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# Study of the hydrocarbon-oxidizing activity of bacteria of the genera *Pseudomonas* and *Rhodococcus*

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**Abstract**

The selective activity of hydrocarbon-oxidizing microorganisms with regard to the degradation of alkanes, cycloalkanes, arenes was presented. The hydrocarbon-oxidizing activity of microorganisms of the genera Rhodococcus and Pseudomonas such as heptane, cyclohexane, toluene within the hydrocarbons destruction was determined. The growth rate for various hydrocarbons differs. Thus, the average specific growth rate of hydrocarbon-oxidizing microorganism (HOM) of the genus Rhodococcus is twice more than these substrates.



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*Keywords:* hydrocarbon-oxidizing microorganisms (HOMs); bacteria of the genus Pseudomonas; bacteria of the genus Rhodococcus; biokinetics.

## 1. Introduction

In recent decades, the extensive contamination of the environment by petroleum products and polymer wastes occurs as a result of human activities[1-3]. It has already been determined that the microorganisms, capable of utilizing hydrocarbons, are widespread and can be extracted from any field, forest and meadow soils. The first studies on the microbial oxidation of hydrocarbons of various classes began in the 70s of the XX century[4-5]. The list of

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microorganisms capable of hydrocarbons oxidation is presented in the reviews by E.P. Rozanov[6-7], J.W. Foster[8-9], M.J. Klug, A.J. Markovetz[10], and others[5, 10-11].

The ability to destruct petroleum hydrocarbons is inherent to microorganisms presented by different taxonomic groups. These include various species of micromycetes, yeasts and bacteria[4,6]. The most active oil destructors are found among bacteria of the following genera: *Pseudomonas, Arthrobacter, Rhodococcus, Acinetobacter, Flavobacterium, Corynebacterium, Xanthomonas, Alcaligenes, Nocardia, Brevibacterium, Mycobacterium, Beijerinkia, Bacillus, Enterobacteriaceae, Klebsiella, Micrococcus, Sphaerotilus*[4,7]. They are characterized by their ability to destruct a wide spectrum of hydrocarbons, including aromatics, as well as to have a high growth rate. Therefore, they are of great practical interest.

Thus, the most perspective methods to study the degradation of oil hydrocarbons are the biological methods. They are based on the natural process of hydrocarbons destruction. Hydrocarbon-oxidizing microorganisms (HOMs) are greatly included in this process[12-15]. By their nature, the microbial oxidation processes are enzymatic reactions occurring in multienzyme systems[16-18]. The autocatalytic process promotes increasing in microorganisms biomass and the total concentration of enzymes in the system with the population development as well. Therefore, during the development of new products containing strains-destructors, the influence of taxonomy on biokinetics is being discussed[6,19-21]. The current study is based on the chemical and kinetic modeling of microbial growth in the hydrocarbons oxidation. It has a significant and practical value. In this regard, the study of the culture growth rate and its hydrocarbon-oxidizing activity in the destruction of hydrocarbons is very significant. The purpose of the work was to study the hydrocarbon-oxidizing activity of bacteria of the genera *Pseudomonas* and *Rhodococcus* of certain hydrocarbons.

## 2. Experimental

### *2.2. Materials and methods*

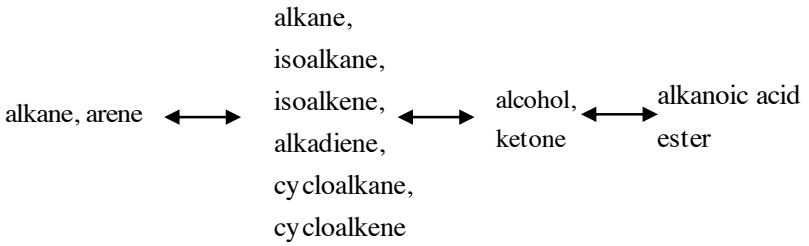
The hydrocarbon-oxidizing microorganisms of the genera *Pseudomonas* and *Rhodococcus* were examined. The samples for analysis were isolated from the soil and water reservoirs of Zapadno-Katylginskoe field in Strezhevoy (Tomsk Region) by the company "Ecoil" (Tomsk) during the remediation of oil-contaminated lands[22]. The taxonomy of pure isolated cultures was determined on the basis of the sequence analysis of nucleotides in 16S pRNA, their modification and primary bioinformatical analysis (laboratory "Genotech" Ltd., Moscow). To study the activity of hydrocarbon-oxidizing bacteria, the cultures (48 h) were inoculated into liquid mineral Adkins' medium with the addition of heptane (puriss, TS 2631-023-4449317-99), cyclohexane (puriss p.a., TS 2631-029-44493179-99), toluene (puriss p.a., AUSS 5789-78) in the amount of 5% as a sole carbon and energy source. The cultivation was carried out at a speed of 85-90 rev/min and a temperature of 27-32° C within 152 hours. Every 24 hours the samples were taken for the microbiological and gas chromatographic analyses. During the microbiological analysis the biomass growth was determined. During the chromatographic analysis the residual hydrocarbons in the course of biodegradation of heptane, cyclohexane, toluene were used. The number and growth dynamics of hydrocarbon-oxidizing microorganisms in the biodestruction process were determined by the McCready[23] and Koch's [23] methods, using the culture media of the meat-and-peptone broth (MPB) and meat-and-peptone agar (MPA) as well. Hydrocarbons were extracted from the water surface by carbon tetrachloride, and the content of residual hydrocarbons was analyzed by gas chromatography.

## 3. Results and Discussion

Hydrocarbon-oxidizing microorganisms of the genera *Rhodococcus* and *Pseudomonas* are widely used in biological products. It indicates their ability to metabolize harmful environmental contaminants such as toluene, naphthalene and herbicides[16]. According to the relevant research data[18] the molecules of alkanes, isoalkanes and naphthene first undergo the destruction as a result of the unsaturated structure of carbon chain. Their destruction occurs due to the substance biotransformation of hydrocarbon-oxidizing microorganisms under the influence of the reaction system. Therefore, isomerases catalyze the structural conversion of isomers and dehydrogenases catalyze the proton transfer and hydrogen abstraction. During further destruction stages the oxygen-containing organic

compounds such as ethers and alcohols are formed in the system[18].

In total the hydrocarbon biotransformation process can be schematically presented as follows:




During the study of hydrocarbon-oxidizing microorganisms' activity of the genera *Pseudomonas* and *Rhodosochkus* in liquid mineral Adkins' medium with heptane, cyclohexane, toluene, the microbiological analysis according to the McCready's method was used. This analysis revealed that each culture individually transformed the studied substrates. The results of changes in the number of microorganisms in the course of biodegradation are presented in figures 1-4.

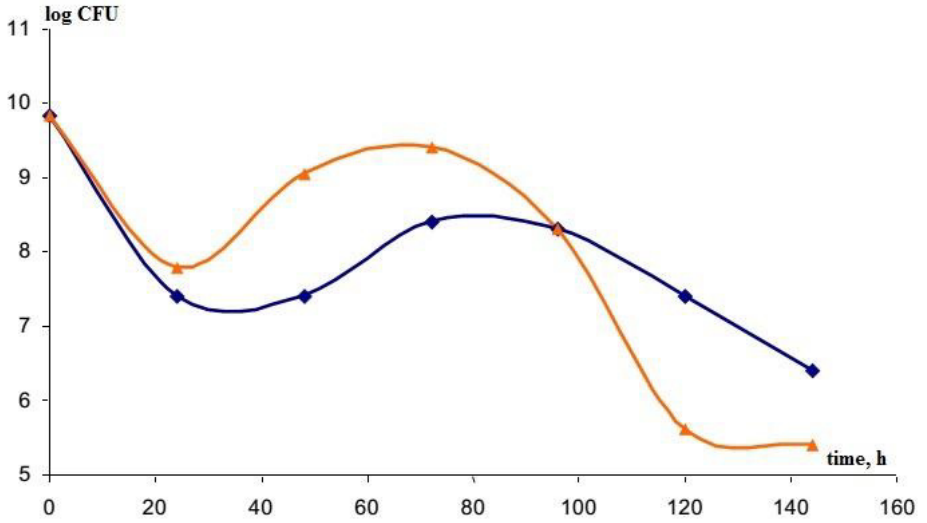



Fig. 1. Changes in the number of HOMs of the genus *Pseudomonas* during the heptane and cyclohexane destruction.

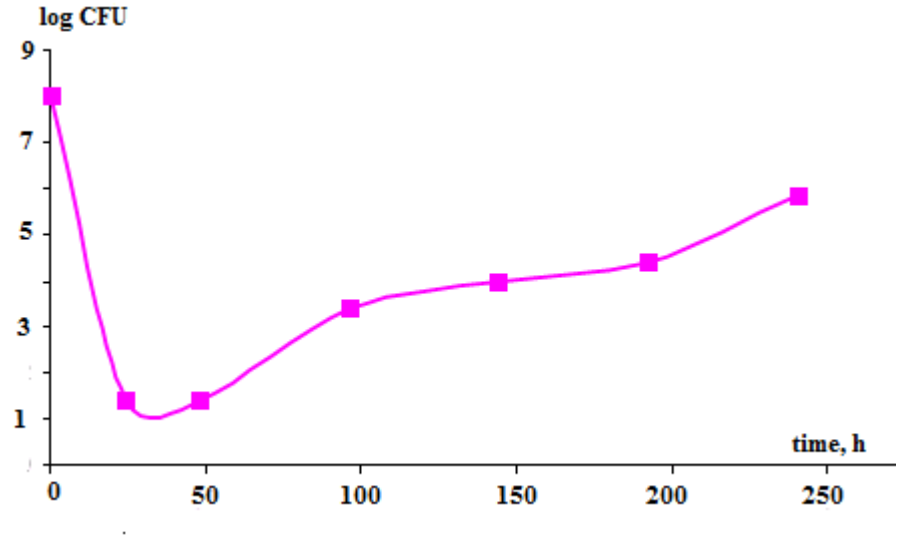



Fig. 2. Changes in the number of HOMs of the genus *Pseudomonas* during the toluene destruction.

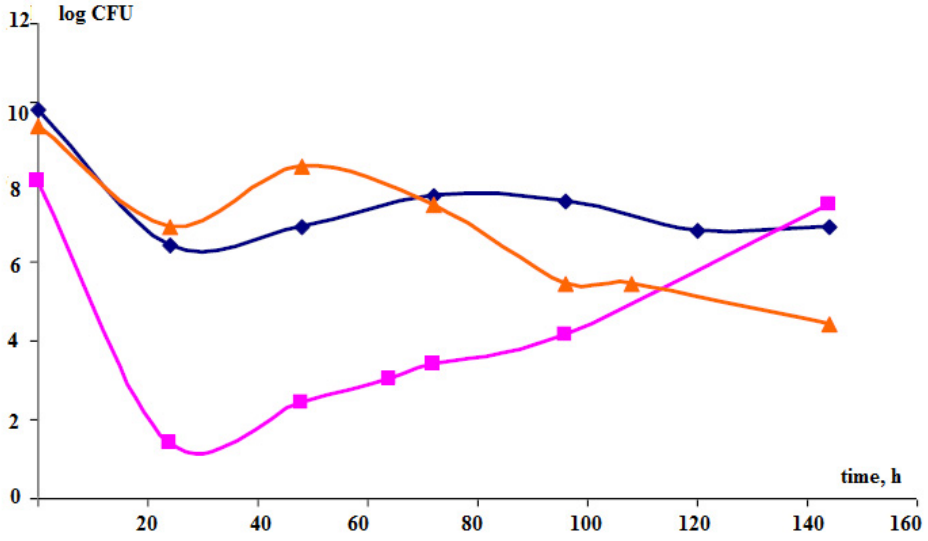



Fig. 3. Changes in the number of HOMs of the genus *Rhodococcus* during the heptane , toluene and cyclohexane destruction.

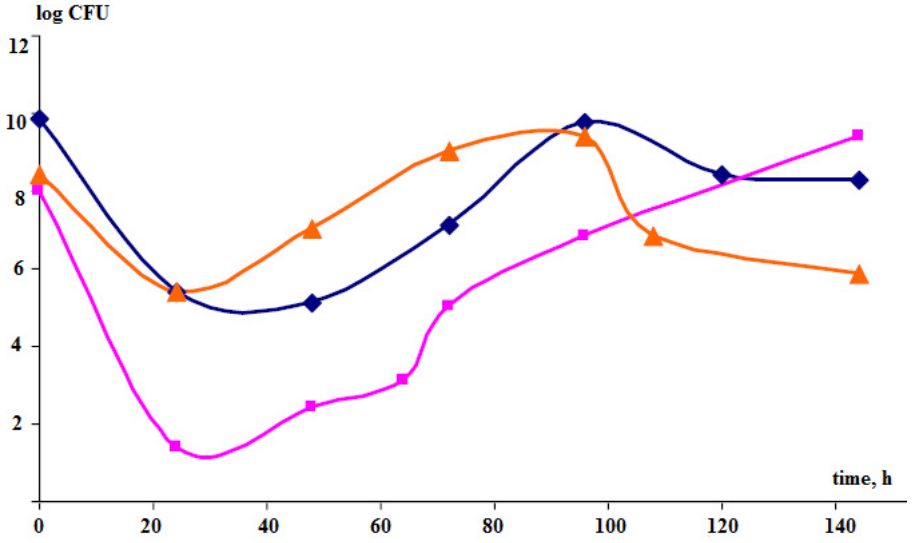



Fig. 4. Total change in the number of HOMs of the genera *Rhodococcus* and *Pseudomonas* during the heptane , toluene and cyclohexane destruction.

According to the figures, the current kinetic curves are complex. There are some phases of the culture development: induction, exponential, stationary, and death.

According to Figure 1, the phase of physiological adaptation of a culture to the medium lasts 36 hours. It is mainly in this phase the destruction of alkanes and isoalkanes occurs. Oxygenates and alkenes are formed under the influence of the enzymatic complex of dehydrogenase. Further, within the next 36 hours (36-72 hours) more complex products (arenas, esters) are formed in the system. This stage is characterized by the maximum rate of cell division (exponential growth phase). The period of 72-144 hours corresponds to the stationary phase, in which the availability of essential nutrition substances becomes limited. The balance between the cell growth and division, as well as the process of cell death, is established. After 96 hours (when the arene concentration reaches 80-100%) the phase of death begins as a result of the accumulation of toxic aromatic metabolites.

The specificity of each culture to a substrate can be analyzed on the basis of the dependencies summarized in Table 1.

Table 1. Periods of the kinetic growth phases for HOMs within the hydrocarbon destruction.

| Cultures | *Pseudomonas* | | | *Rhodococcus* | | | *Rhodococcus, Pseudomonas* | | |
|---|---|---|---|---|---|---|---|---|---|
| Hydrocarbons | Phases, h | | | | | | | | |
| | I | E | S | I | E | S | I | E | S |
| heptane | 0–36 | 36–72 | 72-96 | 0-32 | 32–72 | 72–90 | 0–48 | 48–96 | 96–120 |
| toluene | 0–36 | 28–60 | 48-78 | 0–28 | 32–76 | 46–60 | 0–30 | 30–84 | 84–98 |
| cyclohexane | 0–48 | 48-240 | – | 0–30 | 30–144 | – | 0–32 | 32–144 | – |

where, I – induction phase, E – exponential phase, S – stationary phase.

The culture of the genus *Rhodococcus* has the lowest induction period. The induction period is characterized by the cell metabolism restructuring, the synthesis of enzymes that are specific to using the substrates and protein biosynthesis activation as well. Bacteria of the genus *Pseudomonas* demonstrate a high utilization rate of heptanes. The exponential phase lasts 36 hours. It should be noted that the phase of death starts for the monocultures in about 2-3 days, e.g. for heptanes – in 3 days and for cyclohexane – in 2 days. In the case of the destruction of unsaturated hydrocarbons of two genera, depending on the substrate, the exponential phase lasts 48 h, 54 h, and 112 h. It is probably connected with the formation of a wide range of metabolites or their complex interaction.

The specific growth rate and generation time for monocultures (Table 2) were calculated with regard to the obtained dependences.

The average specific growth rate $K$ for a certain period of time ($t_1$-$t_0$) is calculated by the equation 1:

$$K = \frac{2.303(\log m_1 - \log m_0)}{t_1 - t_0} \quad (1)$$

where, $m_0$ and $m_1$ – initial and final mass of cells.

Generation time ($T_g$) – the time required to complete the cell cycle. It is inversely related to the growth rate (Equation 2):

$$T_g = \frac{0.693}{K} \quad (2)$$

Table 2. Specific rate and time generating for HOMs within the heptane and cyclohexane destruction.

| Culture | heptane | | cyclohexane | |
|---|---|---|---|---|
| | K, $h^{-1}$ | g, h | K, $h^{-1}$ | g, h |
| Rhodococcus | 0.22 | 3.1 | 0.21 | 3.3 |
| Pseudomonas | 0.06 | 10.8 | 0.11 | 5.9 |

The obtained data lead to the conclusion that the average specific growth rate for HOMs of the genus *Rhodococcus* is twice more with regard to the data of substrates. The time interval between two consecutive cell divisions is 3 hours.

## 4. Conclusions

Thus, the selective activity of hydrocarbon-oxidizing microorganisms to the destruction of alkanes, cycloalkanes, arenes was shown. The rate constants and regeneration time of microorganisms of the genera *Rhodococcus* and *Pseudomonas* during the biodestruction of heptanes, cyclohexane, toluene were determined. It was established that the growth rate for each culture in various hydrocarbons is specific and individual. The obtained data during the experiment will be used in the development of new biological products, containing the studied strains of hydrocarbon-oxidizing microorganisms, targeted to specific physical-chemical parameters of pollution.

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