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Biosorbents based on esterified starch carrying immobilized oil-degrading microorganisms

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ABSTRACT

The complex sorbents based on hydrophobized starch, which contain oil-degrading microorganisms, have been proposed for effective sorption and utilization of petroleum-related pollutants. The sorbents were made on the base of benzoic, lauric and stearic acid esters of starch with degrees of substitution of 0.4–1.1. The esterification of starch was carried out by the reaction with acyl chlorides of the corresponding acids in an aqueous-organic medium. The structure of the esters was studied by SEM, IR and NMR spectroscopy. As a result of porous hydrophobic structure, these sorbents are capable of binding and retention of petroleum products on the water surface, and keeping the flotation for at least 30 days after the petroleum products sorption. The test of biodegradability of the obtained samples revealed that the modified starches can be degraded by microscopic fungi, therefore they do not cause secondary pollution. The cultures of yeast *Rhodotorula glutinis VKM Y-2993D* and bacteria *Pseudomonas libanensis VKM B-3041D* immobilized on the sorbent facilitate the rapid utilization of accumulated petroleum products.

1. Introduction

Various types of sorbents are used widely in processes of remediation of soils and water bodies for collecting and utilization of petroleum products. Many of these sorbents are based on artificial polymers, which can act as a source of secondary pollution after they have been used without specialized recycling. Another type of sorbents is based on products of processing of activated sludges of water treatment facilities (Dobele et al., 1996; Sobgaida et al., 2011). However, the use of these sorbents can lead to the problem of contamination with heavy metals and other xenobiotics (Zhou et al., 2016; Volesky, 1987; Ostman et al., 2017) accumulated in the activated sludge mass after recovery from the treatment facilities. The use of various peats as sorbents is a more attractive variant (Novoselova and Sirotkina, 2008; Cojocaru et al., 2011), but it raises the problem of their subsequent biodegradation due to the presence of hardly hydrolyzable components (lignin, bitumens). Additionally, the increased acidity of this type of raw material requires its special preparation.

The promising direction is the use of complexes consisting of the sorbing carrier and oil-degrading microorganisms (Chugunov et al., 2000). The use of such complexes can significantly accelerate the process of biodegradation of petroleum-related pollutants in water and soil (Filatov et al., 2013). Adapted microorganisms isolated from oil-contaminated soils can have a wide range of abilities for biodegradation of various oil components (Archegova et al., 2012). The oil-degrading bacteria, which were effective even at the temperature of 5 °C, have been isolated from marine sediment (Lin et al., 2009). In addition, microorganisms that were able to utilize petroleum products have been found in natural oil seeps (Bryanskaya et al., 2015).

It is known that representatives of many species of oil-degrading microorganisms have the ability to synthesize biosurfactants, which can include lipopolysaccharide complexes for accumulating hydrophobic substances of petroleum products and for further diffusion of these substances into cells (Cho et al., 2001; McClements and Gumus, 2016; Satpute et al., 2010; Kuyukina and Ivshina, 2010). Microbial lipopolysaccharides are prone to biodegradation in natural environment to non-toxic compounds, this fact stimulates the researches in the field of development of bio-inspired polysaccharide structures to solve the problems of collecting and utilizing oil pollutants.

Cellulose and starch are some of the most promising

Abbreviations: BAC, benzoyl chloride; LAC, lauroyl chloride; SAC, stearoyl chlorid; St-BAE, starch benzoate; St-LAE, starch laurate; St-SAE, starch stearate; SEM, scanning electron microscopy; PP, petroleum products

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polysaccharides to produce the analogues of natural surfactants. They are available, and the methods of their modification are well developed in the industry. In some studies, it has been proposed to use cotton fibers (Lin et al., 2014) or plant wastes (Yu et al., 2016; Patel, 2012; Chakresh et al., 2016) as natural cellulose-containing carriers. However, cellulose-based materials have some disadvantages related with their insolubility in water. In some cases, the need of insertion of hydrophilic groups in addition to the hydrophilic groups makes the modification for obtaining the structures similar to biosurfactants more difficult and expensive. Starch, the polysaccharide widely used in many spheres, is a more suitable raw material for this kind of modification. In order to obtain sorbents and carriers of microorganisms, starch can be hydrophobized by various methods (Qiao et al., 2006). One of the most reasonable methods, in terms of subsequent utilization of the sorbent in natural environment, is the method of preparation of long-chain fatty esters of starch, which have new properties in comparison with the initial polysaccharide (Vanmarcke et al., 2017; Aburto et al., 1999). In several of studies, the esterification of starch was carried out in organic solvents using a variety of condensing systems (Grote and Heinze, 2005; Gao et al., 2012; Winkler et al., 2013). In this way, the esters with different degrees of substitution can be obtained, including the highest degree. In aquatic medium, starch esterification is carried out using acyl chlorides, in the presence of bases (Namaz et al., 2011; Stojanović et al., 2002). The method is simple, but the achieved degrees of substitution are lower.

Thus, the sorbents based on modified starches combined with oil-degrading microorganisms are of great importance to solve the problem of remediation of oil-contaminated environment, as they provide fixation and enhanced stability to the specialized microorganisms, sorption of petroleum products on the surface, contact of fixed microorganisms with substrate, and subsequent safe disposal of the biosorbents.

The aim of this work was to develop the complexes of starch hydrophobized by carboxylic anhydrides with immobilized oil-degrading microorganisms for sorption and biological utilization of petroleum products.

2. Materials and methods

2.1. Acylation of corn starch with acyl chlorides

The corn starch (PubChem CID: 24836924) used in this work had the following composition: 25% amylose, 75% amylopectin, and 8% moisture content by mass. The hydrophobic modifiers of starch, benzoyl chloride (PubChem CID: 7412), lauroyl chloride (PubChem CID: 8166) and stearoyl chlorid (PubChem CID: 8212), were purchased from Alfa Aesar. Ethanol (PubChem CID: 702), chloroform (PubChem CID: 6212) and sodium hydroxide (PubChem CID: 14798) were purchased from Vecton (Russia).

In 350 cm^3 of water, 5.0 g (30.9 mmol) of corn starch was suspended and stirred for 45 min at 50 °C. After the dissolving of starch, the solution was cooled to 10 °C, and NaOH (1.1 mol excess relative to the amount of an acyl chloride) in 50 cm^3 of water was added. Then, the starch alkaline solution was slowly mixed for approximately 10 min. The selected amount of an acyl chloride (see Table 1) in 50 cm^3 of $CHCl_3$ was added dropwise into the reaction container, under stirring at 4000 rpm. The mixture was allowed to react at 10 °C for 60 min, and then at 25 °C for 2 h. The polymer was isolated by precipitation in 600 cm^3 of ethanol, filtered, and washed three times with 500 cm^3 portions of hot ethanol (60 °C). Then it was air-dried over night and kept for 12 h in vacuum at 60 °C.

2.2. Characterization of starch esters

Starch benzoate DS: 1.2, FTIR (KBr): 3600–3300 (ν OH), 2921 (ν C—H, methylene), 2852 (ν C—H, methyl), 1745 (νst C═O), 1468 (ν C—O—C), 1415 (ν C—O—C), 1376 (δ C—H), 1170–1035 (νas C—O—C) cm-1; 13C NMR (CDCl3): d = 173.6 (C-7), 96.1 (C-1), 74.0–67.6 (C-2, 3, 4, 5), 60.5 (C-6), 34.3 (C-8), 32.3 (C-9), 30.1–29.8 (C-10- 20), 25.1 (C-21), 23.1 (C-22), 16.0 (C-23), 14.5 (C-24) ppm.

Table 1
Characteristics of starch samples after hydrophobic modification.

Modifications	St-BAE	St-BAE	St-LAE	St-LAE	St-SAE	St-SAE
	1	2	3	4	5	6
Elementary unit-to-acyl chloride ratio	1:1	1:3	1:1	1:3	1:1	1:3
Degree of substitution in starch	0.42	1.08	0.38	1.10	0.40	0.95
Bulk density, g/cm^3	0.5	0.4	0.2	0.15	0.15	n.d.*

*Not determined.

Starch laurate DS: 1.2, FTIR (KBr): 3600–3300 (ν OH), 2921 (ν C—H, methylene), 2852 (ν C—H, methyl), 1745 (νst C═O), 1468 (ν C—O—C), 1415 (ν C—O—C), 1376 (δ C—H), 1170–1035 (νas C—O—C) cm-1; 13C NMR (CDCl3): d = 173.6 (C-7), 96.1 (C-1), 74.0–67.6 (C-2, 3, 4, 5), 60.5 (C-6), 34.3 (C-8), 32.3 (C-9), 30.1–29.8 (C-10- 20), 25.1 (C-21), 23.1 (C-22), 16.0 (C-23), 14.5 (C-24) ppm.

Starch stearate DS: 0.4, FTIR (KBr): 3600–3300 (ν OH), 2921 (ν C—H, methylene), 2852 (ν C—H, methyl), 1745 (νst C═O), 1468 (ν C—O—C), 1415 (ν C—O—C), 1376 (δ C—H), 1170–1035 (νas C—O—C) cm-1; 13C NMR (CDCl3): d = 173.6 (C-7), 96.1 (C-1), 74.0–67.6 (C-2, 3, 4, 5), 60.5 (C-6), 34.3 (C-8), 32.3 (C-9), 30.1–29.8 (C-10- 20), 25.1 (C-21), 23.1 (C-22), 16.0 (C-23), 14.5 (C-24) ppm.

The degrees of substitution of the starch esters were determined by potentiometric titration with preliminary alkaline hydrolysis of the samples (Freire et al., 2005).

^{13}C NMR spectra were acquired using a spectrometer Bruker Avance II 300 (operating frequency of 75 MHz) in DMSO-d_6 or $CDCl_3$.

IR spectra were recorded on an IR Fourier spectrophotometer Shimadzu IR Prestige 21 using KBr pellet technique.

Microstructure of the samples were studied by scanning electron microscopy. The SEM images of the specimens were recorded in secondary electrons mode using TESCAN scanning electron microscope (Vega3 SBU, TESCAN, Czech Republic) operating at an acceleration voltage of 5 kV. The specimens for the SEM were sputter coated with platinum (thickness ≈ 5–10 nm).

2.3. Biodegradability test

The evaluation of biodegradability of the samples was done using the common microscopic fungus *Aspergillus niger VKM F-1119*. For this purpose, the 1–2 × 10^6 CFU/cm^3 suspension of spores of the fungus *Aspergillus niger* was prepared. The spores of the fungus were transferred from a test tube containing the pure culture to a flask containing 25 ± 5 cm^3 of sterile distilled water. The fungal spore suspension was thoroughly mixed by shaking to break all spore clumps and filtered through four layers of sterile gauze to get rid of pieces of mycelium, agar and spore clumps. The concentration of fungal spores was evaluated using a photoelectric concentration colorimeter (KFK-3, Russia), a light filter λ = 400 nm, and a 50 ± 0.5 mm cuvette. The optical density of the solution corresponding to the concentration of the spores of the fungus *Aspergillus niger* of 1–2 × 10^6 CFU/cm^3 was 0.220-0.440. A 0.5 g sample was placed in a Petri dish, 10 cm^3 of Czapek liquid medium without carbohydrates were added, then, 10 drops containing spores of the microscopic fungus *Aspergillus niger* were placed therein, and their germination was assessed at 25 °C.

The strains of soil yeast *Rhodotorula glutinis VKM Y-2993D* and bacterial culture *Pseudomonas libanensis VKM B-3041D* were used in this work. The strains of microorganisms were cultivated in 250 cm^3 flasks at 20 °C on a sterile semisynthetic medium containing mineral salts (3 g of $NaNO_3$, 1 g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.5 g KCl, and 0.01 g of