Bioconversion of Bottom Sediments Using Mesophilic Prokaryotes of the Genus *Bacillus spp*.

N.A. Sidorova* and *A.A.* Kucko

Petrozavodsk State University, Petrozavodsk, Russian Federation

Abstract. The aim of the study was to develop a scientific and technical justification for the bioconversion of bottom sediments using mesophilic prokaryotes. The main component of the bottom sediment bioconversion technology includes microorganisms of the genus *Bacillus spp*., isolated in a pure culture from the microbial community of bottom sediments. Bacilli are highly resistant to natural (climatic) conditions, anthropogenic factors, elevated concentrations of pollutants and are able to adapt to their effects. As a result of the laboratory experiment, work was carried out on the selection of factors that ensure the intensity of microbial bioconversion of bottom sediments. In order to achieve the maximum effect from the enzymatic and biochemical activity of bacilli, kinetic characteristics associated with the achievement of maximum values of optical density, microbial cell concentration and growth rate were studied. After analyzing a number of the above indicators, it is concluded that the method of processing bottom sediments using mesophilic prokaryotes of the genus *Bacillus spp.* will allow in the future to obtain an environmentally friendly and mineral-saturated product for further use in the practice of crop production.

1 Introduction

The main types of reclamation of freshwater reservoirs subject to anthropogenic pollution are those that involve the use of mechanized and compost systems capable of selecting and processing bottom sediments. According to recent studies devoted to the problems of restorative ecology, surface reservoirs are a priority structure that requires constant spatial and temporal monitoring to prevent their degradation [1]. This process can be defined as a set of phenomena that determine the functioning and stability of this ecosystem [4, 7]. Comprehensive studies of both agricultural and biotechnological processes of reclamation and sapropel processing are necessary for the development and implementation of effective bioconversion systems to control not only the existing volume of bottom sediments, but also those entering the system during a long period of economic use of the reservoir. This is especially true for reservoirs experiencing not only anthropogenic stress, but also variability from climatic and geographical features. Such reservoirs are considered to be the waters of

* Corresponding author: fagafon@yandex.ru

the north-western region. They are located in areas of natural accumulation of humic compounds with impaired pH-homeostasis, coupled redox reactions, which leads to a violation of the intensity of mineralization processes of a complex complex of organic pollutants. Violation of the intensity of biogeochemical cycles in such reservoirs leads to a constant accumulation of organic matter, which is accompanied not only by an increase in the trophic capacity of reservoirs, but also by a change in their saprobity from oligosaprobic to polysaprobic. Research in the context of the above problem is constantly being conducted. Not only large lakes are under control, but also flowing ecosystems, such as rivers, river communities [3, 10-12].

The uniqueness of the study is associated with the use of catalytic processes caused by the enzymatic activity of microorganisms capable of causing the destruction of insoluble organic phosphorus compounds as the main component of bottom sediments in the zone of functioning of fisheries cages. The use of bioconversion technology will optimize the known methods of reclamation of aquatic ecosystems, which is extremely important for preserving the ecological status of reservoirs located in the northwest.

The main objective of this study was focused on studying the biochemical and kinetic activity of mesophilic cultures of the genus *Bacillus spp.,* and their further applicability for the purposes of bioconversion of a complex organomineral substrate in the form of bottom silt deposits formed under hypoxic environmental conditions.

2 Materials and methods of the studies

The research was carried out on the basis of the Aquaculture Research Center of Petrozavodsk State University using the resources of the Microbiology Laboratory of the small innovative enterprise Microbiom LLC.

A module consisting of bottom sediments and a nutrient medium for cell culture was used as a substrate. Bottom sediments were used as a source of single-component nitrogen compounds (ammonium salts, nitrates, urea, amino acids), macronutrients 10^{-3}–10^{-4} mol (salts K, Ca, Mg, Fe, S, P) and trace elements 10^{-6} –10^{-8} mole (salts of Zn, Cu, Na). The averaged spectra of the elemental composition of bottom sediments were studied using a scanning electron microscope-lithograph with a biological prefix "HITACHI SU1510".

The Reverse-Spin bioreactor (RST) was used as hardware support for the biotechnological fermentation process of bottom sediments, which provides stationary conditions for periodic fermentation under specified parameters and modes. The monitoring and control system is automated by Grant Instruments "Labvise" software, which made it possible to set optimal parameters for fermentation, register and record all the studied kinetic parameters (mixing rate, process temperature, optical density, cell concentration, growth rate, etc.).

Oxygen mode, temperature mode and acidity were selected as the technological mode of bioconversion. The oxygen regime was selected based on the type of respiration of microorganisms *Bacillus spp.* (facultative anaerobes). The temperature regime was selected based on the ecological characteristics of microorganisms living in water – they are mesophiles, i.e. the temperature optimum for their growth lies in the range from 20 to 40 °C [3, 5]. The acid fermentation regime was selected based on the biochemical activity of microbes and the direction of their biochemical transformations, established according to the analyzed literature sources: for *Bacillus spp.* – 4.5 min, 6.8 optimum, 8.5 max [9, 13].

A pure culture of bacilli was isolated from the microflora of bottom sediments using the Methodology of studying biogeocenoses of inland reservoirs [2]. The choice of these microorganisms for use in the bioconversion of bottom sediments is justified by the fact that they are easily cultivated on standard nutrient media meat-peptone agar (MPA) and meat-peptone broth (MPB) and are good indicators of the content of organic substances in

the reservoir. The crops were carried out from tenfold dilutions of bottom sediments. To do this, a 100 mg suspension of bottom sediments was made on a sterile watch glass, which was placed in a flask with 100 ml of sterile saline solution. After mixing, 1 ml of liquid was taken from the flask and transferred to a test tube with 9 ml of sterile saline, etc. until the tenth dilution. Of the last three dilutions, 1 ml of liquid was inoculated by a "deep" method on meat-peptone agar. For morphological typing of isolated cultures of microorganisms, a method was used to determine the morphology and tinctorial properties of isolated pure bacterial cultures by applying Gram staining technique. For staining, a set of dyes was used: "Micro-GRAM-NITSF" (produced by the Research Center for Pharmacotherapy (NITSF), Russia), designed for differential diagnostic staining of microorganisms by sequential processing of a smear taken from biological material with the components of the set: gentian violet, basic fuchsin, Lugol and 96 °alcohol. Microscopy was used to assess the presence of bacteria with a gram-positive type of cell wall, stained dark purple with gentian violet [7-8]. Microscopy was performed using immersion technology at magnification ×1000 on a biological microscope "Motic B1-220E-SP" (manufactured in China), equipped with a digital camera "Moticam T" and software "Motic Images Plus 2.0" (manufactured in China) to display an image of the observed object on a computer screen in real time and documenting the obtained results of microscopic examination. Identification to the genus was performed according to a number of phenotypic traits regulated in the Determinants of Bergi bacteria [5].

Depending on the set parameters of fermentation of bottom sediments using microorganisms of the genus *Bacillus spp.* enzymatic hydrolysis of the feedstock was performed under laboratory conditions. During the experiment, objective criteria for changing the state of microbial cells were investigated:

1. morphological and cultural (visible bacterial changes);
2. physiological (optical density, cell concentration, growth rate).

For the experiment, the volume of the substrate was 29 ml, and the volume of the introduced microorganisms was 1 ml. °°The parameters of the optical density of the reaction mixture and the growth kinetics of the studied microbial cultures were measured at a given measurement range of 0-8 OD at λ- 850 nm, measurement accuracy ± 0.3 OD and a constant incubation temperature of 37 °C (temperature stability ± 0.1 °C).

3 Results and discussions

As a result, an analysis of the biochemical activity of microbial cultures was carried out under the conditions of a model experiment with specified intervals of parameters of the technological regime. The obtained results of the experiments performed with interdependent factors are presented in the form of a graph (Fig. 2). The results of the experiments are graphically displayed to analyze the complex interrelated processes of cell growth during fermentation, as well as to determine the optimal reproducible parameters necessary to obtain cellular material.

The dependence of the growth rate on time, shown in the graphs, is based on data obtained during the fermentation process, which lasted from 60 to 90 hours. Optical density measurements were made at intervals of 10 minutes. The optical density was determined by the monolayer of growing cells, which was formed from the grown medium as a result of a vortex (as described in the diagram (Fig. 1). When calculating the length of the optical path for a rotating tube, the volume of the culture medium was taken into account, which made it possible to calculate the optical density in standard units describing the biotechnological process (X = 600 nm, optical path: 10 mm).

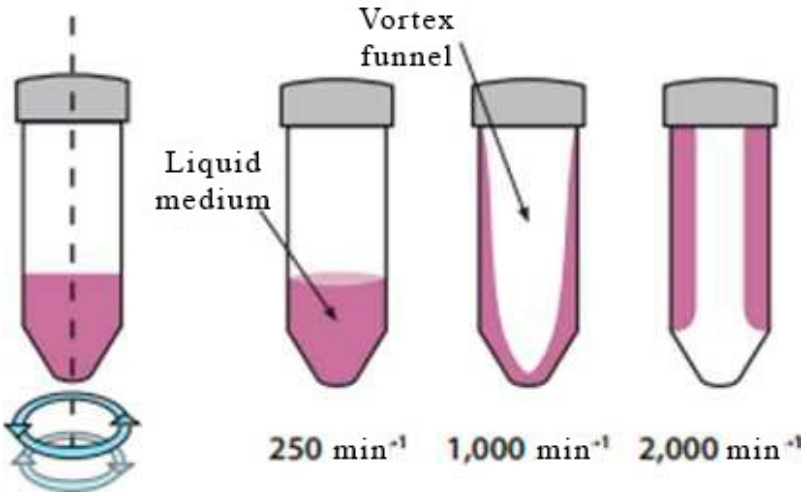


Fig. 1. The principle of noninvasive vortex mixing

Based on the analysis of the data obtained, it was found that the growth of a bacillary culture in the presence of a substrate consisting of bottom sediments and a nutrient medium obeys the laws described for microbial cultures during periodic fermentation and is expressed by a sequence of 6 phases of cell growth: 1. lag phase - adaptation of cells to the conditions and synthesis of enzymes necessary for growth, 2 – short intermediate phase I, 3 – exponential phase – cell growth is described by a kinetic equation of the 1st order, 4 – intermediate phase II – slowing of cell growth due to depletion of the nutrient medium, accumulation of toxic metabolites, high density the population is described by the Monod equation for growth limited by the amount of substrate, 5 – stationary phase means a constant number of cells and the formation of biotechnological products in the form of secondary metabolites, 6 – death phase, the processes of stopping metabolism in the cell

Bacterial growth in the exponential phase obeys the kinetic equation of the first order:

$$\mu = \frac{1}{X} \times \frac{dx}{dt} \quad (1)$$

Where:
μ (h^{-1}) – specific growth rate;
X (g/l) – biomass;
t (h) – fermentation time

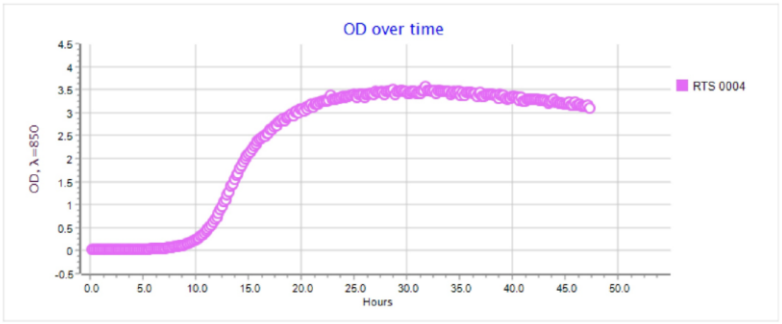
Bacterial growth during transition phase II obeys the Monod equation for growth limited by the amount of substrate:

$$\mu = \mu \max \times \frac{S}{Ks + S}, \quad (2)$$

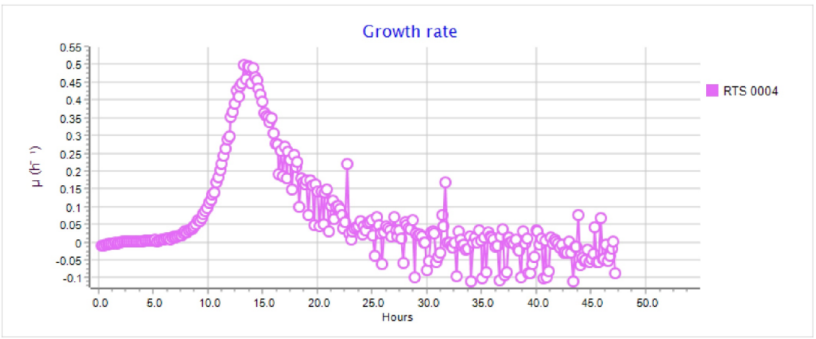
Where:
μ (h^{-1}) – specific growth rate;
μ max (h^{-1}) – maximum specific growth rate;
S (mg/l) – the concentration of the nutrient substrate;
Ks (mg/l) – the concentration of the nutrient substrate limiting cell growth (S), at which the specific growth rate (μ) is below the maximum value

Taking into account the revealed patterns, custom graphs reflecting the kinetics of the growth of the studied crops have been created. Physiological activity of *Bacillus spp.* was

investigated within 48 hours of the experiment. °°The pattern of development and growth of the strain is constructed according to 291 points (Fig. 2) at a temperature range of measurements from 28.0 °C (5 measuring point) to 38.1 °C (184 measuring point). The adaptation of the microorganism to the cultivation conditions lasted for 9 hours. From 10 to 20 o'clock, an exponential growth stage was observed, followed by a transition to a stationary growth stage or "plateau", which corresponded to 3.39 ODU and values of μ (h^{-1}) – 0.02 at the 135 measurement point. The maximum values of the optical density were fixed by 32 o'clock – the OD values were 3.57 ODU at μ (h^{-1}) – 0 (189 measuring point). By the end of the experiment, the optical density remained at the level of 3.10 ODU (Table 1) and did not change significantly in the same way as the growth rate (Table 1).



a.



b

Fig. 2. Kinetics of growth of the experimental strain of *Bacillus spp*: a. – optical density; b – growth rate

The study of morphological and cultural changes in *Bacillus* cells after fermentation using an organomineral substrate revealed no significant changes. The bacilli retained the integrity of morphological, tinctorial and cultural properties.

4 Conclusions

The obtained data on the cultivation of bacilli in the presence of bottom sediments as a substrate for biconversion should be considered as primary data, on the basis of which it can be argued that the microorganisms used are promising for creating a mineral-saturated product based on them for further use in crop production practice. When added to the bottom sediments, they are able to multiply, probably due to the ability to extract from the bottom sediments a complex of macro- and microelements found in the studied samples of bottom sediments: K, Ca, Mg, Fe, S, P, Na; due to enzymes, cause their degradation and ensure the availability of compounds of nitrogen, phosphorus, macro- and microelements for plants.

According to the obtained positive effects of the kinetics of bacillus growth in the presence of bottom silt deposits, it can be argued that the preliminary microbial fermentation of bottom sediments due to the biochemical activity of mesophilic cultures of the genus *Bacillus spp*. will cause an increase in the content of micro- and macroelements necessary for plant nutrition in the organomineral supplement, which will cause an increase in the growth-stimulating activity of the drug being developed.

Acknowledgments

The work was carried out with the support of the grant of the Head of the Republic of Karelia for 2022, project No. KGRK-21/H3-12.

References

1. V. G. Drabkova, M. Ya. Prytkova, O. F. Yakushko, *Restoration of ecosystems of small lakes*, 144 (St. Petersburg: Nauka, 1994).
2. Methods of studying biogeocenoses of inland reservoirs, 239 (Science, 1975).
3. E. G. Nabeeva, Assessment of restoration and self-purification of diverse aquatic ecosystems according to macrozoobenthos indicators: Dissertation abstract of the candidate of biological sciences: 03.02.08, 24 (Nizhny Novgorod, 2010).
4. C. Auel, R. M. Boes, Sediment bypass tunnel design – Revie, ICOLD Symposium «Dams under ChangingChallenges», 403-412 (Taylor and Francis: Lucerne, Switzerland, 2011).
5. Bergey's Manual of Systematic Bacteriology, J. of Syst. Bact., 408 (1985).
6. S. Cajot, A. Schleiss, T. Sumi, S. Kantoush, Reservoir sediment management using replen-ishment: A numerical study of Nunome Dam, Proceedings of the (on CD) of the Inter-national Symposium on Dams for a changing world-80th Annual Meeting and 24th Con-gress of CIGB-ICOLD, Kyoto, Japan, 2-6 June, 2-131 (2012).
7. A. E. East, G. R. Pess, J. A. Bountry, C. S. Magirl, A. C. Ritchie, J. B. Logan, T. J. Randle, M. C. Mastin, J. T. Minear, J. J. Duda, Large-scale dam removal on the Elwha River, Washington, USA: River channel and floodplain geomorphic change, Geomorphology, **228**, 765-786 (2015).
8. A. Foldvik, A. Silva, I. Albayrak, K. Schwarzwälder, R. M. Boes, N. Ruther, Combining Fish Passage and Sediment Bypassing: A Conceptual Solution for Increased Sustainability of Dams and Reservoirs, Water, **14**, 1977 (2022).
9. D. Krier, F. Caporuscio, A. Lavine, J. Gardner, Stratigraphy and geologic structure at the SCC and NISC building sites, technical area 3, Los Alamos National Laboratory, New Mexico, Los Alamos National Laboratory technical report LA-13507-MS (1998).
10. A. D. Kshirsagar, Bioremediation of wastewater by using microalgae: An experimental study, International Journal of Life Sciences Biotechnology & Pharma Research, 24-32 (2013).
11. I. Rodushkin, T. Moiseenko, L. Kudravsjeva, Aluminium in the surface waters of the Kola peninsula, Russia, Science Tot Environ, **163(1-3)**, 55-59 (1995).
12. N. Surian, M. Rinaldi, Morphological response to river engineering and management in al-luvial channels in Italy, Geomorphology, **50**, 307-326 (2003).
13. J. I. Young, E. P. Hong, J. Castle, J. Crespo-Barreto, A. B. Bowman, M. F. Rose, D. Kang, R. Richman, J. M. Johnson, S. Berget, H. Y. Zoghbi, Inaugural article:

Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc. Natl. Acad. Sci., **102**, 17551-17558 (2005).