


Elimination of Oil Pollution

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Keywords: Industry, oil refining, petrochemistry, nature pollution, biological cleaning methods, oil decomposition.

Abstract: In recent years, there has been an increase in the industry in the field of oil refining and petrochemistry, which contributes to an increase in the volume of pollution entering the natural environment; the produced petroleum products, as well as, in the first place, the crude oil itself, are dangerous pollution. Biological methods are considered to be the most promising methods of cleaning from oil pollution. They are based on the natural mechanisms of oil decomposition. This is what will be discussed in this scientific work.

1 INTRODUCTION

When it comes to cleaning up oil pollution, oil-oxidizing bacteria play a key role in this process. Coryneform bacteria are the most effective group of microorganisms used in the destruction of oilfield waste and the elimination of the consequences of spills and oil pollution. They do not lose their potential even at high concentrations of contaminants. Also, coryneforms are widely represented in various fields of biotechnology (Bendinger, 1992).

At present, bioremediation of soil and water from oil pollution, based on the use of microorganisms-destroyers of oil hydrocarbons, shows the best results. Thus, the high biotechnological and biodegradative potential indicates the relevance of research in the study of this genus of bacteria.

In the past, bacterial cultures such as micrococci, pseudomonads, bacilli, etc. were used. However, these groups of microorganisms are not able to survive in an anhydrous environment containing only oil hydrocarbons.

In this connection, their biodegradative activity is manifested only superficially. To the number of disadvantages already listed, there is also a limited range of hydrocarbons that these bacteria are able to assimilate, which is generally expressed in the lower efficiency of cleaning measures carried out with their use in comparison with coryneform bacteria.

Coryneform bacteria are able to develop directly in the oil column, which gives them an advantage


over other oil-oxidizing bacteria. They play an important role in cleaning up oil spills into the environment: participating in the initial stage, they create favorable conditions for the growth of oil-oxidizing microorganisms of other genera.

These bacteria are of great ecological importance, but they are also used as biological agents in other areas: biotechnology, agriculture, and medicine (Lin, 2022).

Bacteria of the genus *Arthrobacter* are typical inhabitants of the soil microflora, but are also found in other sources: in water bodies, peat, inside or on the surface of other organisms as a symbiont. Thus, some species of this genus are symbionts of the chironomid larva *Polypedilum vanderplanki*.

Ammonium salts and some organic substances containing nitrogen are used as a source of nitrogen. Individual representatives of the genus *Arthrobacter* are associative nitrogen fixers.

Arthro bacteria can utilize phenol and 2,4-dichlorophenol, which can bring tangible benefits in the treatment of wastewater from plywood, pulp and paper industries. Also, they noted the ability to oxidize a wide range of oil components, including mono- and polycyclic aromatic hydrocarbons. Bacteria *A. globiformis* destroy 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids, surpassing other microorganisms in this; breaking down such compounds as chlorine derivatives of benzoic acid, halogen derivatives of polycyclic aromatic

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hydrocarbons, acridine orange, are able to metabolize carbohydrates, alcohols, carboxylic acids, and other organic substances.

The genus *Arthrobacter* is extremely valuable from the point of view of biotechnology since its representatives are used as producers for the synthesis of such important groups of organic substances as enzymes, amino acids, and peptides, for the production of porphyrins, vitamin B12.

In agriculture, the Mizorin P preparation based on *A. mysorens* is widely used. Seed treatment with its use has a positive effect on soybean seedlings. Some strains of this genus are resistant to cadmium, which opens the way to new promising ways to protect plants from soil pollution by heavy metals, in particular cadmium. *Arthrobacteria* is able to accelerate plant growth by synthesizing growth substances.

Gordonia is another genus of coryneform microorganisms used for bioremediation due to their ability to metabolize hydrocarbons, a wide range of environmental pollutants, various xenobiotics, and difficult to decompose substances.

Members of the genus *Gordonia* play an important role in biofiltration and wastewater treatment. Some other species of *Gordonia* are able to decompose or convert aliphatic and aromatic hydrocarbons, halogenated aromatic compounds, benzothiophene, nitrile, polyisoprene, xylene, etc. The presence of long-chain mycolic acids in the composition of the cell wall makes it waxy and highly hydrophobic, which plays an important role in the destruction of also hydrophobic pollutants. An example of this is the growth of some strains of bacteria of the genus *Gordonia* on the surface of the rubber and the metabolization of hydrophobic hydrocarbons by some species of this genus.

2 MATERIALS AND METHODS

In the process of studying an object, difficulties often arise: the inaccessibility of the original or the inappropriateness of its use, or the involvement of the original requires the use of a large number of resources. All these problems can be solved with the help of simulation. The model can serve as an almost complete or complete replacement of the object under study. With the help of mathematical models, it is possible to evaluate the behavior of microorganisms under certain conditions, based on the principle that the reaction of microorganisms to environmental factors is reproducible. The ability to predict the growth of microorganisms and their survival is of

great importance for all branches of microbiology (Pepper, 1995).

Before introducing a mathematical model into production or starting to use it in a laboratory, it is necessary to verify its ability to adequately reflect the features of the life processes of microorganisms. There are many equations and models in the literature that are used as growth functions. The main difference between them is the complexity and number of parameters of the equation. Some authors compared the behavior of growth models from different points of view: mathematical indicators of the quality of fit and other statistical criteria. Quality of fit scores for comparing models in these studies are calculated by calculating bias (Bf) and precision (Af) indices as done by Ross, coefficient of determination (R²), standard error, or by performing an F-test. Other authors have focused on direct comparisons of individual growth parameters. All these studies led the authors to different conclusions, which means that there is no clear consensus in the literature about which model is the most optimal for predicting bacterial growth (Buchanan, 1997).

A group of scientists led by Osipenko M.A. built a probabilistic mathematical model of the morphogenetic development of bacteria of the genus *Rhodococcus*. Within the framework of this study, theoretical images simulated on the basis of mathematical formulas were created and compared with real microphotographs obtained during the experiment. A comparison of them showed that, in general, the model is correct, both in a qualitative and quantitative aspect, but it has a drawback in the form of somewhat increased sizes of cells of great length (Dalgaard, 2001).

Vodopyanov V. in his research work, analyzing the progress made in the field of modeling the decomposition of pollutants in the soil, notes that they cannot properly take into account the change in the number of bacteria. Subsequently, the mathematical model he created made it possible to establish that the only way that can increase the efficiency of biodegradation in the soil is to stimulate the native microflora by introducing appropriate additives or carbon-assimilating associations of bacteria.

Biosurfactants released by some bacteria have a very promising future in pharmacology, the food industry, and bioremediation. Therefore, a mathematical model of biomass accumulation and production of biosurfactants by *Nocardia amarae* bacteria was created. The model presented by them gave an adequate picture of the growth process. At the same time, the average error in the production of surfactant during the fermentation process was 5.74,

and the error in the concentration of microorganisms was 10.172%. Another group of scientists studied and modeled the kinetics of surfactant formation by various strains of *Bacillus* sp. As a result, the productivity of biosurfactant formation and the optimal fermentation conditions, in particular the ratio of carbon and nitrogen, as well as the surface tension force, were established (Hesty, 2017).

Understanding how a complex set of phenotypic traits depends on individual molecules and their various interactions is a top priority in bioinformatics. Carr and colleagues developed a comprehensive computer model of the cell. Its greatest importance among similar works is great in connection with the ability to predict the phenotype based on the genotype. The phenotype of a particular microorganism in the model is made up of its molecular components, as well as the processes occurring inside the cells.

3 RESULTS AND DISCUSSION

Of great interest to applied science is a mathematical model simulating nitrogen regulation in the bacterium *Corynebacterium glutamicum*. This species is widely used for the synthesis of lysine. It is based on piecewise linear differential equations and, due to the high degree of knowledge of the simulated processes, even with a small amount of input data, allows obtaining adequate information about the state of the biological system.

Fractal analysis and modeling of the growth of colonies of biological objects were studied in detail by Sletkov D.V. He created a computer software package for visualizing a model in which the shape of cells is closely related to their so-called fractal dimension. In this study, he managed to obtain a qualitative correspondence between the model and natural experiment, which allowed him to conclude that the model adequately describes various growth processes and the dynamics of their course.

To date, there is a problem of increasing antibiotic resistance of bacteria. This is happening against the background of the lack of new developments in the field of industrial production of antibiotics and the irrational use of the latter in the treatment of people and in agriculture. With this formulation of the question, it is important to understand the mechanism of the adaptation of bacteria to the action of antibiotics. To this end, Serovaisky S. Ya. and colleagues resorted to the creation of a mathematical model for reducing sensitivity to antibiotics for microorganisms such as *Chlamydia pneumoniae*,

Haemophilus influenzae, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*. The created model allows you to dynamically track the sensitivity and resistance of the studied bacteria, which to a certain extent helps to correct the tactics of treating patients with pneumonia.

Predicting the concentration or number of microorganisms, taking into account growth conditions, using mathematical models of growth rate and temperature dependence is an effective tool that finds very wide applications in many areas of biotechnology (in particular, bioremediation) and the food industry. In the work of Zwietering M. H. and co-authors, the suitability of various mathematical models existing at the time of the study was considered. The models were compared using the F-test. Modified forms of the Ratkowski model were chosen as the most suitable growth rate models. An important condition for growth is the presence of biogenic elements in the environment. Thus, a model is proposed that describes the growth of microorganisms in the soil, taking into account the exchange of carbon and nitrogen. The approach combines modified classical equations for microbial growth by introducing a new state variable (r) that determines microbial activity. The activity factor, in turn, controls microbial growth and mortality rates, as well as the rate of decomposition of insoluble organic matter. The model was tested for conditions of periodic and continuous application of the substrate at various amounts of biomass and the content of carbon and nitrogen in the soil. The proposed structure of the mathematical model makes it possible to reproduce such features of the vital activity of microorganisms in the soil as the transition of the microbial population to a dormant state in the case of a lack of carbon or nitrogen, the primary effect on the decomposition of soil organic matter, and a decrease in the efficiency of microbial biosynthesis in the case of nitrogen deficiency (Blagodatsky, 1998).

Evaluating the effect of temperature on microbial growth was the goal of the work of scientists led by Lihan Huang. They developed a new model based on the Eyring and Arrhenius equations and compared the results of its calculations with experimental data published in the literature for such microorganisms as *Pseudomonas* spp., *Listeria monocytogenes*, *Salmonella* spp., *Clostridium perfringens*, and *Escherichia coli*. Another team of scientists attempted to develop mathematical equations to calculate the maximum increase in biomass as a function of temperature for a sigmoid empirical growth model. Here we compared the performance of two models based on empirical parameters and two more based on

biological parameters. The considered models were adapted to the experimental data for *Lactobacillus Plantarum* under six isothermal conditions.

The role of software in microbiology.

Computer software, based on mathematical formulas, is able to visually visualize and even predict many important aspects of the dynamics of growth and development of bacterial microorganisms and their communities, both those that actually exist in wildlife and artificially created ones (Parish, 1979; Shi, 2020).

For example, a software system such as "AgentCell" models biochemical processes, as well as the movement of cells in a three-dimensional environment. It is designed to study stochastic fluctuations. This software was tested on the example of the chemotaxis response of *E. coli* cells in relation to a chemoattractant gradient.

The software complex "Haploid Evolutionary Constructor" allows you to simulate genetic mutations, transfer, and loss of genes, as well as fix these genetic changes. Using this software, it is possible to simulate phage infection and the functioning of gene networks.

The AQUASIM computer simulation system (Wanner and Morgenroth, 2004) creates a model of bacterial biofilms in aquatic ecosystems, which, in turn, makes it possible to analyze the sensitivity of the model and evaluate its parameters.

In his scientific work, Davydov A. A. developed mathematical models and a set of programs called "Biomod", which serve to simulate the dynamics of the interaction of microorganisms used in the production of raw smoked sausages in order to ensure their stable quality and biological safety.

The software "Biodestructor", which is used for simulation modeling, allows you to simulate the dynamics of the spread of oil pollution in the sea, taking into account several phenomena at once: diffusion, convective transfer, oil fractionation, biological decay, and bacterial population.

Material and research methods.

This work on the study of oil pollution was carried out on the basis of the Department of Genetics, Microbiology, and Biochemistry of the Faculty of Biology of Kuban State University. As part of the study, data were also obtained on the dynamics of the process of biological treatment of oil-contaminated soil at the site of the Tikhoretskaya oil depot from the Biotechnology Research Center of the Kuban State University.

Object of study.

The objects of study in this work were the oil-contaminated soil of the Tikhoretskaya tank farm and

bacterial strains of *Arthrobacter globiformis* AC1112, *Gordonia alkanivorans* K9. *A. globiformis* culture cells are gram-variable (gram-positive in the stationary phase; gram-negative, in the exponential growth phase). obligate aerobes. Motionless, non-sporing. During growth, the colony changes color from yellow to white. During growth, a bacillus-coccus cycle is observed.

G. alkanivorans culture cells, Gram-positive. Aerobes. Non-spore-forming. Colonies are orange. The growth cycle is two-stage: bacillus-coccus.

To examine the morphological features of the cells of the studied cultures, a phase-contrast microscope CX41 (Olympus, Japan) with a 1000x magnification was used. First, the pre-prepared preparation is placed on the object stage, then the microscope is adjusted, focused, and then aimed at different fields of view, using a standard and micrometric eyepiece.

To determine the increase in cell biomass (optical density) of microorganisms in culture, a KFK-2MP photoelectrocolorimeter (Zagorsk Optical and Mechanical Plant, Russia) was used.

When working with a photoelectric colorimeter, first of all, calibration is carried out. This happens by establishing the OD of the BCH liquid medium, into which no biomass was introduced, as a reference. Then, a small amount of culture content is added to another cuvette and its OD is measured; the resulting value is recorded in the logbook.

In the framework of this work, a special preparation consisting of living cells was used to examine the cell morphology. The procedure for its preparation: a small drop of water is placed on a sterile glass slide, then a small amount of live bacterial mass is introduced into it using a loop; The resulting suspension is covered with a coverslip.

The pre-prepared preparation is microscopied using a phase-contrast microscope with an immersion objective. Look through 10 fields of view. For each of them, the total number of cells and the number of individual cell forms are fixed, then for each group of cells, a percentage of the total is calculated. The final indicators of the determined value are the arithmetic mean of the indicators obtained for each field of view.

In the work, we used nutrient media produced by the FBSI SRC PMB (Federal Budgetary Institution of Science "State Scientific Center for Applied Microbiology and Biotechnology") - the standard nutrient medium of MPA, as well as MPB.

The composition of the MPA medium (per 1000 ml): pancreatic hydrolyzate of fish meal (12.0 g); enzymatic peptone (12.0 g); sodium chloride (6.0 g); microbiological agar (10.0 g); distilled water.

The composition of the MPB medium (per 1000 ml): dry enzymatic peptone (10.0 g), meat extract (11.0±1.0 g), sodium chloride (5.0 g), and distilled water.

The MPA medium was used to obtain isolated colonies, which subsequently served for the preparation of "crushed drop" preparations. Studying the preparations using a phase-contrast microscope, the morphological changes in the cells of the studied microorganisms were recorded, as well as the sizes (length and width or diameter, depending on the shape of the cells) of the cells. In this case, special attention was paid to the ratio of cell shapes in a variety of fields of view of the preparation. These manipulations were carried out with a frequency of 3 hours, during the morphogenetic cycle of the development of microorganisms. In parallel, in order to assess the growth of bacteria and build growth curves, cultivation was carried out in a liquid medium of the MPB. OD was measured with the same frequency; the value was entered into the journal.

Thus, serious work has been done to prepare the results of the study, with the help of which it is possible to build objective mathematical and biological models. This solution will significantly automate the research process and increase its practical significance.

4 CONCLUSIONS

Summing up, we can conclude that the construction of models and the use of software systems in the field of microbiology plays a very significant role, being the basis of various methods of observation and knowledge of the processes of development of microorganisms and phenomena. The issue of pollution of natural resources is more relevant than ever. And only the combination of traditional methods of studying and solving global problems with advanced information tools - can answer many questions and allow you to develop a whole apparatus for responding to changing environmental conditions. Therefore, it is so important to develop research in this direction, to develop biological and mathematical models and special software that could track the cycles and phases of the development of microorganisms capable of cleaning up oil pollution. The results obtained can play a fundamental role in solving this type of problem.

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