococcus erythropolic BT 3	-	++	-	-	-	+	I
Serratia marcescens BT 4	++	+++	++++	+++	-	+++	I
Arthrobacter nicotinovorans BT 5/1	-	+	+++	+	-	++	I
Pseudomonas fluorescens BT 5/2	+	+++	+++	-	++	++	I
Serratia marcescens BT 6	-	+	-	+	-	+++	I
Enterobacter cloacae BT 7	+	++	-	-	-	+++	I

Note: AnA – antagonistic activity; PMA – phosphate-mobilising activity; PA – proteolytic activity; LA – lipolytic activity; AmA – amylolytic activity; NA – nitrifying activity; SA – sensitivity to antibiotics (S – high sensitivity, I – medium sensitivity); ++++ very high activity; +++ high activity; ++ medium activity; + weak activity; - negative result.

As a result of the studies, 2 most promising strains were selected - *Arthrobacter nicotinovorans* BT 2 and *Serratia marcescens* BT 4, which have a number of biological properties (Figure 2). Cultural and physiological-biochemical properties of strains:

- the strain *Arthrobacter nicotinovorans* BT 2 is represented by small, round colonies of beige color, with smooth edges, soft consistency, convex profile, shiny, opaque colonies with a diameter of 0.5-1.5 mm. The strain *Arthrobacter nicotinovorans* BT 2 has antibacterial activity against *Salmonella enteriditis, Enterococcus faecium, Pseudomonas taiwanensis*, has proteolytic activity, dissolves phosphates, absorbs organic and mineral form of nitrogen as a source of nutrition, forms catalase, is resistant to antibiotics from groups of β-lactams, macrolides, tetracyclines.

- the strain *Serratia marcescens* BT4 is represented by round colonies of beige color, with smooth edges, soft consistency, translucent, convex, shiny colonies with a diameter of 1.0-2.0 mm. the strain of *Serratia marcescens* BT4 shows antagonism to such opportunistic pathogens as *Staphylococcus aureus, Aeromonas punctata,* has proteolytic, lipolytic activity, dissolves phosphates, assimilates organic and mineral

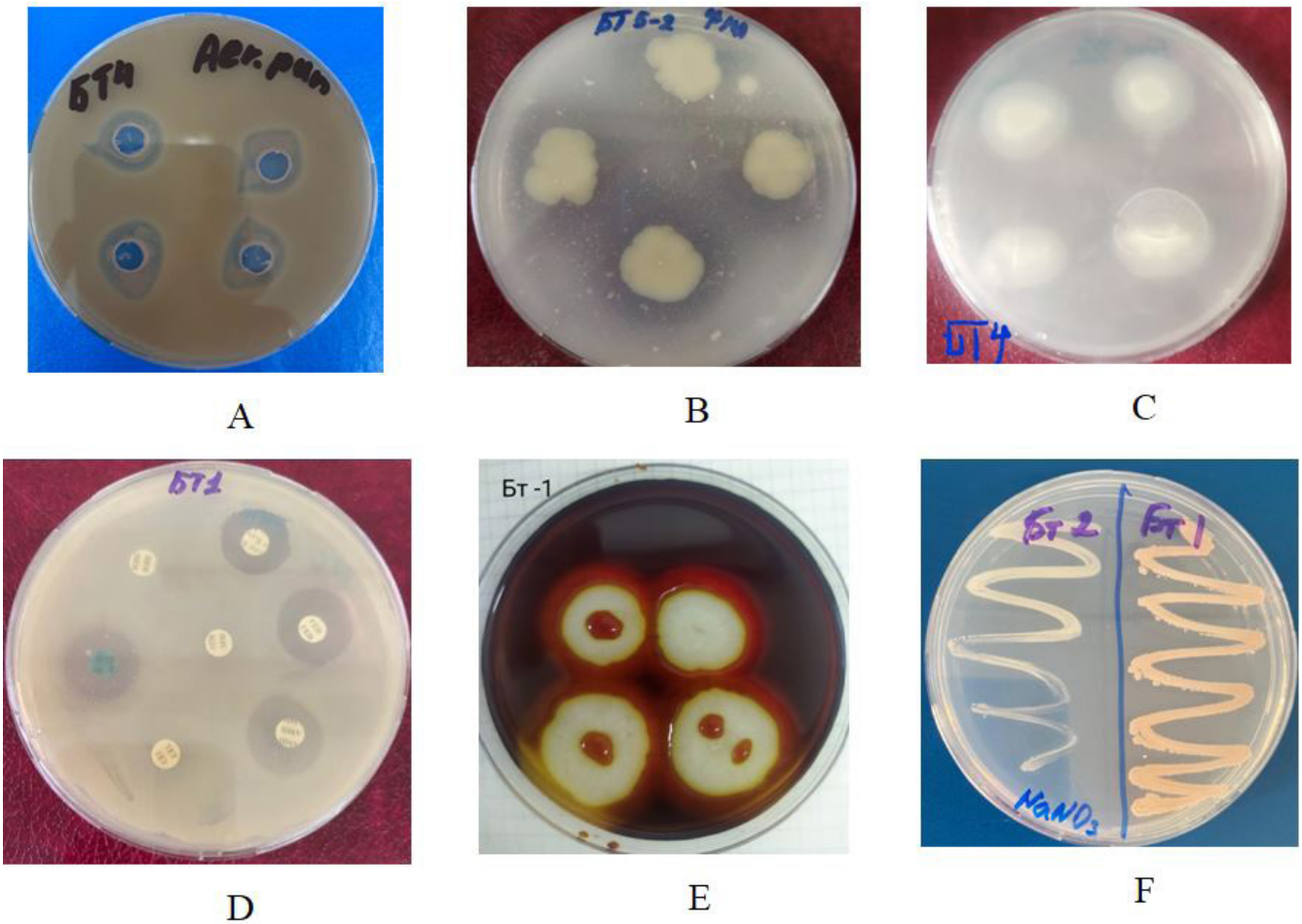


Figure 2 - Biological activity of isolated strains: A) antibacterial activity; B) phosphate-mobilizing activity; C) proteolytic activity; D) sensitivity to antibiotics; E) starch hydrolysis; F) nitrification activity

form of nitrogen as a source of nutrition, forms catalase, is resistant to antibiotics from groups β-lactams, macrolides, tetracyclines.

The most active bacterial strains were tested for pathogenicity in the Nutritest company (Almaty), which showed that these strains belong to the 4th hazard class. The strains were deposited in the Biobank of Industrial Microorganisms of the Republican Collection of Microorganisms under the numbers: B-RKM 0956 – *Arthrobacter nicotinovorans* BT 2, B-RKM 0955 - *Serratia marcescens* BT 4.

The culture of green microalgae *Chlorella sp.* SV-5 possesses resistance to many antibiotics from the groups of cephalosporins, aminoglycosides, β-lactams, tetracyclines, as well as antagonistic activity against *Escherichia coli*, a member of the *Escherichia coli* group. The isolated culture of green microalgae belongs to the genus *Chlorella*.

DISCUSSION

Under the conditions of a large population of cities, there is a characteristic excess in terms of biogenic elements in wastewater, i.e. pollution is anthropogenic. When treating wastewater, the greatest attention must be paid to the removal of nitrogen and phosphorus, which cause biological fouling of pipelines, sewers, and other sewage equipment, and also lead to the development of corrosion processes.

An important role of microalgae and microorganisms in the restoration of aquatic technogenic ecosystems is photosynthetic aeration, the production of biologically active substances that have a stimulating or inhibitory effect, as well as the ability to take a direct part in the utilization of some pollutants through accumulation, transformation, and mineralization [17].

Therefore, the use of biological methods of bioremediation of water bodies, namely microorganisms-destructors of biogenic elements, is currently necessary due to the deterioration of the environmental situation not only in the country, but also in the world.

CONCLUSION

Thus, monitoring of pollution of water bodies in the city of Nur-Sultan was carried out, promising strains of native microorganisms have been obtained, which have a number of biological properties necessary for the purification and post-treatment of polluted water bodies from pollutants of various origins, namely biogenic elements, which are mainly found in surface waters in large quantities due to human activities. As is known, the use of biological methods for cleaning polluted aquatic ecosystems with the help of microorganisms is quite economical, expedient and justified, since no environmental damage is caused.

On the basis of the studies carried out, strains of active microorganisms were selected, with the help of which further studies on their destructive activity in relation to biogenic elements will be carried out.

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ЖЕР ҮСТІ СУ ҚОЙМАЛАРЫН БИОГЕНДІ ЭЛЕМЕНТТЕРДЕН ТАЗАРТУ ПЕРСПЕКТИВАЛАРЫ

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ТҮЙІН

Су тапшылығының қаупі және су ресурстарын тиімсіз басқару Қазақстанның тұрақты экономикалық өсуі мен әлеуметтік дамуы үшін басты кедергі болуы мүмкін. Әдетте, ластаушы заттар жер үсті су нысандарына негізінен адамның антропогендік іс-әрекетінің нәтижесінде түседі. Ағынды суларда болатын әртүрлі заттардың ішінде азот пен фосфор қосылыстары ерекше назар аударуға тұрарлық. Олар су нысандарынатүскеннен соң, эвтрофикацияны тудырады, содан бұлжалпы экожүйе және адамдар үшін де қауіпті болуы мүмкін. Ағынды суларды тазартудың әртүрлі әдістерінің ішінде биологиялық әдістер ең перспективті және тиімді болып саналады.Осыған байланысты су нысандарын биогенді элементтерден тазарту үшін белсенді микроорганизмдер мен микробалдырларды іздеу ерекше өзекті болып табылады. Зерттеу нысаны Нұр-Сұлтан қаласының маңызды су объектілерінің бірі, Талдықөл сарқынды су жинағыш-буландырғышты қайта құнарландыру нәтижесінде қалпына келтірілген Үлкен Талдықөл көлі. Соңғы жылдары елорданың су нысандарын ластайтын негізгі заттар жалпы фосфор, кальций, магний, хлоридтер, сульфаттар, жалпы темір, минералдануы және оттегінің химиялық қажеттілігі (ОХҚ) екені анықталды.Үлкен Талдықөл көлінен алынған су сынамаларындағы химиялық талдауыхлоридтер, сульфаттар, ОХҚ, жалпы темір, өлшенген заттар, құрғақ қалдықтың артық мөлшерін көрсетті. Биогенді және органо-минералды ластаушы заттардың деструкторлары болып табылатын микроорганизмдерді қолдану перспективалары қарастырылады. Үлкен Талдықөл көлінің суынан микроорганизмдер мен жасыл микробалдырлар культуралары бөлініп алынды.Зерттелетін микроорганизмдерді қолдану биогенді элементтердің санын азайтып, олардың эвтрофикациялану мүмкіндігін азайтады.

Кілт сөздер: су айдыны, ластану, микроорганизмдер, культивациялау, микробалдырлар, биотазарту, биогенді элементтер, деструкция.

ПЕРСПЕКТИВЫ ОЧИСТКИ ПОВЕРХНОСТНЫХ ВОДОЕМОВ ОТ БИОГЕННЫХ ЭЛЕМЕНТОВ

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АБСТРАКТ

Угроза дефицита воды и неэффективное управление водными ресурсами может стать основным препятствием для устойчивого экономического роста и социального развития Казахстана. Загрязняющие вещества поступают в поверхностные водоемы в основном в результате антропогенной деятельности человека. Среди различных веществ, присутствующих в сточных водах, наибольшего внимания заслуживают соединения азота и фосфора. Попадая в водоемы, они вызывают эвтрофикацию, что может быть опасным для экосистемы в целом и для человека. Среди различных методов очистки сточных вод наиболее перспективными и эффективными считаются биологические. В связи с этим, поиск активных микроорганизмов и микроводорослей для очистки водных объектов от биогенных элементов является особенно актуальным. Объектом исследования являлось озеро Большой Талдыколь, воссозданное в результате рекультивации накопителя-испарителя сточных вод Талдыколь и являющимся одним из важных водных объектов г. Нур-Султан. Выявлено, что в течение последних лет основными веществами, загрязняющими водные объекты столицы являются общий фосфор, кальций, магний, хлориды, сульфаты, общее железо, минерализация и химическое потребление кислорода (ХПК). Химический анализ проб воды озера Большой Талдыколь показал превышения по хлоридам, сульфатам, ХПК, железу общему, взвешенным веществам, сухому остатку. Рассмотрены перспективы использования микроорганизмов, которые являются деструкторами биогенных и органно-минеральных поллютантов. Из воды озера Большой Талдыколь выделены культуры микроорганизмов и зеленой микроводоросли. Применение исследуемых микроорганизмов может уменьшить количество биогенных элементов, тем самым снизив их потенциал к эвтрофикации.

Ключевые слова: водоем, загрязнение, микроорганизмы, культивирование, микроводоросли, биоочистка, биогенные элементы, деструкция.