Жұбанова Ажар Ахметқызы

Биология ғылымдарының докторы, профессор, Жаратылыстану ғылымдары бойынша Қазақстан Ұлттық академиясының академигі, ЖОО- 2007 Үздік оқытушысы, ірі микробиолог Жұбанова Ажар Ахметқызының 80 –жылдығына арналған

«БИОТЕХНОЛОГИЯНЫҢ ЗАМАНАУИ МӘСЕЛЕЛЕРІ: ЗЕРТХАНАЛЫҚ ЗЕРТТЕУЛЕРДЕН ӨНДІРІСКЕ»

атты Халықаралық ғылыми-практикалық конференция материалдарының

ЖИНАҒЫ

4-5 маусым, 2021 г., Алматы, Казахстан

СБОРНИК

материалов Международной научно-практической конференции

«СОВРЕМЕННЫЕ ПРОБЛЕМЫ БИОТЕХНОЛОГИИ: ОТ ЛАБОРАТОРНЫХ ИССЛЕДОВАНИЙ К ПРОИЗВОДСТВУ»,

посвященной 80-летию крупного ученого-микробиолога, академика Казахстанской Национальной Академии Естественных Наук, Лучшего преподавателя ВУЗа-2007, доктора биологических наук, профессора

Жубановой Ажар Ахметовны

4-5 июня, 2021 г., Алматы, Казахстан

COLLECTION

of the International scientific and practical conference

"MODERN PROBLEMS OF BIOTECHNOLOGY: FROM THE LABORATORY RESEARCHES TO PRODUCTION",

dedicated to the 80th anniversary of outstanding scientist, microbiologist, academician of Kazakhstan National Academy of Natural Sciences, the best teacher of the university - 2007, doctor of biological sciences, professor

Zhubanova Azhar Akhmetovna

4-5 June, 2021 y., Almaty, Kazakhstan

Международная научно-практическая конференция «Современные проблемы биотехнологии: от лабораторных исследований к производству», посвященная 80-летию крупного ученого-микробиолога, академика Казахстанской Национальной Академии Естественных Наук, Лучшего преподавателя ВУЗа-2007, доктора биологических наук, профессора Жубановой Ажар Ахметовны, 4-5 июня, 2021 г.– Алматы: Қазақ университеті, 2021. – 231 с.

В сборник вошли научные статьи научных сотрудников НИИ, преподавателей ВУЗов, студентов, магистрантов, докторантов участвовавших в Международной научно-практической конференции «Современные проблемы биотехнологии: от лабораторных исследований к производству» (Казахстан, Алматы, 4-5 июня, 2021 года).

УДК 579

A.M. Malik, G.Zh. Abdieva, P.S. Ualieva, A.A.Zhubanova
Al-Farabi Kazakh National University
Almaty, Kazakhstan
E-mail: Azhar.malikkyzy@gmail.com

MICROBIAL DIVERSITY OF ENVIRONMENTAL OBJECTS IN THE TERRITORY ADJACENT TO THE BURIAL SITES OF PESTICIDES AND THE STUDY OF BIOCOMPATIBILITY OF DESTRUCTOR STRAINS

Annotation: Among the various chemical ecotoxicants of anthropogenic origin, organochlorine pesticides are among the most stable and dangerous for the environment and humans. One of the major environmental problems is the contamination of natural objects with organic pesticides, which are highly toxic and persistent. In addition to places of intensive use of pesticides, the potential danger to the environment and humans is caused by their burial sites – special underground concrete bunkers or wells. Toxic substances can enter the environment from storage facilities and pose a threat to all living organisms, including soil and aquatic microbial populations. Most of the conducted studies are devoted to the study of the effect of pesticides on the populations of microorganisms in the soils of agrocenoses, while the issues of studying soil microbial complexes in the areas of pesticide burial are not sufficiently covered. At the same time, microorganisms isolated from ecosystems exposed to long - term exposure to pesticides have the potential for faster decomposition of these compounds, which makes it necessary to study microbial communities of soils contaminated with pesticides, both for assessing biological risk and for selecting promising destructor microorganisms for bioremediation technology of natural objects.

Key words: *microorganisms, strains, destruction, bioremediation, persistent organic pollutants, pesticides.*

Introduction. Pesticides are chemical compounds used to control pests, weeds and plant diseases in agriculture, as well as pests of wood, products made of wool, leather, cotton, ectoparasites of domestic animals, vectors of human and animal diseases. The use of pesticides is primarily due to the desire to ensure maximum efficiency of agriculture. It is believed that if successful pest control in the world, it would be possible to annually collect an additional 200 million tons of grain, which would be enough to feed 1 billion people. However, the downside of the use of pesticides was the serious negative consequences for the environment in general, and human health in particular.

Organochlorine pesticides are widely used in agriculture. They are poorly soluble in water, highly soluble in organic solvents and fats, and extremely stable in the environment. Pesticides such as dichlorodiphenyl-trichloromethylmethane (DDT), aldrin, and heptachlor can be detected in soil 10 years or more after their use. They linger for a long time in the upper layers of the soil, slowly migrate to the depth, accumulate in products of plant and animal origin [1].

It is generally recognized that the main factor causing the conversion of pesticides in the soil is the action of microorganisms that are capable of destroying a wide range of compounds. The reverse process is also natural - the effect of pesticides introduced into the soil on the composition and vital activity of the microflora.

The direct effect of pesticides on soil microbial communities depends on the chemical properties of the preparation and the species of microorganisms. However, according to the available data, it is not of decisive importance, since the concentrations of agrochemicals that are critical for such an impact are tens or hundreds of times higher than the doses used in practice [2].

Thus, pesticides, entering the soil, have a direct or indirect impact on the communities of microorganisms. According to modern concepts, microbiological monitoring is one of the priority areas of environmental quality control. Currently, the best developed methods for assessing the impact of pollutants are based on the study of the structural rearrangements of communities and taking into account different groups of microorganisms [3].

Materials and methods

Methods for studying the microbial diversity of environmental objects in the territory adjacent to the sites of pesticide disposal.

The determination of the number of different groups of soil microorganisms to identify physiological groups resistant to the pollutant and to compare the microbiological composition of the

soil and water microflora in the areas adjacent to the storage facilities was carried out by the method of successive dilutions of the soil suspension on dense nutrient media. The number of cells was determined by the Koch method.

The essence of the method consists in seeding a certain volume of the studied suspension of microorganisms on a dense medium in Petri dishes and counting the colonies grown after incubation. Sowing is carried out on agarized media in Petri dishes. To determine the total number of microorganisms, meat - peptone agar (MPA) is used, to determine the content of fungi in the soil-wort-agar (CA), to determine the number of different physiological groups of microorganisms, appropriate nutrient media are used. Mold fungi were taken into account on the agarized medium of Chapek–Doxa, ammonifying bacteria were detected on GRM agar, nitrogen-fixing bacteria-on Ashby medium, aerobic cellulolytic bacteria were taken into account on the dense nutrient medium of Hetchinson and Clayton.

Methods study of biocompatibility of destructor strains

The vast majority of representatives of the microbial system realize the potential for biodegradation in natural conditions in combination with other microorganisms. An important condition for the effective transformation of xenobiotics is the absence of antagonism between destructive organisms [4].

N. A. Glushanova and others modified the drop method as follows: a daily strain culture grown on a liquid nutrient medium is applied to the surface of a dense medium in Petri dishes with a bacteriological loop with a diameter of 2-3 mm and left at room temperature until the drop is completely absorbed. After that, retreating 1-2 mm from the edge of the first spot, apply a drop of the daily test culture grown on the same nutrient medium. Spreading out, the second drop enters the culture spot about half the diameter. In the superimposed part, cultures develop in mutual presence (co-cultivation), competing with each other.

The free parts of the spots of each crop serve to control the viability of each crop and the germination of the nutrient medium. After drying, the drops of the second culture of the cup with the crops are incubated with the lid down at the optimal temperature. Preliminary accounting of the results is carried out after 18-20 hours of incubation, the final accounting - after 48 hours. The result of the experiment is taken into account visually by the presence of signs of suppression of one culture by another. The antagonistic activity of bacteria is evaluated by the number of strains of the tested microorganisms that they suppress [5].

Results and discussion

In the work, soil samples were taken from the following points: Amangeldy No. 1, Amangeldy No. 2, Belbulak, Kyzylkairat, Beskainar settlements adjacent to the pesticide burial sites of Talgar district of Almaty region. Soil samples from the village of Basshi, Kerbulak district, Almaty region, served as a control.

The results of the study of the qualitative and quantitative composition of the native microbiota in the studied water and soil samples are shown in Figure 1.

The microbial diversity of soil samples contaminated with pesticides was studied. Data on the quantitative and qualitative composition of the microflora of the studied soil samples were obtained. The total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) of the control sample of the soil and water of the village of Basshi was $1.8x10^6$-$4.\ 7x10^8$ CFU/g.

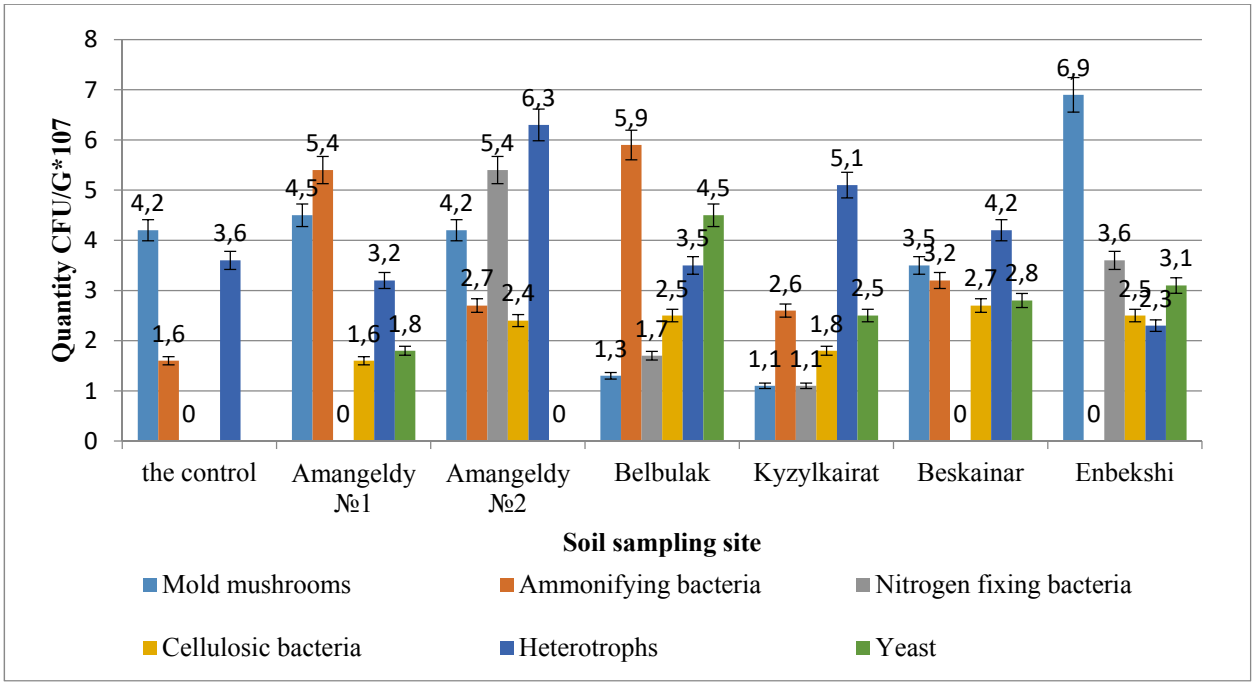


Fig. 1 - Microbial diversity of soils in Amangeldy village-Brigade 1 (warehouse 1 and warehouse 2), Belbulak village, Kyzylkairat village, Beskainar village

According to the analysis of the microbial diversity of the soil in the samples of the village of Amangeldy No. 1, the number of heterotrophic bacteria was 3. 2x108 CFU/g, the number of spore-forming bacteria of the genus *Bacillus* and bacteria of the genus *Pseudomonas*, as well as ammonifying bacteria was $5.4x10^5$ CFU/g,while the number of aerobic cellulose - decomposing bacteria and mold fungi was an order of magnitude lower. In the soil samples of Amangeldy No. 2, the number of heterotrophic bacteria was 6.3 x 10^7 CFU / g,including representatives of ammonifying bacteria in the amount of 2.7 x 10^6 CFU/g.

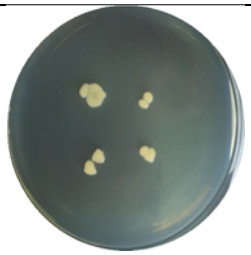
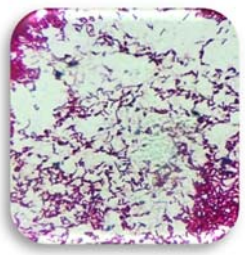
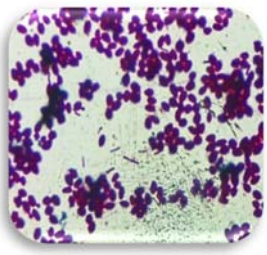
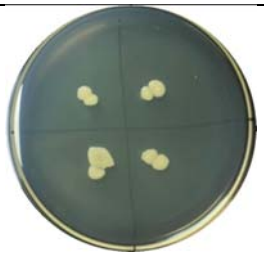
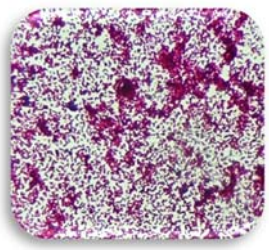
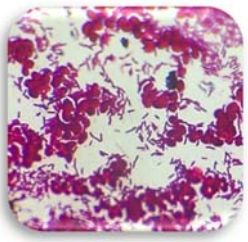
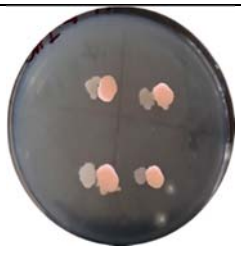
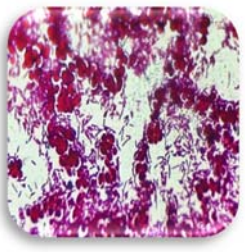
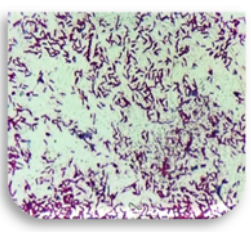
As a result of studying the microbial diversity of the soil of the village of Belbulak,microorganisms of the following physiological groups were identified: heterotrophic bacteria - 3. $5x10^7$ CFU/g, nitrogen – fixing bacteria – 1. $7x10^5$ and ammonifying bacteria – 5. $9x10^5$ CFU/g,as well as mold fungi-1. $3x10^7$ CFU/g and yeast-4. $5x10^7$ CFU/g. In soil samples, p. Kyzylkairat revealed heterotrophic bacteria in the amount of 5.1 x 10^7 CFU/g,as well as microorganisms of the following physiological groups: nitrogen – fixing bacteria – 1.1 x 10^5, ammonifying bacteria – 2.6 x 10^7 CFU/g, as well as mold fungi-1.1 x 10^7 CFU/g and yeast-2.5 x 10^7 CFU/g,aerobic cellulolytic bacteria-1.8 x 10^5 CFU/g. In the soils of Beskainar, the number of heterotrophs was $4.2x10^7$ CFU/g, aerobic cellulolytic bacteria-2.7 $x10^7$ CFU/g, ammonifying bacteria-$3.2x10^7$ CFU/g, yeast -2.8 $x10^7$ CFU / g, and mold fungi - $3.5x10^7$ CFU / g .

The vast majority of representatives of the microbial system realize the potential for biodegradation in natural conditions in combination with other microorganisms.

An important condition for the effective transformation of xenobiotics is the absence of antagonism between destructive organisms. This was the basis for the next stage of work – the identification of biocompatibility between bacterial and yeast cultures, taking into account their further compatibility in the consortium.

In this regard, the antagonistic relationships of the destructor strains in relation to each other for the purpose of joint cultivation were determined. Figure 2 shows the results of joint cultivation of bacterial and yeast strains.

Figure 2 - Biocompatibility of microbial strains isolated from contaminated soils by pesticides

№	Culture	Joint growth on nutrient media	Cell morphology
1	*Bacillus amyloliquefaciens AK3 - Pseudomonas koreensis AK1*		
2	*Bacillus pumilus AK4 – Rhodotorula sp.*		
3	*Alkanindiges illinoisensis БР7 - Pseudomonas plecoglossicida K2*		
4	*Bacillus aryabhattai K3 - Rhodotorula sp.*		
5	*Bacillus paramycoides CA1 - Rhodotorula sp.*		
6	*Bacillus subtilis AK 5 - Pseudomonas plecoglossicida K2*		

As can be seen from Figure 41, antagonism between bacterial and yeast strains was evaluated by the value of the diameter of the inhibition zone. Antagonism between *Bacillus aryabhattai K3, Bacillus pumilus AK4, Bacillus paramycoides CA1* and yeast cultures of *Rhodotorula sp.* he was absent.

On culture media, antagonism between bacterial strains *Bacillus amyloliquefaciens AK3-Pseudomonas koreensis AK1, Alkanindiges illinoisensis BR7 - Pseudomonas plecoglossicida K2, Bacillus subtilis AK 5-Pseudomonas plecoglossicida K2* was not established.

The study of antagonistic properties in these strains did not reveal inhibitory properties in relation to each other. This allows them to be cultured together and used in a consortium to create a biological product.

Conclusion. The characteristic of the diversity of microbial and fungal flora of the studied soil and water samples is given. The total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) of the control sample of the soil and water of the village of Basshi was $1.8x10^6$-4. $7x10^8$ CFU/g. As a result of the study of the qualitative and quantitative composition of the microflora, mold fungi dominated in the soil samples of Amangeldy No. 1 (31%), and heterotrophic bacteria dominated in the soil samples of Amangeldy No. 2 (34%). In the soils of Kyzylkairat, the number of heterotrophic bacteria was 34%, ammonifying bacteria 23%, and in the soils of P. Belbulak the number of ammonifying bacteria was -35%.

Micro-organisms of the following physiological groups were detected in the water microflora of Kyzylkairat, Beskainar, Amangeldy No. 1, Belbulak, Brigade – 2-Almaty Plemzavod JSC, Basshi (control): micromycetes, actinomycetes, heterotrophic bacteria, nitrogen-fixing and ammonifying bacteria.

During the screening, 25 promising strains were selected, and as a result of molecular genetic analysis, up to a species was identified.

The biocompatibility of destructor strains selected from contaminated soils with pesticides was studied. Antagonism between *Bacillus aryabhattai K3, Bacillus pumilus AK4, Bacillus paramycoides CA1* and yeast cultures of *Rhodotorula sp.* he was absent.

This allows them to be cultured together and used in a consortium to create a biological product.

References:

1. Thyssen, Wodageneh. FAO requires financial support for the disposal of obsolete pesticides in Africa and the Near East // 1998. – Vol. 11. - P. 19–23.
2. Natalia Rodríguez Eugenio, AdelaideDaniel Pennock. /Soil Pollution: // a hidden reality. Rome, FAO. 2018. 142 pp.
3. Nowak and Sigmund. Evolution of Indirect Reciprocity by Image Scoring // 1998. – Vol. 23. - P. 9–15.
4. Nelson K.J., Hoagland K.D., Siegfried B.D. Chronic effects of atrazine on tolerance of a benthic diatom // Environ. Toxicol. and Chem. 1999. V. 18. № 5. P. 1038–1045.
5. Feng Y., Minard R.D., Bollag J.-M. Photolytic and microbial degradation of 3,5,6-trichloro-2-pyridinol // Environ. Toxicol. and Chem. 1998. V. 17. № 5. P. 814–819.

А. М. Мәлік, Г. Ж. Абдиева, П. С. Уалиева, А.А. Жубанова
Казахский национальный университет им. Аль-Фараби Алматы, Казахстан
Электронная почта: Azhar.malikkyzy@gmail.com

МИКРОБНОГО РАЗНООБРАЗИЯ ОБЪЕКТОВ ОКРУЖАЮЩЕЙ СРЕДЫ НА ТЕРРИТОРИИ, ПРИЛЕГАЮЩЕЙ К МЕСТАМ ЗАХОРОНЕНИЯ ПЕСТИЦИДОВ И ИЗУЧЕНИЕ БИОСОВМЕСТИМОСТИ ШТАММОВ ДЕСТРУКТОРОВ

Аннотация: Среди различных химических экотоксикантов антропогенного происхождения хлорорганические пестициды являются одними из наиболее стабильных и опасных для окружающей среды и человека. Одной из основных экологических проблем является загрязнение природных объектов органическими пестицидами, которые являются высокотоксичными и стойкими. Помимо мест интенсивного применения

пестицидов, потенциальную опасность для окружающей среды и человека представляют места их захоронения – специальные подземные бетонные бункеры или колодцы. Токсичные вещества могут попадать в окружающую среду из хранилищ и представлять угрозу для всех живых организмов, включая почвенные и водные популяции микроорганизмов. Большинство проведенных исследований посвящено изучению влияния пестицидов на популяции микроорганизмов в почвах агроценозов, в то время как вопросы изучения почвенных микробных комплексов в районах захоронения пестицидов освещены недостаточно. В то же время микроорганизмы, выделенные из экосистем, подвергшихся длительному воздействию пестицидов, обладают потенциалом для более быстрого разложения этих соединений, что делает необходимым изучение микробных сообществ почв, загрязненных пестицидами, как для оценки биологического риска, так и для отбора перспективных микроорганизмов - деструкторов для технологии биоремедиации природных объектов.
Ключевые слова: *микроорганизмы, штаммы, деструкция, биоремедиация, стойкие органические загрязнители, пестициды.*

А. М. Мәлік, Г. Ж. Абдиева, П. С. Уалиева, А.А. Жубанова
Әл-Фараби атындағы ҚазҰУ Алматы, Казахстан
Электронды почта: Azhar.malikkyzy@gmail.com

ПЕСТИЦИДТЕРДІ КӨМУ ОРЫНДАРЫНА ЖАҚЫН АУМАҚТАҒЫ ҚОРШАҒАН ОРТА ОБЪЕКТІЛЕРІНІҢ МИКРОБТЫҚ АЛУАНТҮРЛІЛІГІН ЗЕРТТЕУ ЖӘНЕ ДЕСТРУКТОР ШТАММДАРЫНЫҢ БИОСӘЙКЕСТІГІН ЗЕРТТЕУ

Аннотация: антропогендік әр түрлі химиялық экотоксиканттардың ішінде органохлорлы пестицидтер қоршаған орта мен адамдар үшін ең тұрақты және қауіпті болып табылады. Негізгі экологиялық проблемалардың бірі-табиғи нысандардың өте улы және төзімді органикалық пестицидтермен ластануы. Улы заттар қоршаған ортаға қоймалардан түсіп, барлық тірі организмдерге, соның ішінде микроорганизмдердің топырақ және су популяцияларына қауіп төндіруі мүмкін. Жүргізілген зерттеулердің көпшілігі пестицидтердің агроценоз топырағындағы микроорганизмдер популяциясына әсерін зерттеуге арналған, ал пестицидтерді көму аудандарындағы топырақ микробтық кешендерін зерттеу мәселелері жеткілікті түрде қамтылмаған. Сонымен бірге, пестицидтердің ұзақ әсеріне ұшыраған экожүйелерден оқшауланған микроорганизмдер осы қосылыстардың тез ыдырауына мүмкіндік береді, бұл пестицидтермен ластанған топырақтың микробтық қауымдастықтарын биологиялық қауіпті бағалау үшін де, табиғи объектілерді биоремедиациялау технологиясы үшін перспективті деструктивті микроорганизмдерді таңдау үшін де зерттеуді қажет етеді.
Түйінді сөздер: *микроорганизмдер, штаммдар, деструкция, биоремедиация, тұрақты органикалық ластағыштар, пестицидтер.*

UDC 579.66

V.V. Martynenko [1,2], A.A. Kurmanbayev [1]
[1]RSE «National Center for Biotechnology» SC MES RK, Kazakhstan, Nur-Sultan city
[2]L.N. Gumilyov Eurasian National University, Kazakhstan, Nur-Sultan city
vitalya2497@gmail.com

BIOSYNTHESIS OF ALGINATE BY BACTERIAL STRAINS AZOTOBACTER CHROOCOCCUM

Abstract. *In the present work, the ability of fifteen strains of Azotobacter chroococcum for biosynthesis of alginic acid polysaccharide was investigated. As a result, 6 most promising bacterial strains capable of alginate synthesis were selected. They are A. chroococcum 1/3 Ac, A. chroococcum 1/5 Ac, A. chroococcum 10, A. chroococcum 13, A. chroococcum 22 and A. chroococcum 37. The proportion of capsular alginate was determined during the work. It varied from 4.4% to 30% of the total amount of synthesized alginate.*
Keywords: *Azotobacter, alginate, polysaccharide, biosynthesis, biopolymer*

Introduction

Alginate is a linear unbranched biopolymer. This polysaccharide consists of two monomers - mannuronic and guluronic acids, which are linked by a glycosidic bond [1]. The main producers of alginates are various types of brown algae of the class Phaeophyceae, as well as bacteria of the genus *Pseudomonas sp*. and *Azotobacter sp*. [2].