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Modeling a new form of oil-oxidizing preparations in the form of cell agglomerates of mixed cultures of microorganisms stabilized by polyelectrolytes and salts of higher fatty acids

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Abstract

The efficiency of application of cationic polyacrylamide and its hydrophobized derivative at the stage of cell biomass separation from the cell cultures fluid is estimated. Cells of oil-degrading microorganisms obtained from mixed culture fluid by flocculation and flotation retain their viability for further use in the treatment of oil-contaminated soils under non-sterile conditions. The application of a new form of oil-oxidizing preparation resulted in a 40 % reduction of total petroleum hydrocarbon content in the contaminated soil.

Keywords:

hydrocarbon-oxidizing microorganisms, oil pollution, flocculation, polyelectrolytes

Разработка новой формы нефтеокисляющих препаратов в виде агломератов клеток смешанных культур микроорганизмов, стабилизированных полиэлектролитами и солями жирных кислот

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Аннотация

Проведена оценка эффективности применения катионного полиакриламида и его гидрофобного производного на стадии отделения биомассы клеток от культуральной жидкости. Клетки микроорганизмов-нефтедеструкторов, полученных из смешанной культуры с помощью флокуляции и флотации, сохраняют жизнеспособность для дальнейшего использования при обработке нефтезагрязненных почв в нестерильных условиях. Применение новой формы нефтеокисляющего биопрепарата приводило к снижению содержания нефтепродуктов в загрязненной почве на 40 %.

Ключевые слова:

нефтеокисляющие микроорганизмы, нефтяное загрязнение, флокуляции, полиэлектролиты

Introduction

Both yeast and bacteria can act as biological agents in the composition of biopreparation to eliminate oil pollution [1–4]. Highly concentrated cell biomass from culture liquid can be obtained using microporous membranes in special, rather expensive installations where tangential flow filtration is implemented [5, 6]. The methods of centrifugation and separation [7] are widely used but they can result in additional energy consumption. A method for the rapid separation of yeast using magnetic nanoparticles is also described [8]. The spray drying method leads to the death of a significant number of living cells due to the thermal denaturation of cellular proteins. Thus, the development of microbial biopreparations consisting of specialized biological agents for the prompt solution of the problems of protecting and restoring the environment is relevant and economically feasible. The aim of the work is to obtain a biopreparation with oil-oxidizing properties in the aggregated form using flocculants, as well as to assess its biotechnological potential on sample of oil-contaminated soils.

Materials and methods

The soil yeast strain *Rhodotorula glutinis* VKM Y-2993D and bacterial strain *Pseudomonas libanensis* VKM B-3041D were used in this work. The strains of microorganisms were cultivated in 250 cm^3 flasks at 20°C in the sterile semisynthetic medium containing mineral salts (3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, and 0.01 g $FeSO_4$ per dm^3 water) and sucrose (20 g per dm^3 water) as a carbon source. The volume of cultivation medium was 150 cm^3. The cell titer for *R. glutinis* culture on the fifth day was $1÷3×10^7$ CFU cm^3. The cell titer for *P. libanensis* culture on the fifth day was $1÷5×10^9$ CFU cm^3. The fluorescent photos were obtained using the Axiovert 200 M microscope equipped with the digital camera

AxioCam ERc 5s under the hundredfold magnification. Before the fluorescence microscopy manupulations, the specimens were stained with acridine orange and fluorescein diacetate, and then washed with distilled water through a nozzle equipped with a 0.25 μm microporous membrane. The cationic polyacrylamide (cPAA) C-8380 Specfloc (China) was used as a flocculant. The sodium salt of stearic acid was used as hydrophobic modifiers of cPAA.

The efficiency of biopreparations was evaluated on soil contaminated with oil products. The soil was mainly a sand-gravel mixture sampled in the railway area in Syktyvkar. Two flasks (volume 250 cm^3) were filled with 20 g air-dry soil samples. 100 cm^3 tap water, 50 mg $NaNO_3$, 20 mg K_2HPO_4, and 10 mg $MgSO_4$ were added to each flask. The new form of oil-oxidizing preparation (0.3 g) was added to one flask. The flasks were placed in an orbital shaker to mix the contents for 8 days at 150 rpm. At the end of the treatment process, the residual content of total petroleum hydrocarbons (TPH) in soil samples was evaluated. All variants of the experiments were performed three times. The content of TPH was determined by the gravimetric method [9]. The initial TPH concentration in samples was 15±1.6 mg/g air-dry soil. Statistical calculations were performed using the standard Excel tools.

Results and discussion

To optimize the yeast cell obtaining process we proposed the use of a polyelectrolyte – cPAA. Polyelectrolytes are widely used for wastewater treatment, and the choice of one or another flocculant is determined by the surface charge of polluting particles [10]. The surface charge of R. glutinis cell is negative, which is confirmed by the formation of large flocculated complexes after the addition of cPAA. After water-dilution of 100 cm^3 *R. glutinis* culture fluid sampled on the fifth day of cultivation with water to 200 cm^3, 0.1 % solution of cPAA was added to the mixture in 1 cm^3 portions until cell flocculation was complete. Finally, we obtained 45÷50 cm^3 of polymer solution. The yield of air-dry *R. glutinis* biomass from 100 cm^3 of culture fluid was 0.64±0.05 g. Large flakes formed by the process were well filtered through the fibrous polyester material.

There are certain difficulties in obtaining concentrated biomass with the use of such polyelectrolytes as cPAA for the *P. libanensis* bacterial culture. Some species of the *Pseudomonas* genus and many other oil-degrading bacteria are known to be able to synthesize biosurfactants that facilitate the diffusion of hydrocarbons into cells [11]. Biosurfactants are usually glycolipids or lipopolysaccharides [12], shielding from charged particles, and therefore the cells are sufficiently inert to polyelectrolytes like hydrophilic ionic polyacrylamides. To form the concentrated bacterial biomass, we proposed to use combinations of cPAA and salt based on alkali metal and higher fatty acid (SAF). The cPAA contains usually a tertiary amine as a cationic group, a chloride or methyl sulfate group as an anion. If the polymer used is mixed with SAF, ion exchange occurs resulting in cPAA acquirement of fatty acid residues and formation of water-insoluble complex with bacteria cells captured. Due to the hydrophobic nature of complexes obtained it is more reasonable to use the flotation method. In our case, solution of sodium salt of stearic was used as the commercially available hydrophobic modifier of cPAA.

50 cm^3 0.5 % solution SAF and 20 ml 0.1 % solution cPAA was added to 100 cm^3 *P. libanensis* culture fluid after five cultivation days. The suspension was stirred until the bacterial complexes appeared and then bubbled with air until the foam completely removed. The resulting yield of the bacterial complex was 0.25±0.04 g from 100 cm^3 of the culture fluid.

The similar procedure was carried out with a mixed culture obtained after five days of cultivation. At the first stage (Figure 1), a positively charged polyelectrolyte interacted with oppositely charged functional groups of the cell wall of yeast cells. Bacteria with lipophilic surface properties did not participate in the formation of primary agglomerates. The unused part of the flocculant, due to the introduction of salts of higher fatty acids, was subjected to hydrophobic modification. As a result, hydrophobic micelles were formed with the capture of bacteria together with the final form of the biological product with the primary agglomerates of yeast cells.

The yield of mixed agglomerates (MA) was 0.67 ± 0.04 g from 100 cm^3 culture fluid. The high product yield from the mixed culture fluid *via* flotation indicates that the yeast com-

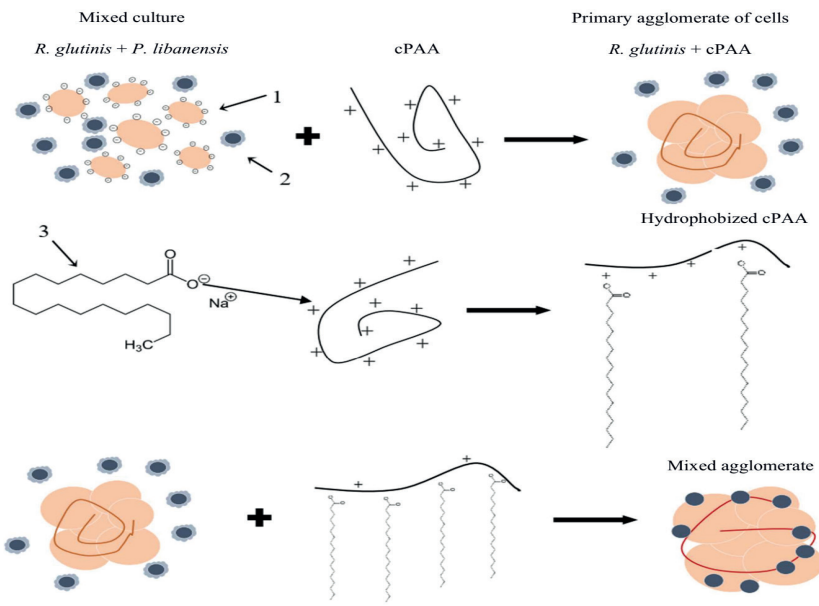

Figure 1. Scheme of the process of molecular design of a new type of oil-oxidizing biological products based on the principles of the interaction of polyelectrolytes and hydrophobic aggregation (1 – yeast cell; 2 – bacterial cell surrounded by hydrophobic biosurfactant; 3 – sodium stearate).
Рисунок 1. Схема процесса молекулярного проектирования нового типа нефтеокисляющих биопродуктов, основанного на принципах взаимодействия полиэлектролитов и агрегации гидрофобных элементов (1 – дрожжевая клетка; 2 – бактериальная клетка, окруженная гидрофобным биосурфактантом; 3 – стеарат натрия

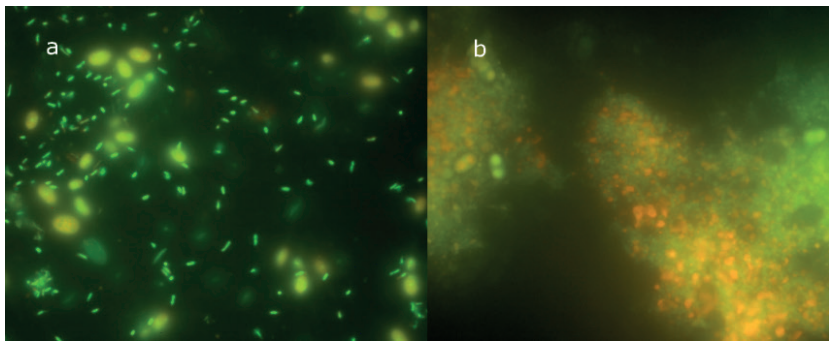

Figure 2. Photo of *R. glutinis* and *R. libanensis* in the mixed culture fluid (a) and MA after flotation (b). Excitation at λ=450÷480 nm, radiation area at λ=500÷600 nm.
Рисунок 2. Микрофотография *R. glutinis* and *R. libanensis* в смешанной культуре (a) и агломераты клеток (b). Возбуждение при λ=450÷480 нм, зона излучения при λ=500÷600 нм.

bined with bacterial strain forms the microbial complex enriched with the cell biomass, in contrast to single strains. It is likely that there is an interaction of cPAA with yeast cells in mixed cultures with formation of large cell aggregates and their subsequent capture together with bacteria into microbial complexes. Fluorescent microscopy revealed the presence of living bacterial and yeast cells in MA (Figure 2).

The efficiency assessment of the obtained biopreparation for the treatment of oil-contaminated soils revealed a significant decrease (≈40 %, $p<0.05$) in content of TPH in the target soil sample compared to the control without MA addition (≈10 %, $p>0.05$). So, this study clearly demonstrates that it is possible to obtain high-value microbial preparations without use of expensive and complicated equipment. The methods developed can be used to remediate accidental oil spills on or near the soil surface.

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