Biosorbents for oil-containing wastewater treatment

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Abstract. Investigated data about the selection of the effective microorganisms – oil destructors from the environmental objects and industrial waste. Founded the most active bacterial strains were isolated from the Caspian Sea bottom sediments. Hydrophobicity’s index and emulsifying activity of the most active oil bacterial destructors were defined. Their efficient use of oil or diesel fuel as the only carbon’s source was shown. The growth dynamics of these strains on nutrient containing oil media was studied. Shown the bacterial strain VGTU-02 possessed the greatest speed of growth and destructive activity concerning oil hydrocarbons. Studied the possibility of the using some crop waste and carbonizate from spent activated sludge as a matrix for biosorbents. The microbial base for these biosorbents was the bacterial strain VGTU-02. Selected microorganisms were attached to the studied carriers from the crop waste and waste activated sludge. Efficiency of purification of model liquid from crude oil at use of the suspended VGTU-02 microorganisms with matrixes was defined. Shown that under similar conditions, the highest efficiency of oil purification was achieved 99.41 % for a matrix based on sunflower husk and 98.46% based on carbonizate. The developed biosorbents can be used to remove oil and petroleum products from wastewater.

The purpose of this study was to develop a technique of biosorbents generating based on crop waste or waste activated sludge as a matrix and oil-oxidizing bacterial strains to use for the treatment of oily wastewater.

Firstly, the screening of the bacterial strains was spended with the greatest destructive activity against crude oil and petroleum products was set. In the course of the research, 19 bacterial strains isolated from environmental objects and industrial wastes. These bacterial cultures contained in the working collection of the Industrial ecology and life safety Department of Volgograd State Technical University Museum cultures.

Selection was carried out by growing microorganisms in a liquid synthetic mineral medium Diana-Voroshilov containing oil or diesel fuel as the only source of carbon in the amount of 20 g / l [1]. Bacterial cultures were grown at 28-30°C in stationary conditions for 14 days.

Activity’s assessment in relation to carbon sources was carried out visually for 14 days. As a control, nutrient medium of Dianova – Voroshilova without microorganisms was used. The results of screening in the point system, compiled by the method of E. R. Faizulina, O. N. Auezov and others [2], were presented in table 1.
Table 1. The results of the determination of oil-degrading activity by the investigated strains.

| The name of the strain   | Oil, activity, score | Diesel fuel, activity, score | The name of the strain   | Oil, activity, score | Diesel fuel, activity, score |
|--------------------------|----------------------|-------------------------------|--------------------------|----------------------|-------------------------------|
| VGTU-02<sup>a</sup>      | 3                    | 3                             | UV-4<sup>a</sup>         | 1                    | 1                             |
| VGTU-03<sup>a</sup>      | 1                    | 1                             | UV-5<sup>a</sup>         | 1                    | 1                             |
| VGTU-05<sup>b</sup>      | 2                    | 1                             | UV-7<sup>a</sup>         | 2                    | 2                             |
| VGTU-06<sup>c</sup>      | 1                    | 1                             | UV-8<sup>a</sup>         | 1                    | 1                             |
| VGTU-07<sup>c</sup>      | 1                    | 1                             | UV-9<sup>a</sup>         | 1                    | 1                             |
| VGTU-10<sup>d</sup>      | 1                    | 1                             | UV-12<sup>a</sup>        | 2                    | 2                             |
| VGTU-22<sup>e</sup>      | 3                    | 1                             | UV-13<sup>a</sup>        | 1                    | 1                             |
| ZSK-DV<sup>a</sup>       | 1                    | 1                             | UV-16<sup>a</sup>        | 1                    | 1                             |
| ZSK-2<sup>a</sup>        | 1                    | 1                             | UV-18<sup>d</sup>        | 4                    | 1                             |
| UVG-11<sup>a</sup>       | 3                    | 1                             |                          |                      |                               |

<sup>a</sup> – bottom sediments and <sup>e</sup> – sea water from the Caspian Sea.
<sup>b</sup> – soil.
<sup>c</sup> – wash from meat processing plant.
<sup>d</sup> – oil sludge.
<sup>f</sup> score: 1- weak growth; 2- moderate growth; 3- well growth; 4-the best growth.

According to the results of screening (table 1), the strain VGTU-02 had the maximum activity in relation to oil and diesel fuel, so it was chosen as the most active destructor of petroleum hydrocarbons from 19 studied bacterial cultures.

The next step was to determine the index of hydrophobicity and emulsifying activity of microorganisms selected for further study. The hydrophobicity index demonstrates the ratio of hydrophilic and hydrophobic components of cells in the surface layers of their shell. This characteristic should be taken into account in biological studies in the study of adhesion processes involving cells and surfaces of different structures, as well as growth on hydrocarbons associated with direct substrate’s using [3]. It is known that oil-oxidizing bacteria, interacting with a hydrocarbon substrate, are capable of direct contact with the hydrocarbon due to the hydrophobic cell surface caused by the presence of lipid components in it [4, 5]. Determination of cell surface hydrophobicity was carried out by the Rosenberg method in Serebryakova modification [6].

Chloroform was used as the hydrocarbon phase during the hydrophobicity index measuring. To determine the hydrophobicity index, a scheme was used: each of the studied cultures was sown with a thinning stroke on the surface of dense nutrient agar in Petri dishes, incubated at room temperature for 3-7 days, depending on the individual growth rate. Bacterial biomass was washed off the nutrient agar with phosphate buffer (pH=7). 1 ml of cell suspension was diluted 10 times, after which the optical density was measured on a photoelectrocolorimeter KFK-2-UKHL-4.2. 4 ml of bacterial suspension with 1 ml of chloroform was shaken for 15 minutes in a 10 ml test tube, then defended for phase separation in a vertical position for 1 hour. After measuring the optical mixture’s density of the bacterial suspension with chloroform, the hydrophobicity index was calculated by the method [6]. Emulsifying activity was determined by shaking in a chemical test tube 4 ml of bacterial suspension with 3 ml of diesel fuel, followed by settling in the vertical position for 1 hour to separate the aqueous and hydrocarbon phases. The calculation of the indicator was carried out by the method [6]. The results of the emulsifying activity and hydrophobicity index determination were presented in table 2.
The results showed the emulsifying activity of the strain VGTU-02 was 66.74%. According to the literature, microorganisms with emulsification activity greater than 50% could be perspective for producing surfactants and utilization of hydrocarbon substrates [7]. Shown the possibility of investigated microorganism to be active producer of surfactants and, therefore, it was characterized by a high destructive potential against petroleum hydrocarbons.

An important criterion for choosing the biosorbtion microbial component is the immobilized culture’s growth rate. To study the growth dynamics of bacterial oil destructors, they were grown in stationary conditions in flasks (200 ml) in the optimal temperature conditions (30 ºC) in a medium (50 ml) containing 2% (vol.) oil or diesel fuel as the only carbon source, the volume of bacterial suspension seeded 5 ml (10⁹ microbial cells/ml). After 1, 2, 3, 5, 7 days culture liquid was seeded on the dense medium in Petri dishes to determine the concentration of viable cells. The results were presented in figures 1 and 2, respectively.

**Table 2.** The results of the determination of the hydrophobicity’s index and the emulsifying activity.

| The name of the strain | the index of hydrophobicity, % | the emulsifying activity, % |
|------------------------|--------------------------------|----------------------------|
| VGTU -02               | 25,0                           | 66,74                      |

The dependences shown in figures 2 and 3 demonstrated the strain VGTU-02 actively had used organic components as food sources. The bacterial strain VGTU-02 was isolated from the bottom sediments of the Northern Caspian Sea on a selective medium for lipolytic microorganisms [8]. Done visual assessment of changes occurring in flasks with cultured microorganisms. The ability of VGTU-02 to emulsify and recycle oil and diesel fuel was shown, after 7 days the absence of the initially present organic layer and the formation of abundant flakes on the mineral medium surface were observed.

To develop the technology of biosorbtion production at the next stage of research studied the strain VGTU-02’s ability to attach to carriers: vegetable waste of agricultural production-sunflower husk, wheat bran, buckwheat husk or spent active sludge, passed through the procedure of low-temperature pyrolysis-carbonizate.

Plant material was ground and classified on the shakers and selected fraction with a particle diameter less than 0.1 mm. The crushed plant components were sterilized in the hot-cupboard at the temperature...
160°C for 1 h. Three fractions of carbonizate took with the diameter less than 0.6 mm, 0.6-1 mm and 1 mm.

Immobilization of microorganisms on plant carriers and carbonizate was carried out by static adsorption. The suspension of daily culture microorganisms (30 ml) prepared according to the turbidity standard for 10 units in phosphate buffer (pH=7) mixed with 1 g of the carrier on a magnetic stirrer at speed (100 vol./min) and the temperature 30 °C. The initial concentration of microorganisms in suspension was determined by seeding on Petri dishes with MPA. At the end of the mixing time, sedimentation was carried out to precipitate the carrier particles and seeding of the culture liquid at MPA to determine the final concentration of microorganisms in the suspension. The number of immobilized bacterial cells was calculated in percent of the initial concentration of the suspension [9].

During the grown in depth, the culture liquid was diluted with phosphate buffer to a concentration of $10^9$ microbial cells / ml and the same ratio with the carrier was used. The results of the experiment of the carrier selection for the microbial immobilization during stationary and deep cultivation were presented in table 3.

**Table 3. The results of experiments of immobilization efficiency of VGTU-02 on the corp wastes.**

| Sorbent               | Immobilization efficiency, % |
|-----------------------|------------------------------|
|                       | Surface cultivation | Deep cultivation |
| Sunflower husk        | 81,73                      | 83,17            |
| Wheat bran            | 98,2                        | 65,9             |
| The buckwheat husk    | 97,47                       | 8,57             |

The results presented in table 3 indicated about the efficiency of VGTU-02 immobilization on sunflower husks under conditions of deep cultivation and stationary cultivation were more stable in comparison with wheat bran and buckwheat husks.

The results of the VGTU-02 immobilization on different fractions of carbonizate from spent activated sludge were presented in table 4.

**Table 4. Immobilization of the strain VGTU-02 with carbonizate as a matrix.**

| Fraction size f, mm | Immobilization efficiency, % |
|---------------------|-----------------------------|
| f > 1,0             | 98,05 ±2,845                |
| 1,0> f > 0,6 mm     | 93,2 ±4,808                  |
| f < 0,6 mm          | 92,7 ±6,47                   |

Analyzing the data obtained, it can be concluded the most effective for immobilization was using of the carbonizate fraction for 1-0.6 mm [10].

In order to assess the efficiency of the developed biosorbent based on carbonizate for purification of model media from oil, the initial and final content of petroleum products in the media were determined by fluorimetric method (PND F 14.1:2:4.128-98) on the fluid analyzer "fluorate-02-2M".

Model systems were cultured for 10 days, based on the visual assessment of the model systems’ liquid state with using data on the bacterial culture growth dynamics used. Cultivation was carried out at 30 °C in the thermostat [17]. To improve the contact of the sorbent and biosorbent with the pollutant stirring with a magnetic stirrer was additionally used.

The results of residual oil content determination in model systems were presented in table 5.

The data given in table 5 showed the cultivation for 10 days gave the degree of purification from oil with the help of attached microorganisms is significantly higher than using free cells of VGTU-02.

In the course of the conducted studies, screening of bacterial oil destructors was carried out: the oil-oxidizing activity of 19 bacterial strains isolated from various environmental objects was studied. Founded the strain named as VGTU-02 had the maximum activity, the hydrophobicity index and emulsifying activity were determined for it.
Table 5. The results of the experiment of the purification process from oil by the model solutions.

| The model solution composition                                      | Initial oil concentration ± Δ, g/dm³ | Final oil concentration ± Δ, g/dm³ | Purification efficiency from oil, % |
|--------------------------------------------------------------------|--------------------------------------|-------------------------------------|------------------------------------|
| Mineral nutrient medium, oil                                       | 22092 ± 5523                         | 22092 ± 5523                        | -                                  |
| Mineral nutrient medium, oil, carbonizate.                         | 22092 ± 5523                         | 4092 ± 1023                         | 81,47                              |
| Mineral nutrient medium, oil, sunflower husk                      | 22092 ± 5523                         | 956 ± 70                            | 95,67                              |
| Mineral nutrient medium, oil, sunflower husk biosorbent            | 22092 ± 5523                         | 130 ± 36                            | 99,41                              |
| Mineral nutrient medium, oil, carbonizate biosorbent               | 22092 ± 5523                         | 339 ± 85                            | 98,46                              |
| Mineral nutrient medium, oil, microbial suspension of VGTU-02     | 22092 ± 5523                         | 6818 ± 1703                         | 69,14                              |

The ability of VGTU-02 to immobilize on crop waste and activated sludge carbonizate in order to obtain biosorbents was investigated. It was established the most effective use of sunflower seed shells (d< 0.1mm) and hydrocarbon (f>1 mm) as a matrix for the attachment of the studied microorganisms. Developed the method of obtaining biosorbents. Shown the cleaning efficiency model systems from oil was 99.41% with sunflower husks and of 98.45% with carbonizate. The application of the promising biosorbents for purification of sewage, past the main stages of mechanical and physical-chemical purification from oil products can be perspective.

References

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