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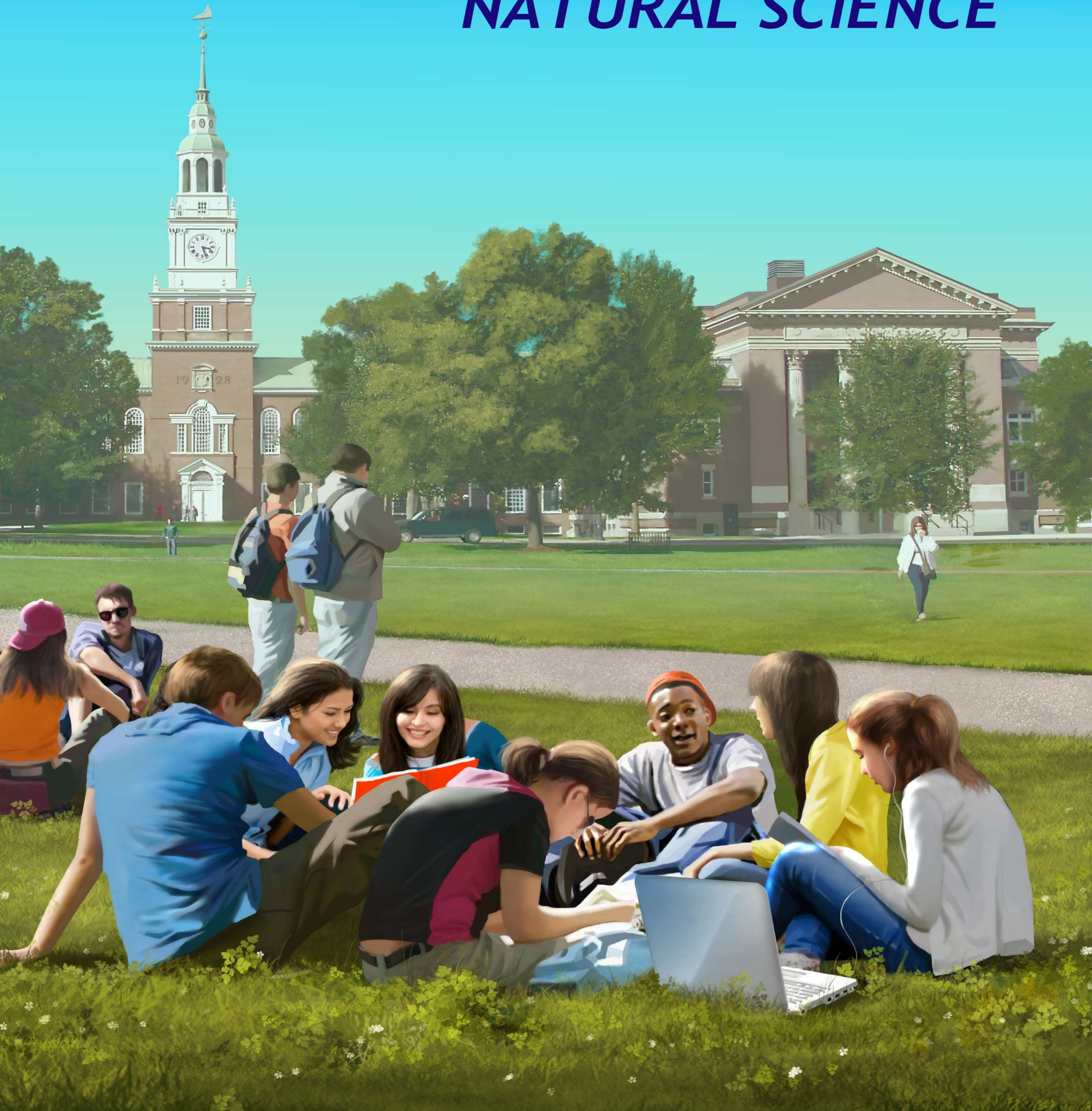

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Y.S.

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Biodegradation of Pyridine by *Arthrobacter sp.*

Fatemat Khasaeva
Leonid Zakharchuk
Alexander Netrusov
Igor Parshikov
Moscow State University of M.V. Lomonosov, Moscow, Russia

Abstract. *During growth of cultures of Arthrobacter sp. KM-P in a medium with a concentration of 2.5 g / L of pyridine, the pyridine was fully utilized in 24 hours. The stability of the process of biodegradation for pyridine with immobilized cells for three consecutive periodic processes was shown. The strain Arthrobacter sp. KM-P is recommended for the treatment of industrial wastewater containing pyridine.*

Keywords: *biodegradation, microorganisms, pyridine, Arthrobacter*

Introduction

As a result of human activities, many toxic compounds have accumulated in the environment. The main sources of pollution of the biosphere are the chemical and pharmaceutical industries. At the same time, methods of biotechnology are now widely applied [1]. The most successful strategy for biological treatment of wastewater is the use of microorganism-destructors, attached to water-insoluble carriers [2]. A serious place among pollutants is occupied by pyridine and its derivatives. The ability of microorganisms to utilize pyridine was discovered for the first time in the early twentieth century [3-5]. The bacteria that were isolated were *Aerobacter aerogenes*, *Serratia marcescens*, *Bacterium herbicola*, which grew in a medium containing 0.1-0.5% of pyridine, using it only as a source of nitrogen [6].

We isolated from the soil the bacteria *Arthrobacter* sp. KM-P, capable of use with pyridine [7], as the single source of carbon, nitrogen and energy.

The purpose of the study was to compare the ability of cells of the strain *Arthrobacter* sp. KM-P immobilized in a gel (calcium alginate) and cells in suspension to destroy pyridine .

Materials and Methods

The strain of *Arthrobacter* sp. KM-P was isolated from pyridine-contaminated soil samples [7]. For the cultivation of microorganisms a medium of the following composition (g/l) was used: KH_2PO_4 – 0.2; $MgSO_4 \cdot 7H_2O$ – 0.2; $FeSO_4 \cdot 7H_2O$ – 0.01; $CaCl_2 \cdot 2H_2O$ – 0.02; $MnSO_4 \cdot H_2O$ – 0.002; Na_2MoO_4 – 0.001; 0.5M 3-(*N*-morpholino)-propanesulfonic acid (MOPS) bicarbonate buffer - 1 L; pH 7.0 – 7.2. The pyridine was added in a concentration of 1.5 - 3.4 g/l. The culture was grown on a shaker at 220 rpm in 750 ml flasks containing 200 ml of

medium with pyridine. Growth of the culture was assessed with the use of a nephelometer. Determination of the residue of pyridine in the medium was conducted with a Hitachi 200-20 (Japan) spectrophotometer (Japan) at λ=255 nm.

To obtain a cell suspension culture, *Arthrobacter* sp. KM-P were grown to stationary phase (18 h), which was assessed by nephelometry. The optical density of the cell suspension in this case was 0.9 units and was in accordance with a concentration of $2x10^8$ cells/ml. The cells were separated from the medium by centrifugation at 6000 g for 10 min.

Cells of *Arthrobacter* sp. KM-P were immobilized in calcium alginate. To do this, the cell suspension *Arthrobacter* sp. KM-P in a volume of 100 ml was poured into 200 ml of sterile 3% sodium alginate solution in distilled water to obtain a 2% solution of sodium alginate with cells. Five hundred ml of 0.2 M solution of $CaCl_2$ in distilled water were prepared separately and sterilized at a pressure of 1 atm. The process of immobilization of bacterial cells was carried out in sterile conditions. For that, a 2% solution of sodium alginate with cells was added by drops to a flask with 0.2 M $CaCl_2$. The resulting granules of calcium alginate (size of 1-1.5 mm) with immobilized cells were kept in a 0.2 M solution of $CaCl_2$ for 10 to 12 hours. Then, the $CaCl_2$ solution was drained and the pellets were placed in 300 ml of sterile 0.9% solution of NaCl, in which immobilized cells of *Arthrobacter* sp. KM-P were kept at 4°C.

To conduct the degradation process with immobilized cells and with cells in suspension, a mineral medium with concentrations of 1.5, 2.5, 3.0 and 3.4 g/l of pyridine was used. To each of the flasks $1.0x10^9$ cells of *Arthrobacter* sp. KM-P were added in the form of 8.3 ml of cell suspension or 25 ml of solution of calcium alginate granules. Samples from the flasks with an initial concentration in the medium of pyridine of 1.5 g/l were taken every 3 hours, and samples from the flasks with other concentrations of pyridine every 6 hours. Granules with immobilized cells were separated from the culture medium by filtration.

Results and Discussion

The initial concentration of pyridine was at a level of 1.5 g/l for consumption during 36 hours in a culture of *Arthrobacter* sp. KM-P immediately after its isolation from the soil [4]. During the time that the culture of *Arthrobacter* sp. KM-P (2007-2013 years) has been done, its activity has significantly increased. At the time of the study, the activity of *Arthrobacter* sp. KM-P almost doubled and during 24 hours 2.5 g/l of pyridine were assimilated. Higher concentrations of pyridine in the medium (3.0 g/l) caused the growth of the culture to slow down, and the content of pyridine in media of 3.5 g/l and over inhibited the growth of culture.

The increase the rate of cleavage of pyridine in a culture of *Arthrobacter* sp. KM-P over a lengthy period can be explained by processes of self-induction cells resulting from multiple passages on a liquid mineral medium containing higher concentrations of the pyridine.

Thus, a strain of *Arthrobacter* sp. KM-P is able to grow in a medium with a high concentration of pyridine, and completely utilize that compound. This is one of the most promising strains-destructors for pyridine. This opens up wide possibilities for the use of this organism in sewage treatment with pyridine. The use of microorganisms in sewage treatment plants under periodic and/or continuous cultivation leads to the accumulation of large amounts of biomass, requiring disposal. The use of immobilized microbial cells for wastewater treatment may eliminate the need for regular disposal of large quantities of biomass [7]. Immobilized microorganisms are protected from adverse impacts. At the same time, granules may be created in any form needed for the required case—granules, drives, fibers, pipes, etc. [8].

Calcium alginate gel was used as a carrier for immobilizing cells of *Arthrobacter* sp. KM-P. This choice is explained by the relatively mild conditions for immobilization of cells and by the possibility of providing nutrients and oxygen. The process of immobilizing cells of *Arthrobacter* sp. KM-P was monitored under the microscope.

The speed of utilization of pyridine by immobilized cells of *Arthrobacter* sp. KM-P was compared with the speed of utilization of pyridine in a suspension of cells. It was established that cells in suspension have a faster speed of utilization of pyridine than immobilized cells. Pyridine at the concentration of 1.5 g/l was fully utilized by cells in

suspension in 6 hours. Immobilized cells assimilate such a concentration in 9 hours. Concentration of pyridine of 2.5 g/l is consumed in 12 hours by suspended cells, and in 18 hours by cells immobilized in calcium alginate. Pyridine in concentration of 3.0 g/l was utilized in 18 hours by suspended cells; pyridine in concentration of 3.4 g/l was utilized in 24 hours. Immobilized cells of *Arthrobacter* sp. KM-P use the same concentrations of pyridine (3.0 and 3.4 g/l) more slowly than cells in suspension (30 and 32 hours, respectively).

The results of the study suggest the possibility in principle of using immobilized cells of *Arthrobacter* sp. KM-P in calcium alginate for the removal of pyridine from wastewater. However, the catalyst system with immobilized cells should provide technological and economic advantages compared with microbiological processes on the basis of suspension cells. Application of the benefits of immobilized cells is only possible when used in bioreactors with batch or continuous mode [7]. In this case, in the reactor it is possible to use higher concentrations of immobilized cells of *Arthrobacter* sp. KM-P. For operation with high concentrations of aerobic bacteria in a fermenter, it is necessary to create high-speed mass transferal with the help of remixing of the catalyst pellets. The mechanical strength of granules of calcium alginate allows for such goals [9, 14].

The most promising approach may to be the model of a flow-through reactor mixing granules of calcium alginate, a variant of the continuous cultivation of independent microbial cells—chemostat. Creation of a bioreactor based on immobilized cells of *Arthrobacter* sp. KM-P for the treatment of wastewater containing pyridine is the next stage of our work.

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