Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Gertsen & al. / Mor. J. Chem. 8 N°2 (2020) 392-399

Utilization of hexadecane by biocomposition based on humic acids of peats and oil-degrading microorganisms of the genus Rhodococcus in aqueous media

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Received 09 Sept 2019, Revised 12 April 2020, Accepted 19 April 2020

Abstract

The concentration of humic acids of peats from the Tula region, equal to 50 mg/l, linking the maximum amount of hexadecane as a model object of the light fraction of oil was revealed. The dependence of the utilizing ability of oil-degrading microorganisms Rhodococcus erythropolis S67 and Rhodococcus erythropolis X5 was studied. It is established that the microorganisms of this strain are the most capable of utilizing hexadecane in a solution of not more than 5% by volume. Humic acids of reed fen peat and sphagnum high-moor peat are capable of binding up to 50% of hexadecane with its content in a solution of 2% by volume. The maximum binding of the model alkane was demonstrated by a biocomposition consisting of humic acids of reed fen peat at 50 mg/l and microorganisms of Rhodococcus erythropolis S67. This biocomposition is capable for utilizing up to 95% of hexadecane with its content in the solution of 2% by volume and up to 35% at 20% vol, which serves as the basis for creating on its basis an effective chemical and biological sorbent for cleaning soil and water environments from oil pollution..

Key words: *humic acids, oil-degrading microorganisms, biodegradation, hexadecane, biocomposition.*

1.Introduction

The use of well-known mechanical and physicochemical methods for the remediation of oil-contaminated areas has several disadvantages: the inability to clear the territory of petroleum products to the level of maximum permissible concentration and the possibility of secondary environmental pollution during their application. Existing methods for cleaning soils from petroleum products are aimed at the degradation of one pollutant and are ineffective in the joint contamination of soil with organic and inorganic pollutants [1]. It is currently important to find effective and environmentally friendly methods for cleaning oil pollution, which include bioremediation using immobilized Rhodococcus and humic acids[2]. Today, microorganisms of the genus Rhodococcus, capable for biodegradation of a wide range of hydrophobic substrates due to the presence of enzyme systems, are most often used to create biologics [3]. Bacteria of this strain predominate in hydrocarbon-oxidizing microbiocenes of hydrocarbon-contaminated soils [4]. This determines the prospects for the use of Rhodococcus as the basis of new oil-oxidizing preparations with enhanced properties. The use of biological products based on microorganisms and humic acids for the disposal of oil spills in soil media allows using to the maximum extent possible of all existing natural self-purification mechanisms. The introduction of drugs helps to reduce the toxicity of hydrocarbons, accelerate the processes of their biochemical decomposition and restore soil fertility and vegetation on the contaminated area [1, 5, 6, 7]. The purpose of this work was to study the degree of utilization of hydrocarbon-hexadecane by humic acids of peats of various genesis and microorganisms by oil destructors *Rhodococcus erythropolis S67* and *Rhodococcus erythropolis X5* with their joint presence and development of a biocomposition on their basis.

2.Materials and methods

The objects of this research were humic acids (HAs) of peats: reed fen peat (RFP), black alder fen (BFP), sphagnum transition peat (STP) and sphagnum high-moor peat (SHP) [8] isolated from peats by the method described previously [9, 10]. The strains of bacteria *Rhodococcus erythropolis S67*, *Rhodococcus erythropolis X5*are obtained from the plasmid laboratory of the Institute of Biochemistry and Physiology of Microorganisms named after GK Scriabin RAS (Pushchino) [3, 11]. Gas chromatography was used to determine the binding capacity of HAsin the relation to hexadecane by a gas chromatograph with a flame ionization detector Kristall 5000.2 (SKB Khromatek, Russia).

Solutions of HAs were prepared by dissolving the exact weights of the preparations in 0.1 M NaOH, followed by dilution with sodium nitrate. Neutral pH values of the solutions were obtained by adding 0.05 M HN03 or 0.05 M NaOH using anion 4154 pH meter. The experiment was carried out in Evans liquid mineral medium [12] with the addition of various concentrations of HAs (25-100 mg / l) and hexadecane (2 -20% by volume). The residual amount of hexadecane was determined after 3 days. Peaks were recorded in the retention time region corresponding to the components of the calibration mixture. According to the obtained chromatograms, the concentration of hexadecane was calculated using the calibration dependence (the dependence of the peak area on the concentration of hexadecane).

3.Results and discussion

The concentration of HAs capable of binding the maximum amount of hexadecane was determined. For this, the concentration of humic acids was varied from 25 to 100 mg / l and the concentration of hexadecane 2% vol. remained unchanged (Fig. 1). The binding capacity of HAs was calculated by the formula 1:

$$Q = \frac{C_0 - C}{C_0} \cdot 100 \quad (1)$$

C_0- initial concentration of hexadecane in solution,%

C-the remaining concentration of hexadecane in solution,%

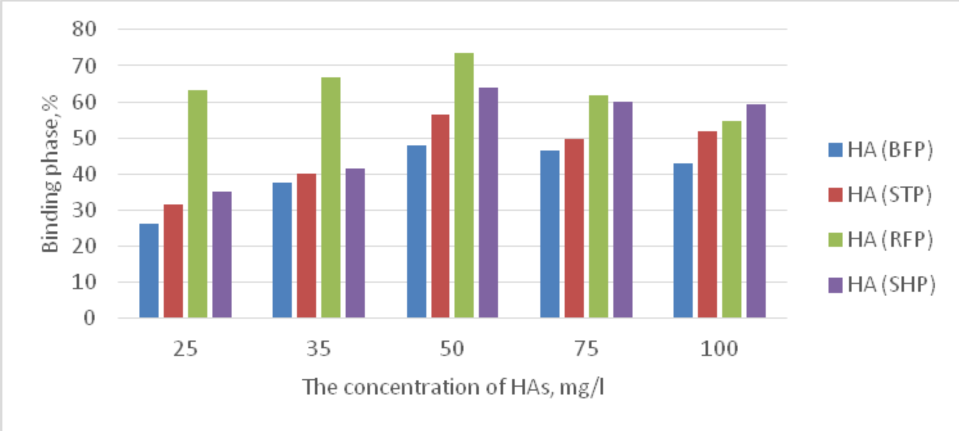

Fig. 1 Binding of Hexadecane with Humic Acids

The maximum binding ability was demonstrated by HAs (RFP) and HAs (SHP) 74 and 64%, respectively, and the most optimal concentration of HAs for binding hexadecane is 50 mg/l. HAs (BFP) have the lowest binding ability with respect to hexadecane all over the range of the analyzed concentration. It is known [5, 13, 14], HAs are complex aggregates of molecules consisting of the aromatic core and the hydrophobic peripheral part. The IR spectra of HAs of peats [15] prove the development of their peripheral part represented by aliphatic fragments, so the valence vibrations in the aliphatic -CH_3 and -CH_2 groups are quite intense and decrease in the order HAs (SHP)> HAs (RFP)> HAs (STP)> HAs (BFP) [16]. Inactivation of hexadecane by humic substances in solution is carried out through the hydrophobic interactions between the developed peripheral part of the hydrophobic fragments of humic acids and the n-alkane molecule (hexadecane) (Fig. 2).

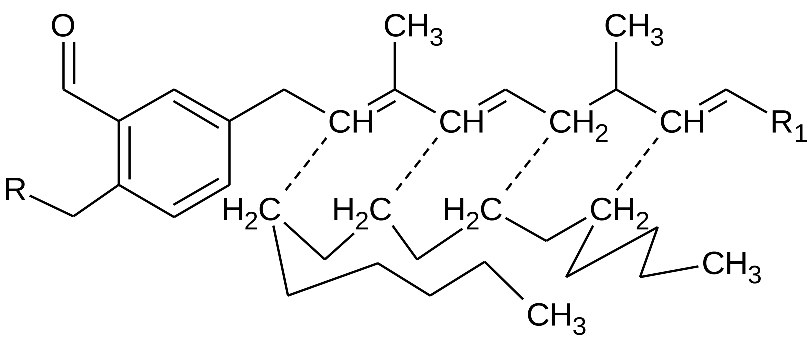

Fig. 2 The interaction of the hydrophobic fragment of humic acids with hexadecane

The binding of dissolved humic acids of petroleum hydrocarbons is similar to the process of removing them from the freely-dissolved state. This process can lead to an increase in the water solubility of petroleum hydrocarbons, a decrease in their bioaccumulation, and an acceleration of photolysis [5]. The task of the next stage of this research was to identify the maximum binding ability of the light fraction of oil, hexadecane, humic acids and microorganisms with their joint presence. The work included two stages: in the first stage, the maximum amount of hexadecane from 2 to 20% by volume in the solution was determined, which can be recycled by oil-degrading microorganisms (Fig. 3); in the second stage, the binding capacity of HAs at a concentration of 50 mg/l was determined relative to hexadecane, the content of which in the solution correlated 2–20% v (Fig. 4). The binding capacity of humic acids with respect to hexadecane was calculated by the formula 1.

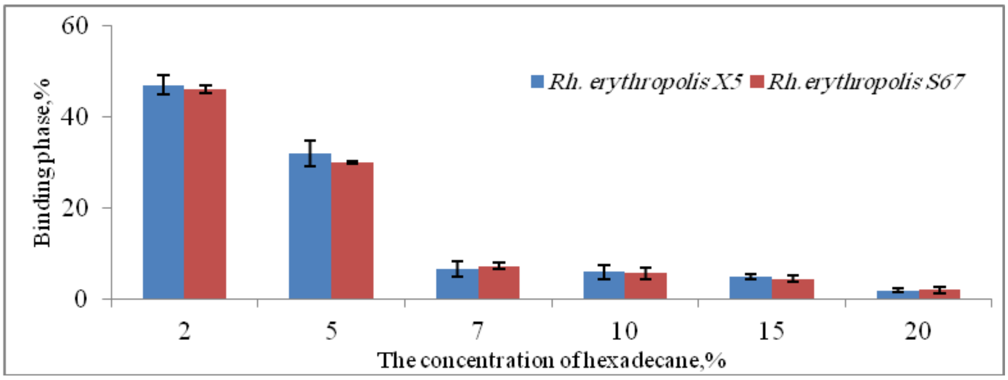

Fig. 3 Phase of hexadecane disposed of by oil-degrading microorganisms.

Rhodococcus erythropolis S67 and *X5* microorganisms are capable of utilizing the maximum amount of hexadecane (30–47%) with its content in the solution of 2–5% by volume (Fig. 3). In order to obtain the most complete information about the ability of these microorganisms to utilize hexadecane, they increased its content in the solution to 20% by volume. There is no information in the literature about the possibility of hexadecane utilization by *Rh. erythropolis S67* and *X5* with its content in the solution of more than 5% by volume [4, 17]. The experiment showed that the tendency to decompose hexadecane with its increased content in the solution by oil-producing microorganisms persists: the proportion of binding of hexadecane by Rh. erythropolis S67 and X5 are also similar (Fig. 3). The ability of bacteria to dispose of hexadecane is reduced by 4 times when the content of hexadecane in the solution is 7% - 10% by volume relative to 5% vol. Increasing the amount of hexadecane in the solution to 20% vol. reduces its consumption by microorganisms of oil destructors by 23 times compared with 2% vol. Such a strong decrease was due to the stress of microorganisms and the loss of cell viability. Thus, for a more complete utilization of hexadecane by oil-degrading microorganisms *Rh. erythropolis S67* and *X5*, its content in the solution should not exceed 5% vol., which is the residual amount of oil and water pollution. The binding capacity of humic acids of peats in the relation to hexadecane, whose content in the solution correlated from 2 to 20% by volume (Fig. 4), was estimated. The residual amount of hexadecane in the solution was determined by gas-liquid chromatography.

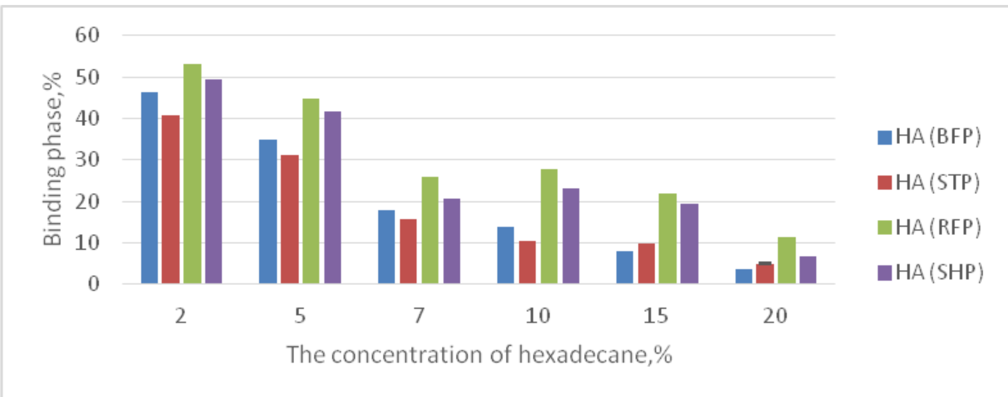

Fig. 4 The binding capacity of humic acids of peats in the relation to hexadecane

The maximum binding capacity of HAs is observed when the content of hexadecane in the solution is 2-5% by volume (Fig. 4). HAs (RFP) and HAs (SHP) showed the maximum binding ability of 53% and 49%, respectively, when the content of alkane in solution was 2% vol. The binding capacity of humic acids decreases by 7–11% with an increase in the content of hexadecane in the solution to 5% by volume. A further increase in the content of hexadecane to 7–15% by volume in solution leads to a decrease in the proportion of its binding in 2 times in all analyzed HAs. The minimum

binding of hexadecane is marked by humic acids with its maximum content of 20% by volume in 5 - 12 times relative to 2% vol. It is established that the binding capacity of both microorganisms and HAs decreases with increasing concentration of hexadecane in the solution. Therefore, the optimal content of hexadecane for further work is 2 and 5%. Determination of the utilization ability of a biocomposition based on humic acids of peat and oil-degrading microorganisms of the genus *Rh. erythropolis S67* and *X5* were performed in solution. 10 ml of Evans medium were added to the tubes at a concentration of 50 mg/l, microorganisms were inoculated with an amount of seed dose of 105-106 CFU / ml and hexadecane was taken in an amount of 2 to 20% by volume (Fig. 5).

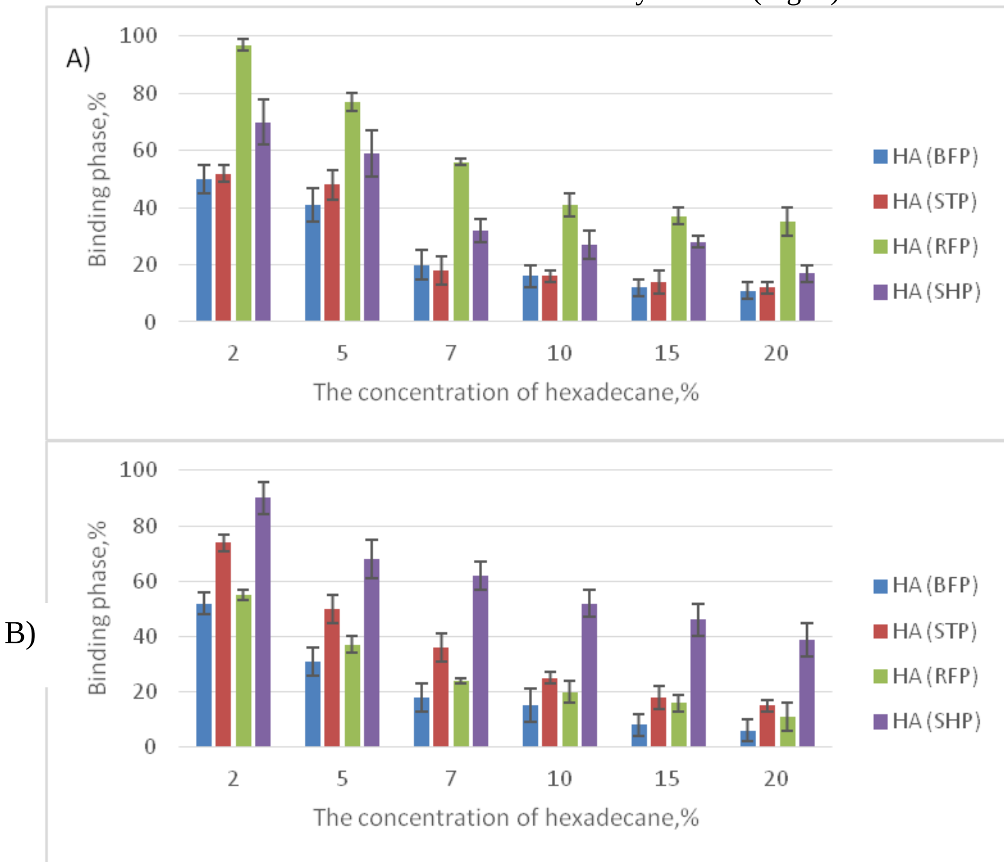

Fig. 5 The binding capacity in the relation to various concentrations of hexadecane A) of *Rh. erythropolis S67* and humic acids B) *Rh. erythropolis X5* and humic acids

Biocomposition on the basis of humic acids of peats and microorganisms *Rh. erythropolis S67* and *X5* increases the utilization of hexadecane over the entire range of its content in a solution of 2–20% by volume. The proportion of binding of hexadecane in the joint presence of humic acids and microorganisms in solution is in 1.1-1.8 times higher than in the presence of only HAs and in 1.1-2 times higher in the presence of microorganisms. The biocomposition on the basis of HAs (RFP) and *Rh. erythropolis S67* has the maximum binding capacity. The proportion of binding of hexadecane reaches 95% with its content in the solution of 2% by volume. It should be noted an increase in 1.5 times the binding capacity of microorganisms with HAs (SHP) than inthe solution that does not contain HAs. The binding capacity of the biocomposition on the basis of HAs (BFP) and HAs(STP) and microorganisms *Rh. erythropolis S67* increases by 4-11% relative to hexadecane. The joint presence in the solution of humic acids and microorganisms *Rh. erythropolis X5* also increases the binding capacity in the relation to hexadecane. The maximum binding capacity was demonstrated by HAs (SHP) and HA (STP) of 90% and 74%, respectively, with a hexadecane content in the solution of 2% by volume. The binding capacity of the biocomposition decreases with increasing content of alkane in the system up to 20% vol, but the proportion of hexadecane binding is higher than in systems containing only HAs or

microorganisms. Many authors [5-6, 18-20] argue about the importance of the sequence of introducing HAs into a system consisting of a toxicant and oil-degrading microorganisms. The positive effect is higher, under the adding HAs to the system prior to the introduction of the toxicant since it has time to form a layer of HAs molecules, which forms an active strainer on the cell surface that can bind toxicants.

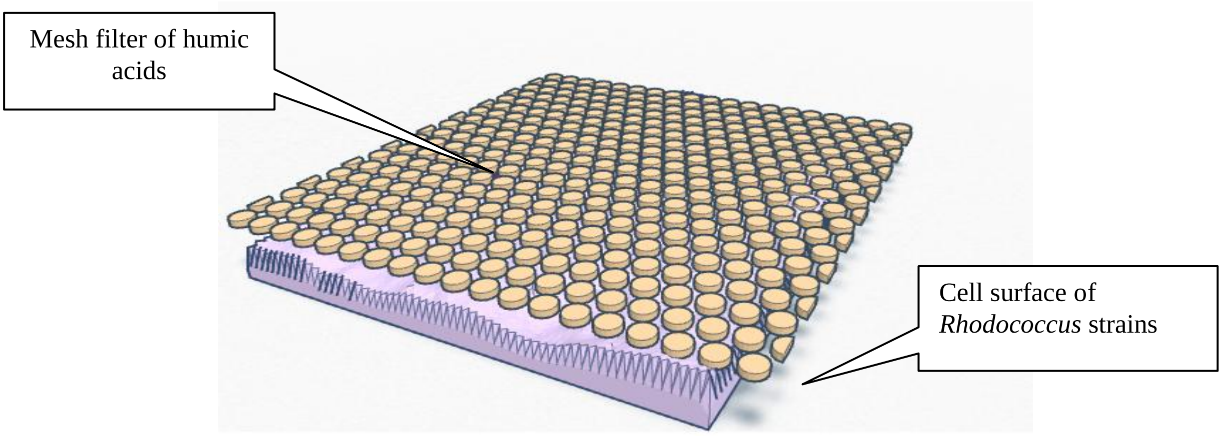

Fig. 6 Mesh filter of humic acids on the cell surface of microorganisms

Humic acids are capable of entering into hydrophobic interactions with hexadecane; microorganisms use alkane as a substrate [16]. Microorganisms *Rh. erythropolis S67* and HAs (RFP) in the joint presence dispose of the maximum amount of hexadecane in the solution relative to other HAs and the microorganism *Rh. erythropolis X5*. In previous experiments, the optimal content of hexadecane in the system (2 and 5% vol.) was determined for its maximum utilization by humic acids and oil-degrading microorganisms. It was decided to compare how much the binding capacity of the system containing HAs and microorganisms relative to a one-component system increases, as according to the results of gas chromatographic analysis, this system utilizes the largest amount of hexadecane in the solution (Fig. 7)

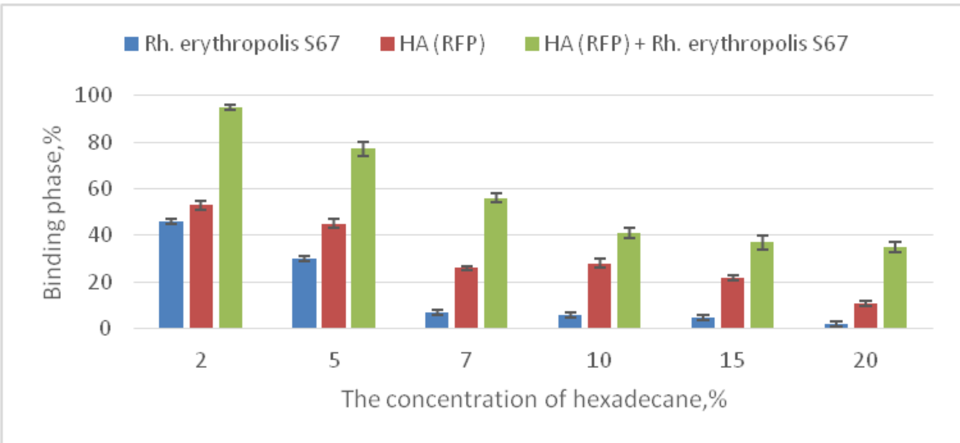

Fig. 7 Binding capacity of microorganisms *Rh. erythropolis S67* and humic acids of reed fen peat in the relation to various concentrations of hexadecane

It was established that the proportion of hexadecane binding increases from 49% with a hexadecane content of 2% by volume up to 33% with a hexadecane content of 20% by volume with the joint presence of HAs and microorganisms, whereas in the presence of only microorganisms hexadecane is practically not observed, and in the presence of HAs only, the binding fraction is only 11%. Utilization of oil contaminations obtained by the biocomposition can proceed in a certain way: HAs molecules capture the heavy and light fractions of oil with their hydrophobic sections and bind

them into non-toxic complexes, with the heavy fractions of oil being bound to the aromatic fragments of HAs, and the light ones from the peripheral part of the hydrophobic fragments of hydrocarbon radicals , the addition of oil-degrading microorganisms is capable of utilizing unreacted oil products from the HAs, as well as oxidizing the available organic pollutants on the surface of humic acids. Thus, the system based on humic acids of reed fen peat and microorganisms *Rhodococcus S67* can be promising to create the biocomposition for utilization of the light oil fraction. In order to identify the optimal concentration of humic acids of reed fen peat when the biocomposition is creating for utilization of the light fraction of oil, the binding capacity of the system in which the HA s concentration varied from 25 to 200 mg/l with a constant content of hexadecane 2% by volume was studied. (Fig.8).

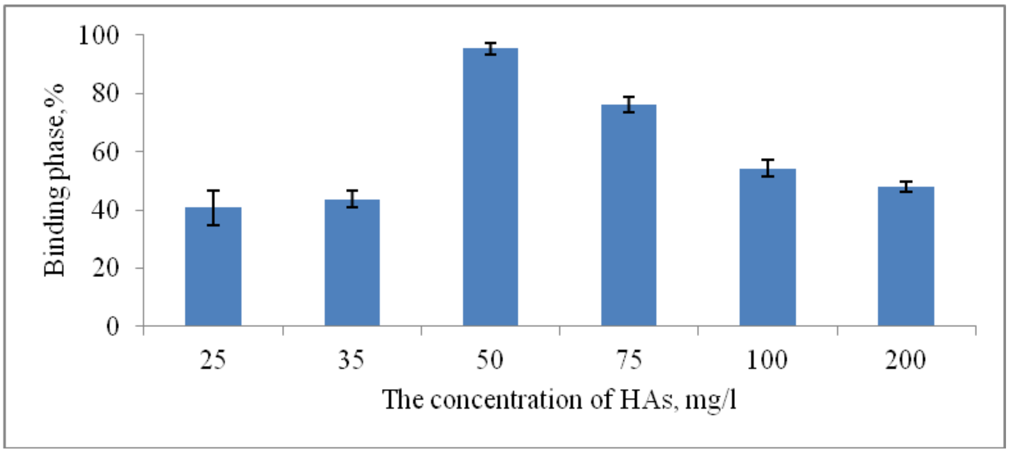

Fig. 8 Binding capacity of microorganisms *Rh. erythropolis S67* and humic acids of reed fen peat in the relation to hexadecane

The maximum proportion of hexadecane binding by the system of HAs-microorganisms, with a concentration of humic acids 50 mg/l. At this concentration, the maximum stimulating effect on the growth of microorganisms of the genus *Rhodococcus* is observed [21]. The resulting monolayer of humic acid molecules on the cell surface is optimal. On the one hand, it does not prevent the penetration of the substrate (hexadecane) through an openwork filter consisting of HAs molecules, but it still minimizes the passage of excess hexadecane into the cell. In addition, the peripheral parts of humic acids themselves act as a nutrient source for microorganisms [18].

4.Conclusion

It was established that the microorganisms of the *Rhodococcus* strain maximally utilize hexadecane in the solution with its content not exceeding 5% by volume. The maximum binding of hexadecane was demonstrated by a complex consisting of humic acids of reed fen peat at the concentration 50 mg/l and microorganisms of *Rhodococcus erythropolis S67*. This system is capable of utilizing up to 95% of hexadecane with its content in the solution of 2% by volume and up to 35% with its content of 20% by volume in the solution.

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