Comprehensive study of the ecological potential of aboriginal microorganisms in the Arctic region

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Comprehensive study of the ecological potential of aboriginal microorganisms in the Arctic region

E V Kramarenko, E V Makarevich and M Y Litvinova

Department of Microbiology and Biochemistry, Institute of Nature and Technology, Murmansk State Technical University, Murmansk, Russia

kramarenkoev@mdtu.edu.ru; makarevichev@mdtu.edu.ru; litvinovsmj@mdtu.edu.ru; vasehamv@mdtu.edu.ru

Abstract. As a result of the conducted research, the chemical composition of bioflocculant-producing microorganisms' habitats was determined, which was characterized by a low content of organic substances and a large number of ammonium salts and phosphates in the studied aquatic ecosystems. Quantitative and qualitative composition of the studied bacteriocenosis in the studied hydro-ecosystems was presented as various physiological groups of bacteria with a quantitative predominance of heterotrophic nitrifying microorganisms, the main representatives of the active sludge. The shift of the optimal C/P ratio due to the low load on the bacteriocenosis of treatment facilities led to a slower growth of microbial biomass, and as a consequence, to a decrease in the degree of wastewater treatment. To achieve the full effect is possible, improving the ability of the sludge to sedimentation, to adsorb oxidized substances inside. The cultures of bacteria *Pseudomonas lundensis*, *Aeromonas eucrenophyla* and *Pseudomonas fragi*, experimentally extracted from the anthropogenically invaded water pools, confirmed the correlation between the primary morphological characteristics of the bacteria's flocculating ability and the high flocculating activity of these microorganisms, as well as the conditions of their cultivation. The developed reference nutrient environments for the cultivation of allocated strains of bacteria differed significantly in the ratio of biogens (C / N, C / P and N / P). It was noted that the growth rate of the culture largely depended on the environmental characteristics that determine the intensity of metabolic processes. The growth rates of cultures on media of different composition varied widely and were most correlated with the relative content of phosphates in the environment. Optimum concentrations of bacteria cells required to implement the bio-potential are directly dependent on the phosphorus concentration load on the microorganisms. The phosphorus concentration did not have a significant effect on the studied bacterial cultures' flocculation, but the limiting factor was the phosphorus nature.

1. Introduction

The Arctic zone is highly vulnerable to pollution of water ecosystems. It has a significantly slowed down rate of self-purification and self-recovery of water resources. To date, in Russia among the major rivers there is not a single one that could be used as a source of drinking water supply, while 90 % of the river flow of the country falls on the Arctic and Pacific oceans’ basins [1].

Dynamics and variability of water ecological systems is an important basis of self-maintenance and self-regulation processes, including self-purification [2]. At the heart of the self-purification processes is the microorganisms’ ability to use high concentrations of organic and inorganic substances in their metabolism. In addition, many scientists have noted the bacteria’s ability to form floccules in the aqueous medium. Thus, in the course of their life, microorganisms can not only oxidize organic and mineral substances, but also bind various pollutants into aggregates and relatively easily separate themselves from the aqueous medium by sedimentation [3, 4, 5]. Given that the bacteria aggregation in the aquatic environment is due to both their ability to synthesize bioflocculants, and physical and chemical conditions of the environment, the detection of bacteria with these properties and the selection of the most favorable conditions for their development and functioning is a priority. The study of the features of aboriginal microorganisms involved in the biological transformation of most pollutants, and the external factors’ impact on the microorganisms’ bio-ecological potential is also very important. It is also essential that in the climatic conditions of the Arctic region the processes of self-purification of water bodies are very weak; and microorganisms that live in extreme conditions have formed adaptations to them and can have unique abilities.

Since the ability to bioflocculate was for the first time found in microorganisms of activated sludge, water samples for research were taken primarily from an artificial ecosystem – an individual local treatment plant for biological purification ILOS "TOPAS-5" by GC TOPOL-ECO (Russia), as well as from the natural hydro-ecosystem – Varnichny stream flowing through the central part of Murmansk city, which collects the roads and construction sites’ drains along the way.

2. Materials and methods

Each component of the aquatic environment is related to other indicators in a certain way. A comprehensive assessment of the water composition of the studied aquatic ecosystems was carried out on the basis of analysis and comparison of the main hydro-chemical parameters, which were determined by standard hydrochemistry methods.

Allocation of different physiological groups of microorganisms participating in transformations of compounds of organic and mineral nature was conducted in parallel with hydro-chemical tests in the search for appropriate aboriginal bioflocculants-producing bacteria. Later in the course of studying bacterial colonies’ cultural and morphological characters, 5 strains of microorganisms possessing the primary symptoms of bioprocesses synthesis were selected and allocated into pure cultures. Identification of the allocated bacteria was carried out by biochemical tests and mass spectrometric analysis.

The strains’ flocculating activity was determined by Kuran’s modified method using a suspension of kaolin clay as a deposited agent; while the phosphate-accumulating activity was determined by photometric measurement of phosphorus concentrations in the reference nutrient media before, during and after cultivation of the strains. The conditions of all laboratory experiments were close to the natural habitat conditions for selected cultures of microorganisms.

Moreover, on the basis of the data obtained on the chemical composition of the analyzed aquatic ecosystems, experimental and reference nutrient media for the cultivation of target strains were developed. As the main characteristics of the culture medium, affecting the intensity and direction of metabolic processes in microorganisms, in one case, the ratio of biogens, in the other – different concentrations of phosphorus of organic and mineral nature were selected. Thus, with the help of the developed reference nutrient media, experiments were carried out to determine the bioecological potential of the allocated strains in the laboratory.

3. Results

Physical and chemical parameters of the habitat of the bacteria under study, which determine the nature of the biochemical processes occurring during sampling, are presented in Table 1.

Table 1. Physics-chemical parameters of the natural (Varnichny stream, polluted with urban wastewater) and artificial water ecosystems (activated sludge, ILOS); treated wastewater). The average air temperature during sampling is 10±2 °C. The average water temperature in the Kola Bay in the waters of Murmansk Seaport during sampling is +4±0,1 °C

Object name	Indicators								
	T, °C	pH	Transparency, cm	Veget. O_2, mg O_2/l	БПК$_5$, mg O_2/l	NH_4^+ mg/l	NO_2^-, мг/л	NO_3^-, mg/l	PO_4^{3-}, mg/l
Varnichny stream	+10±2	5,0±0,2	No data	5,55 ±1,5	113,2 ±12,2	22,7 ±2,6	0,32 ±0,02	1,56 ±0,7	6,8 ±2,4
Activated sludge	+15±2	7,0±0,2	4	No data	65,4 ±2,2	67,0 ±3,1	0,44 ±0,10	3,02 ±0,5	40 ±5,6
Domestic waste water after cleaning	+10±2	7,0±0,1	6	3,8±1,1	19,6 ±3,5	56,0 ±3,4	0,30 ±0,10	5,78 ±1,2	96,0 ±4,1

According to the results of hydro-chemical tests, high concentrations of phosphates were observed in activated sludge and domestic wastewater after purification. The observed phosphate level after purification, which is 96 mg/l and hundreds of times higher than MPC for any type of water reservoir, may indicate insufficient development of activated sludge bacteria that can accumulate phosphorus.

The effectiveness of natural water reservoirs’ self-purification processes depends on the intensity of metabolic processes of microorganisms, leading to the removal of most of the pollutants present in the water. The study of the quantitative and qualitative composition of basic physiological groups of bacteria in active sludge ILOS "TOPAS-5" and of the Varnichny stream showed dominance of heterotrophic nitrifying in these ecosystems ($3,5 \cdot 10^6$ CFU/ml in the activated sludge, $3 \cdot 10^5$ CFU/ml in the Varnichny stream), which are essential participants in the substances’ biotransformation processes in water reservoirs of any origin. The contribution of heterotrophic microorganisms to the oxidation of nitrogen compounds can reach 50% of autotrophic nitrification under certain conditions [6]. A small number of autotrophic nitrifiers can be explained by a sufficient concentration of organic nitrogen for the development of heterotrophs, the main representatives of bacteriocenosis of hydro-ecosystems.

As a result of the study of cultural characteristics of allocated microorganisms’ colonies of the main physiological groups, 5 strains of bacteria with primary signs of synthesis of extracellular polymeric substances were selected. The primary signs of extracellular polymeric substances’ synthesis by bacteria are S-type colonies with a pronounced luster, mucous-like and viscous consistency, the presence of mucous layers, covers, capsules (Figure 1).

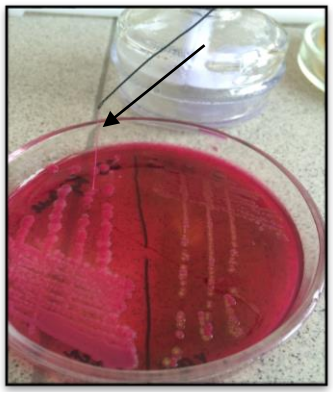

Figure 1. The manifestation of the primary signs of synthesis by bacteria bio-flocculante. The arrow marks the mucous strand stretching behind the bacterial loop from the colony

Isolated from aquatic ecosystems of the Arctic region bio-flocculant-producing bacteria belonged to *Pseudomonas lundensis*, *Aeromonas eucrenophyla* and *Pseudomonas fragi*. All strains are gram-negative nonspore-forming rod-shaped bacteria that can grow at low plus temperatures.

The chemical composition of the reference nutrient media corresponded to the hydro-chemical composition of the habitats of the allocated strains of bacteria. Along with the absolute indicators of the nutrients’ concentrations, the determining value is their ratio, affecting the intensity and direction of metabolic processes in microorganisms. The C / N, C / P and N / P ratios in nutrient media №№ 1-4 were equivalent to the content of biogens in the reference medium YPG (proposed by Chinese microbiologists for the allocation of bioflocculant-producing microorganisms), in the water of the Varnichny stream, in the active sludge ILOS in domestic wastewater after biological treatment, respectively. Glucose was used as a carbon source [7, 8], as a nitrogen source – ammonium sulphate, nitrite and sodium nitrate were used. The yeast extract included in the nutrient media presumably satisfied all the bacteria needs in growth factors.

The results of bacterial cultures’ growth on different ratio of basic nutrients in reference nutrient media varied in a wide range of values and are presented in Figure 2.

The number of bacteria at the end of the cultivation period varied for each species and strain of microorganisms. The maximum concentration of cells was registered in the culture *Aeromonas eucrenophyla* in the reference nutrient medium № 1, which corresponded to the chemical composition of the Varnichny stream water, the growth rate of the strain was 160 thousand cells/h. The minimum concentration was detected in the culture *P. fragi* st. № 2 when cultivated in reference conditions, with a number of $1.1 \cdot 10^6$ C cells/ml its growth rate was 3000 cells/h. Culture *A. eucrenophyla* allocated from the activated sludge also grew well in the reference nutrient medium # 2 close in composition to the source of allocation.

The graphs also show that the number of *Ps. fragi* bacteria strain № 2 on reference ENM, rich in organic substances and biogenic elements, practically did not change. Thus, it can be assumed that the relative phosphorus content, which exceeded the proportion of nitrogen by five times and amounted to 20 % of the carbon content, was a factor limiting the growth of the culture allocated from the activated sludge of the treatment plant.

The ratio of biogens in reference nutrient medium No. 2 was optimal for the growth of two of the three studied *Ps. fragi* strains. At the same time, strain № 3 was growing the most intensively of the three strains of *P. fragi*, showing maximum growth rate and selectivity in the reference nutrient medium № 1. The intensity of *P. lundensis* culture growth varied from 80·thousand cells/h on the reference ENM to 150·thousand cells/h on PS № 1 and 2.

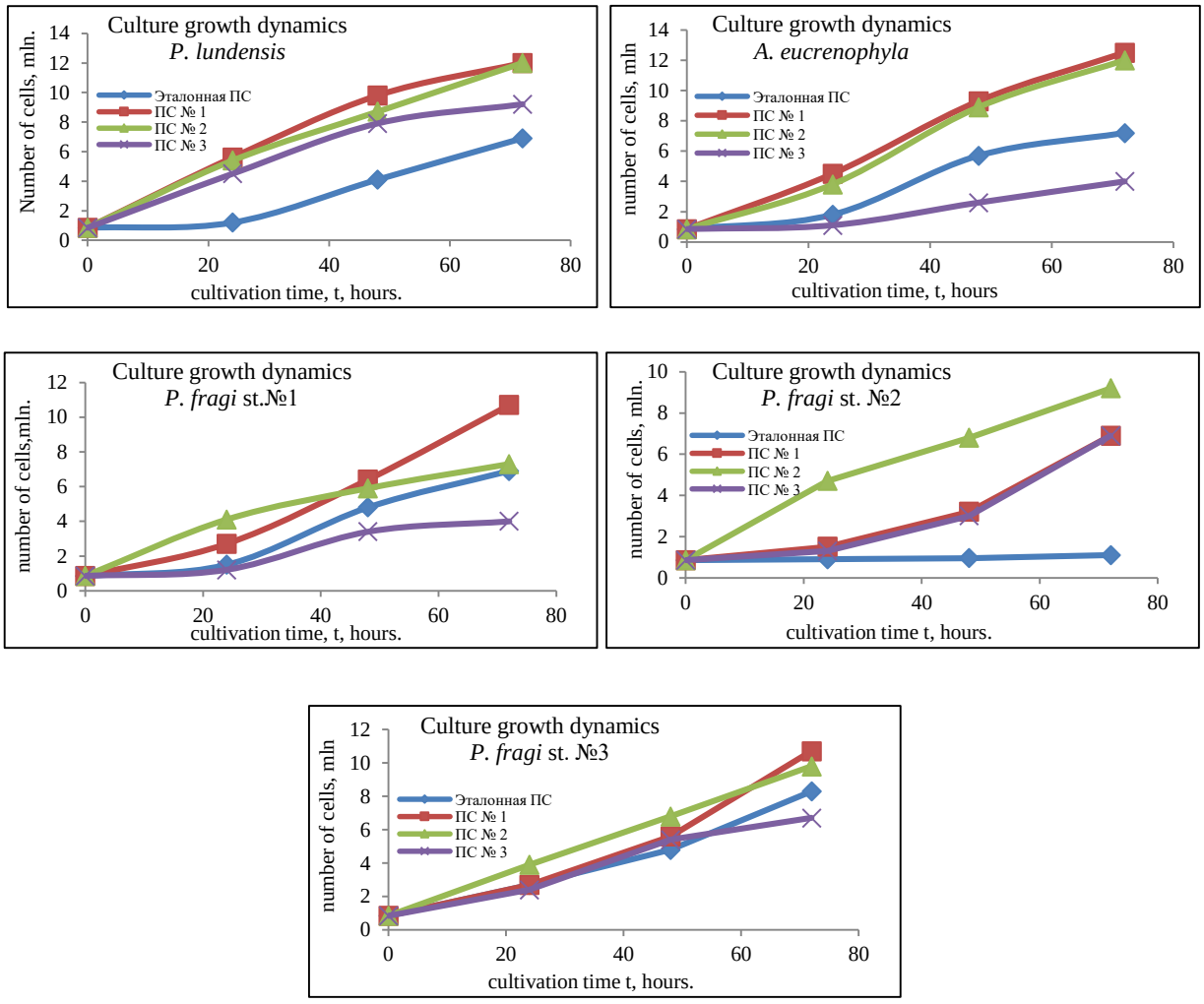

Figure 2. Dynamics of growth of microorganisms’ selected strains in the reference nutrient medium (NM) NM № 1, NM № 2, NM № 3

It is also important to emphasize that the proportion of phosphorus in relation to other nutrients in the reference ENM was maximal in comparison with other nutrient media.

To confirm the assumption of the existence of links between the growth rate of the studied cultures of microorganisms and the ratio of biogenic elements in the reference culture media, the coefficients of rank correlation between these phenomena were calculated. The values of the correlation coefficients indicate a significant impact of the biogenic elements’ content in the elective media on the intensity of crop growth. With an increase in the share of carbon and nitrogen, the growth rate of culture increases, and in the case of phosphorus predominance – it decreases.

In addition to determining the intensity of selected cultures’ growth on reference nutrient media, we conducted an experiment to determine the flocculating activity under the same conditions. The results are shown in Figure 3. It has been found that bacterial suspensions *P. lundensis*, obtained on reference nutrient media of different composition, were able to precipitate a suspension of kaolin clay on average by 41,2 %, *A. eucrenophyla* suspensions – by 64,5 %, *Ps. fragi* st. №№ 1–3 – by 37,4; 50 and 45,2 % respectively.

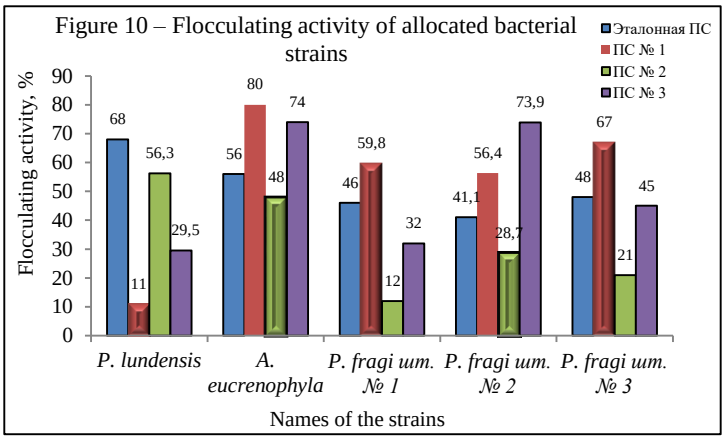

Figure 3. Flocculating activity of allocated bacterial strains. Columns of reference NM corresponding to the chemical composition of the allocation medium for each culture are shown in volume

A. eucrenophyla strain showed the greatest flocculating activity of all the studied cultures, showing a value of 80 % and showing its maximum bio-flocculating potential (in this experiment) on the reference nutrient medium № 1. Reference ENM, and the environment corresponding to the composition of the active sludge (NM No. 2), rich in organic substances and biogenic elements, for strains №№ 1-3 of *P. fragi* culture are less effective than the rest of the media.

The reference NM # 1’s content, maximally similar to the conditions under which strains No. 1 and No. 3 of *P. fragi* culture were allocated, positively affected the microorganisms’ production of bioflocculants.

P. fragi culture strain № 2, according to the results of the study, had the greatest flocculating activity in its cultivation on a nutrient medium corresponding to the chemical composition of domestic wastewater after bio-treatment. The reference nutrient medium, and the medium corresponding to the composition of the activated sludge rich in organic substances and biogenic elements, are less effective than the media as close as possible to the depleted conditions under which the microorganism was allocated.

In contrast to *P. fragi* bacterial culture, strain № 2, bacteria *P. lundensis* showed maximum flocculating ability after their cultivation on organic substances-rich media, showing the greatest flocculating activity during cultivation on reference ENM. It should also be noted that this culture showed low activity against NM No. 1, which is a composition close to the habitat conditions (water of the Varnichny stream).

To study the correlation between the content of biogenic elements, presumably capable of influencing the synthesis of bio-flocculants, and the flocculating activity of bacterial suspensions, a statistical analysis of the data obtained using correlation coefficients was carried out. The correlation analysis revealed no significant correlation between the concentrations of carbon, nitrogen and phosphorus in the nutrient medium (absolute and relative) and flocculating ability of bacteria cultures *Pseudomonas lundensis, Aeromonas eucrenophyla, Pseudomonas fragi* strains №№ 1-3.

Thus, the role of heterotrophs in aquatic ecosystems is not limited to the mineralization of organic matter, it is quite diverse and has not yet been fully studied. However, we have confirmed the relationship between the primary morphological characteristics of bio-flocculant products and the high flocculating activity of target bacteria in cultivation under certain conditions.

In large sedentary water reservoirs, including those used for growing aquaculture, the provision of hydrodynamic processes of self-purification is a difficult task. To optimize the biochemical processes of pollutants’ transformation by microorganisms requires intensive mobility of the environment; otherwise, the process of self-purification slows down. The second group of important factors that determine the effectiveness of biological treatment includes the load on the destructors, depending on the mass of the destructors themselves and the concentration of pollutants. The number of cells of the studied strains of bacteria in reference nutrient media with different phosphorus content

in its different forms (inorganic and organic) was determined as one of the most common contaminants. The results of the experiment are shown in Figure 4.

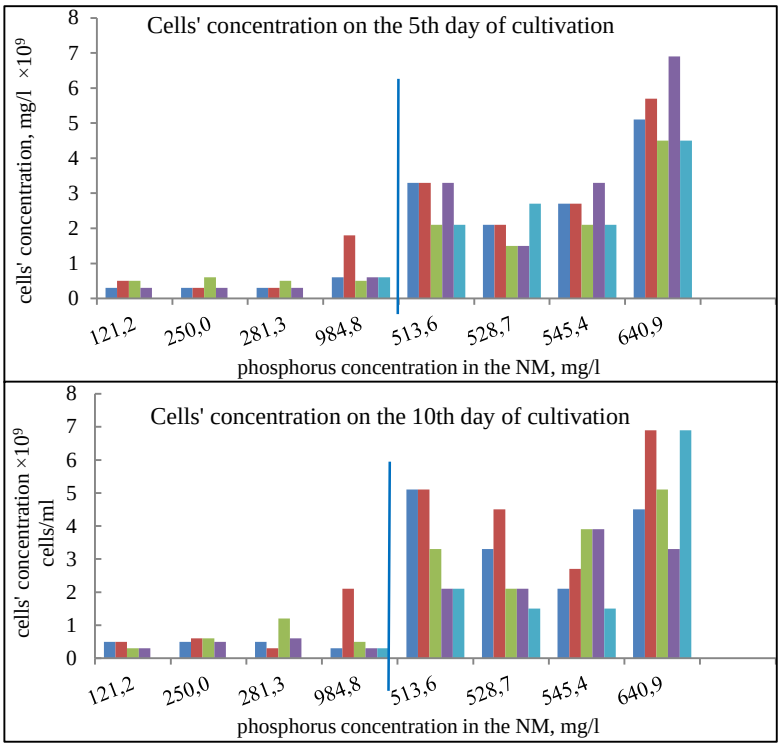

Figure 4. Concentration of cells of studied microorganisms’ strains on the 5th and 10th days of cultivation in model NM. A shows the growth of the cultures in the NM with the content of phosphorus in its inorganic form. B shows the growth of cultures in the NM with the content of phosphorus in its organic form.

In addition to the main nutrients, the main component of the substrates (NM) was inorganic phosphorus in the form of phosphoric acid salts in one case, and in the pure form of organophosphate lecithin in the other.

In the experiment, the number of bacteria reached the required values not earlier than on the 5th day of cultivation. On model nutrient media with the addition of inorganic phosphorus, the cell concentration increased by an average twice regardless of the duration of further cultivation and ranged from $0.3 \cdot 10^9$ to $1.8 \cdot 10^9$ cells/ml. On reference nutrient media with organic phosphorus, the number of bacteria increased 4.6 times. Maximum cell concentration was observed on the 10th day.

In addition to the quantitative characteristics of bacteriocenosis, the ability of bacteria-destructors to form flakes is also important in the process of biological purification. The basic group of main organotrophs was organized into flocculi, the other is dispersed bacterial cells, having less importance in the oxidation processes and gaining advantage in the environment in the suppression of floculli-producing destructors’ life. The increase in the number of free-living bacteria affects the physiological state of other aquatic organisms.

It is known that bacteria of *Pseudomonas* and *Aeromonas* genera can cause *Pseudomonas* and *Aeromonas* in cultivated species of the salmon family fish [9].

On the one hand, the bacteria we study have a high bio-ecological potential, on the other – with the shift of external factors that affect the processes of self-purification, beyond the optimal values, these microorganisms can move into a pool of dispersed bacteria, increasing the risk of susceptible hydrobionts’ infection. Therefore, studies of the external factors’ influence on the ability of these bacteria to aggregate emphasize the practical importance of this scientific direction. During the cultivation of the studied strains of bacteria in the reference nutrient media with different concentrations of phosphorus, the formation of floccules by bacterial suspensions occurred not earlier

than on the 5th day, while the cells’ aggregation depended on the nature of phosphorus contained in the medium. The growth of cultures in reference nutrient media containing organic phosphorus was not accompanied by flocculation.

The time of flakes formation by strains during cultivation did not depend on their initial flocculating activity.

Assessment of biotechnological potential of bio-floccule-producing microorganisms cultures were carried out on the basis of their ability to mobilize inorganic and organic forms of phosphorus (Figure 5). We have found in the study that associated bacteria *Pseudomonas lundensis*, *Aeromonas eucrenophyla* and *Pseudomonas fragi*, when cultured in media containing phosphorus in mineral form in a concentration not exceeding 500 mg/l, reduced its level by an average of 60-75 % on the 5th day.

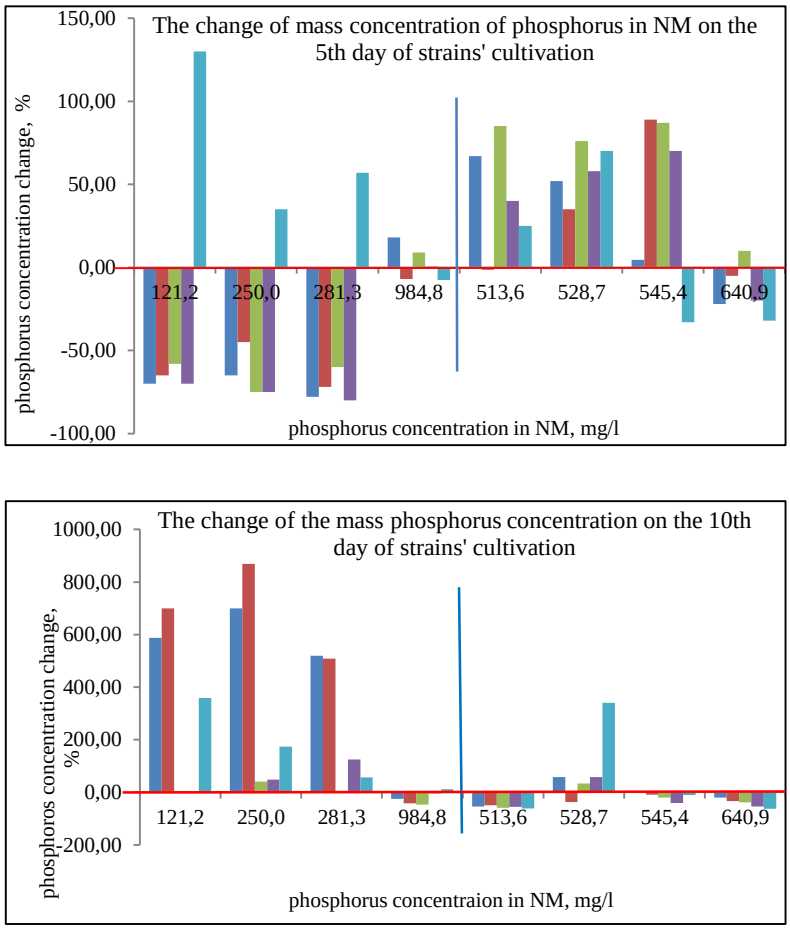

Figure 5. Change in the mass concentration of phosphorus in the referential NM on the 5th and 10th days of strains’ cultivation. A shows the phosphorus release by the cells into the NM when cultivated in the mineral phosphorus containing medium. B shows the phosphorus release by the cells into the NM with the content of phosphorus in its organic form. C shows accumulation of phosphorus by cells when cultured in the NM with the content of mineral phosphorus; D – accumulation of phosphorus by cells when cultured in the NM with the content of phosphorus in its organic form.

The exception was strain № 3 *Pseudomonas fragi* allocated from the waters of the Varnichny stream, which did not show the ability to accumulate phosphate in these conditions, but showed a dephosphating activity at a concentration load of more than 500 mg/l. On the 10th day, there was a significant release of phosphorus by all the above cultures in a medium with an initial phosphorus content of less than 500 mg/l, probably due to cell lysis with continued cultivation in the same nutrient media. It was also noted that on the 10th day of bacteria suspension cultivation in a dispersed state, the level of phosphorus in nutrient media containing its high initial concentrations (more than 500 mg/l) was reduced by an average of 30-45 %.

To assess the correlation between the age of the culture and its ability to accumulate different phosphorus concentrations, a statistical analysis of the data obtained using association coefficients was

carried out. The correlation analysis revealed the presence of a significant direct correlation (0.8) between the age characteristics of the culture and the concentration load on them. Thus, the more "mature" the culture is, the higher its ability to dephosphate the environment is.

4. Results

1) The hydro-chemical composition of the studied aquatic ecosystems was characterized by a low content of organic substances and a large number of ammonium salts and phosphates, tenfold exceeding sanitary and hygienic standards and MPC for surface and fish-economic reservoirs.

2) Referential nutrient media have been developed for the cultivation of indigenous bacteria and those adapted to the extreme climatic conditions with bio-flocculent-producing potential, in the recipe of which the N/P ratio and the nature of the phosphorus should be taken into account.

3) The maximum values of the flocculating activity of the allocated cultures were established, which are 68 % for *Pseudomonas lundensis*, 80 % for *Aeromonas eucrenophyla* and 60, 74 and 67% for *Pseudomonas fragi* strains № 1-3, respectively.

4) It is shown that the growth rate of bio-flocculent-producing cultures vary in a wide range of values (from $1.1 \cdot 10^6$ cells/ml to $12.5 \cdot 10^6$ cells/ml) and depend on the characteristics of media that determine the intensity of metabolic processes, and to the greatest extent correlate with the relative content of phosphates in the medium.

5) It was determined that the concentration of bacterial cells ($1 \cdot 10^9$ cells/ml) necessary for their bio-potential realization reached optimal values after 5 days of cultivation and directly depended on the concentration load of phosphorus on microorganisms.

6) It is established that the initial flocculating activity of strains does not affect the time of floccules’ formation in the process of microorganisms’ cultivation. In nutrient media containing phosphorus in organic form, flocculation is not observed during the entire incubation period.

7) It is revealed that phosphate-accumulating activity depends on the concentration of phosphorus in the medium and on the culture age. The correlation between these characteristics of culture and the processes of accumulation and phosphorus release by bacterial cells is confirmed by mathematical calculations.

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