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Comparison of Biosurfactant Activity for Hydrocarbon-Utilizing Microorganisms.

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ABSTRACT

This article performs the comparison of biosurfactant activity for 12 microorganism strains extracted from oily sludge. The lowest values of surface tension forces during the entire incubation period are determined for 2c, 1c and 4a strains, and made 49 mN/m, 51 mN/m, and 52 mN/m, respectively. The maximum biosurfactant specific activity was observed among 1b, 2b, and 3a strains.

Keywords: biosurfactants, microorganisms, crude oil, surface tension.

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INTRODUCTION

The environmental contamination by crude oil and related products has become a serious problem worldwide. A serious environmental threat is oil wastes besides crude and commercial oil [1]. The most promising of oil sludge utilization technologies are the biological methods based on the use of microorganisms-destructors for oil hydrocarbons [2]. It is known that the majority of microorganisms may synthesize surfactant – biosurfactants. Due to the ability of biosurfactants to reduce the surface and interphase tension, their inclusion in oily waste may improve the mobility and bioavailability of oil components, which improves oil sludge biodegradation processes [3, 4].

Biosurfactants are degradable, environmentally safe, have a more effective action at extreme conditions (temperature, concentration of mineral salts and pH) compared with chemically derived surfactants. They may also be obtained by inexpensive methods. Therefore, they are of great interest among researchers as an alternative to chemical surfactants [5].

In addition to the use in bioremediation biosurfactants may be used for enhanced oil recovery. Biosurfactant-producing microorganisms or a biosurfactant solution is injected into an oil reservoir which reduces oil viscosity and improves its mobility in the reservoir [6, 7].

This article performs a comparative assessment of biosurfactant ability to synthesize the biosurfactants of microorganisms-destructors among oil hydrocarbons.

MATERIALS AND METHODS

The previously extracted strains of hydrocarbon oxidizing microorganisms were used in the article. The cultivation of microorganisms was carried out to determine the biosurfactant activity at 28 °C in a mineral medium of the following composition (g/l): KH_2PO_4 - 3, $MgSO_4*7H_2O$ - 0,2, $NaH_2PO_4*12H_2O$ - 4,5, $(NH_4)_2SO_4$ - 1,0 [8]. Crude oil was used as a sole carbon at the amount of 2% (by volume). The medium was sowed by 1 ml of the test culture and incubated at 28 °C for 21 days. A sterile medium with the oil was used as a control one without bacteria addition. The measurements were performed after 16, 24, 40, 48 hours and during the 7th, 14th and 21st day.

In order to perform measurements the culture liquid was separated from the residual oil in a separatory funnel and then was centrifuged for 10 min. at 8000 rev/min. The supernatant was used to determine the biosurfactant activity of strains. After that the supernatant was discharged and the vial weight was measured. Dry microbial biomass was determined by subtracting the weight of the vial dried to a constant weight with the bacteria of a clean, dry vial weight.

To assess the biosurfactant activity the surface tension of the culture liquid was measured by the Wilhelmy plate method with the tensiometer EasyDyne S K20S.

The measurement of all parameters was performed at least three times. The statistical analysis of the results was performed using Microsoft Office Excel, 2010. The significance of mean value differences was evaluated using the Student coefficient ($p \leq 0.05$). The relationship of a number of data was set using the correlation coefficient.

RESULTS AND DISCUSSIONS

The microbial consortium was extracted preliminary from oily sludge (hydrocarbon content makes 60% by weight) on a liquid salt medium with crude oil as the sole carbon source. During the consortium separation into individual strains based on a solid medium 12 strains of microorganisms were detected.

It was found that during the 10th day the oil emulsion and oil film degradation was observed in the presence of ten strains (strains 1b, 1c, 2b, 2c, 3a, 3b, 3c, 4a, 4b, 4c). These strains were used in further experiments.

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Each of the selected strains for microorganisms was tested on the ability to synthesize biosurfactants based on liquid medium. The ability of surfactant metabolite synthesizing for hydrocarbon oxidizing microorganisms was performed by measuring the surface tension of a culture broth [9, 10].

The method of liquid broth surface tension measuring is a generally accepted one [11]. The advantage of this method is that there is no need to correct the measurement values depending on the measured broth density. Figure 1 shows the measurement results of the surface tension force for the test strains.

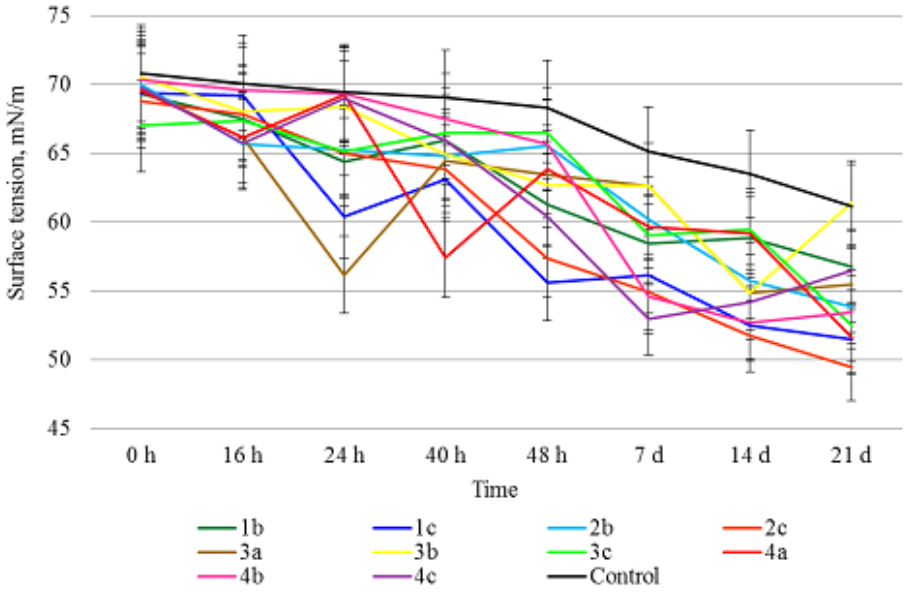

Figure 1: Changing the surface tension forces in the experimental variants

A nonlinear decrease of the surface tension forces was observed in the culture broth during the experiment. It was found that the smallest value for the surface tension forces of 4c strain was recorded on the 7th day of the experiment, for the strains 3a, 3b, 4b, on the 14th day and for the strains 1c, 1b, 2b, 2c, 3c, 4a on the 21st day. The lowest values of surface tension forces during the entire incubation period were recorded for the strains 2c, 1c and 4a and made 49 mN/m, 51 mN/m and 52 mN/m, respectively.

For an easy interpretation of the obtained values of the surface tension change in the presence of biosurfactants of the studied strains the specific activity of biosurfactant synthesis calculation was performed during the next phase. For this purpose, the difference in surface tension values for control and test sample was brought to the microorganism weight according to dry matter.

According to the obtained data the specific activity of the biosurfactant synthesis was performed. The obtained data are presented in Table 1.

Table 1: Specific activity of biosurfactant synthesis by extracted strains, mN/m*g

Strain name	Incubation period							
	0 h	16 h	24 h	40 h	48 h	7 d	14 d	21 d
1b	18,34	**52,58**	51,87	14,53	53,88	51,20	28,43	27,00
1c	14,78	2,56	21,38	21,36	**28,76**	18,87	18,26	21,12
2b	9,78	45,14	36,60	43,72	28,02	49,93	**59,61**	40,00
2c	11,14	8,02	13,73	18,67	**30,43**	15,21	12,23	18,78
3a	12,17	31,64	**56,19**	15,17	8,53	2,79	8,76	7,70
3b	3,35	10,34	3,69	13,55	11,15	3,63	**25,03**	0,71
3c	8,72	5,96	6,98	4,28	3,53	8,41	6,35	**24,09**
4a	15,90	21,84	1,06	27,37	8,34	10,75	7,82	**38,80**
4b	6,11	1,80	0,61	6,48	8,10	22,14	**38,84**	36,07
4c	10,19	16,55	1,66	7,87	18,69	**21,22**	15,04	10,57

Note: Maximum specific activity of the biosurfactant synthesis for this strain mN/m*g is selected by bold type.

The table shows that the highest values of the specific surface tension forces, reflecting the synthesis of biosurfactants are observed for different strains at different times. Thus, the highest specific activity of biosurfactant synthesis is observed during the 1st day of incubation for 1b and 3a strains, during the 2nd day of incubation for 1c and 2c strains, 21st day for 4c strain, the 14th day for 2b, 3b, 4b strains, the 21st day for 3c and 4a strains.

It is known that *Bacillus subtilis* strain *B30* had its maximum activity during the 8th hour of incubation period [12]. *Bacillus sp*. strain had its peak of biosurfactant synthesis activity on the first day of incubation [8].

Pseudomonas sp. bacteria showed its maximum activity during the 4th day of incubation period [6].

When the maximum specific activity of biosurfactant synthesis was compared it was found out that the highest values of the specific activity are observed among the strains the peaks of which are observed during the 16th and the 24th incubation hour. These are 1b (53 mN/m*g) and 3a (56 mN/m*g) strains and 2b (59 mN/m*r) strain, the highest specific activity peak of which occurred on the 14th day of incubation. The effectiveness of these strains was also observed visually - they created stable emulsions of "oil in water". There is evidence in the literature that *Pseudomonas sp*. strain reduces the surface tension of mineral medium to 32 mN/m, the specific value was high - 19 mN/m*g [13]. Approximately the same results were obtained by Shavandi et al. [14]. *Rhodococcus sp*. strain of reduced the surface tension to 30 mN/m and had a specific index of 22 mN/m*g. Rodrigues et al. [15] *Lactobacillus sp.* strains reduced the surface tension to 39-42 mN/m, but had a low specific indicator value - 1.5-7.5 mN/m*g.

CONCLUSIONS

Thus, 10 of the available 12 hydrocarbon oxidizing microorganisms were able to synthesize biosurfactants.

A nonlinear decrease of the surface tension forces in the culture broth was observed in the experiment. The lowest values of surface tension forces during the entire incubation period were recorded for 2c, 1c and 4a strains and amounted to 49 mN/m, 51 mN/m and 52 mN/m, respectively.

It was found out that 1b, 2b and 3a strains actively extracted biosurfactants during the surface tension changes analysis. The maximum specific activity for these was 53 mN/m*g, 56 mN/m*g and 59 mN/m*g, respectively. The extracted strain identification is planned in the future.

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