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Decontamination of Oil-Polluted Soils: Power of Electronic Bioinformatic Databases

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Abstract. The main idea was to solve the problem related to oil contamination of soil using bioremediation with bioaugmentation with modeled microorganism strains. The paper aimed to develop a bacterial consortium for petroleum hydrocarbon degradation during the biological treatment of oil-contaminated soils using electronic databases. The research methodology included an analysis of the mechanisms and metabolic pathways of petroleum hydrocarbon degradation and an assessment of the possible reaction modulus and enzymatic systems for the degradation of aromatic compounds. The taxonomic classification and review of oil compound transformation metabolic pathways were carried out using electronic KEGG, MetaCyc, and EzTaxon databases. The KEGG database was used to create a microbiological consortium of certain strains of bacteria that improved hydrocarbon degradation process performance. Identification of bacteria's complete genome using Island Viewer 4 allowed to create of a consortium of oil-destructive bacteria consisting of such strains: *Pseudoxanthomonas spadix* BD-a59, *Rhodococcus jostii* RHA1, *Rhodococcus aetherivorans* IcdP1, *Pseudomonas putida* ND6, *Pseudomonas stutzeri* 19SMN4, *Pseudomonas fluorescens* UK4, *Acinetobacter lactucae* OTEC-02, *Bacillus cereus* F837/76.7.9. The ratio between the mentioned strains of microorganisms in the consortium was set at 20 % : 20 % : 15 % : 10 % : 10 % : 5 % : 5 % : 15 %. This bacterial consortium for aromatic hydrocarbons was created according to the metabolic information of basic enzymatic systems and the predominant transformation of particular oil compounds using the BacDive database.

Keywords: soil pollution, bioremediation, oil biodegradation, synergism.

1 Introduction

The microbiological degradation of organic substances after the ingress of oil and oil products into the soil is very relevant from the point of view of ensuring the environmental safety of oil-producing territories. At the same time, the decomposition of linear hydrocarbons is much easier than aromatic hydrocarbons due to the complexity of the chemical structure of arenes, which requires more thorough study to develop approaches to intensify the process. In this regard, the main focus of this article is the study of polycyclic aromatic hydrocarbons (PAH) and their biodegradation processes. It is known that *Pseudomonas* [1], *Sphingomonas*, *Mycobacterium* [2], *Bacillus* [3, 4], and *Rhodococcus* [5] genera consist of bacteria with the capacity for PAH degradation. Jain and Bajpai [6] claimed that genetically modified microorganisms have exhibited enhanced destructive capabilities encompassing a large number of chemical

pollutants, in particular petroleum hydrocarbons and even polycyclic aromatic hydrocarbons. Consequently, it could be potentially used for the biological treatment of oil-contaminated soils and other environments.

Scientists have studied the patterns and mechanisms of chemical reactions with the participation of microorganism's strain *Aspergillus glaucus* belonging to the genus *Aspergillus*. The final and intermediate oxidation products of naphthalene and anthracene have been determined in the study [7].

Heterotrophic aerobes bacteria get carbon and energy from petroleum hydrocarbons during oxidative processes resulting in the degradation of the aforementioned organic substances [8]. Developing a complex of special enzymes promotes the absorption of hydrophobic substrate and microorganisms' decomposition of organic matter. Bacteria-producing enzymes from the oxygenase group could destroy substances with hydrophobic properties. The enzyme group of dehydrogenases acts in the oxidative

processes of hydrophilic compounds [9]. According to a study by the authors [10], *Bacillus* sp. strain X6 produced dehydrogenase with the capacity to biodegradation rate of 50 % and other enzymes had a lower value.

2 Literature Review

According to plenty of research, the enzymatic activity of the soil directly depends on the oil content. In this point, the paper [11] indicates that the concentration of oil up to 10 % positively affects catalase, dehydrogenase and urease activity; up to 17 % – on phosphatase and lipolytic activity. However, the amount of oil in the soil above these values inhibits the activity of all types of enzymes.

The maximum effective result can be achieved if it is used a consortium of bacteria capable of the destruction of organic substances of certain chemical composition and nature [12]. According to the obtained results by [13], bacterial consortium consisting of *Stenotrophomonas acidaminiphila*, *Bacillus megaterium*, *Bacillus cibi*, *Pseudomonas aeruginosa* and *Bacillus cereus* showed a good degradation ability for oil wastes as well as biosurfactant production capacity, compared with isolates. The problem of creating favorable living conditions for consortium is partially solved by bioaugmentation, biostimulation and the use of biologics, primarily, mentioned above biosurfactants in the implementation of the In-situ method that means without removing the soil, which enhances the metabolism of microflora [14–17].

Strains of microorganisms capable of oxidizing oil produce biosurfactants that provide the dispersion process of oil and oil products and increase the selective membrane permeability, which has a positive effect on the entry of PAHs into the cell from the outside. Such substances must be a high-molecular-weight bioemulsifier such as extracellular biosurfactant of lipopolysaccharide nature produced by *Acinetobacter calcoaceticus*, *Acinetobacter radioresistens* KA53 [18].

Despite the presented research results on the pathways of decomposition of aromatic substances, poor structuring of information and gaps in methodological approaches to creating appropriate bacterial consortia exist. Most of the publicly available databases do not contain the results of experimental studies of protein sequences of microorganisms [19].

The present research has been focused on the electronic bioinformatic databases, aiming to develop bacterial consortium for petroleum hydrocarbons degradation during bioremediation of oil-contaminated soils.

To accomplish the aim, the tasks have been set as follows:

- 1) to analyze the mechanisms and metabolic pathways of aromatic petroleum hydrocarbons degradation based on electronic KEGG databases use;

- 2) theoretically substantiate and simulate the composition of the oil-degradable bacterial consortium capable for bioaugmentation in the case of soil bioremediation.

3 Materials and Methods

Electronic KEGG databases (the Kyoto encyclopedia of genes and genomes), MetaCyc and the EzTaxon database were used for taxonomic classification and study of metabolic pathways for the decomposition of petroleum compounds. Bacterial Diversity Metadatabase BacDive was used to study the application and interaction of oil degradable microorganisms, their physiology, morphology, isolation, culture and growth conditions, and sampling and environmental information.

Well-known and widespread among specialists, webserver IslandViewer 4 (<http://www.pathogenomics.sfu.ca/islandviewer/>) was used to predict and visualize genomic islands “(GIs, regions of probable horizontal origin) in bacterial genomes”. GIs contain information about medically or ecologically necessary adaptation factors that increase the ability to compete with microorganisms in the ecological niche, including different factors [20].

“The exploration of the catalytic activities of microbial communities involved in the degradation of persistent PAHs in contaminated sites was carried out using MAGICPAH stands for Molecular Approaches and MetaGenomic Investigations for Cleanup of Polyaromatic Hydrocarbon” (<http://aromadeg.siona.helmholtz-hzi.de/>). MAGICPAH is a database on a core catabolic enzymes that allows an adequate scheme of reactions and enzymes responsible for the destruction of aromatic hydrocarbons. MetaCyc (<https://MetaCyc.org>) is a massive database including practically identified metabolism reactions of all living beings. MetaCyc contains the pathways related to metabolism and reactions, enzymes, metabolites, and genes [21].

The subject of research was the study of metabolic pathways for the decomposition of polycyclic aromatic products by certain microorganisms, with the release of important biosurfactants that affect the efficiency of a given process. KEGG database (<https://www.kegg.jp/kegg/pathway.html#xenobiotics>) was used as specific research to achieve this goal. KEGG is a computer visualization of the living systems, including genomic information (molecular structure sets of genes and proteins) and chemical information (different chemicals). Genomic information is combined with systems information (dates on schemes for combining networks of interactions, reactions and relationships at the molecular level) and increase biochemical reaction speed [22].

Thus, various ecological and trophic groups of microorganisms can be part of a bacterial consortium under the conditions of a specific ratio to achieve the set goal of the destruction of specific hydrocarbons [13].

4 Results and Discussion

4.1 Mechanisms and metabolic pathways of petroleum hydrocarbons degradation

For today various databases have simplified the process of constructing metabolic pathways for various organic substances. Particularly, the KEGG database simulates metabolic pathways for different substances, petroleum hydrocarbons, to determine the types of involved microorganisms and enzymatic systems. For instance, the result of such modeling for toluene conversion to benzoic acid is shown in Figure 1.

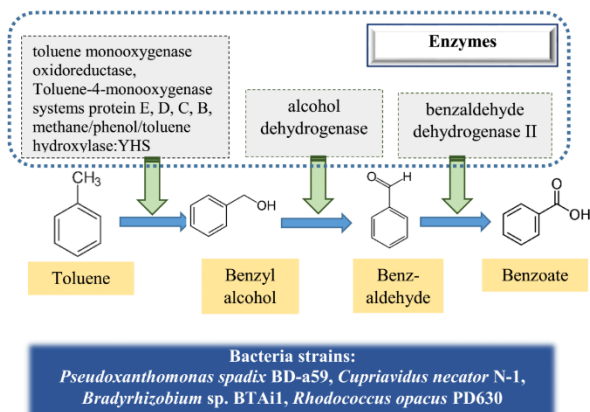


Figure 1 – Toluene degradation, toluene => benzoate: methyl to carboxyl conversion on the aromatic ring

It is known that microorganisms can include in their metabolic reactions not only nutrients but also various anthropogenic chemicals, including aromatic compounds, which contribute to their degradation in the environment [23]. The capacity of toluene degradation by *Rhodococcus opacus* sp. has been confirmed in the research [24]. The diagram of the degradation of PAHs demonstrates complex models of reaction pathways for biodestruction of aromatic substances, including three basic types of ring dihydroxylation modules, followed by meta- or ortho-cleavage modules and joint dihydroxylation and cleavage of aromatic ring, together with an optional pretreatment modulus to convert a methyl group to a carboxyl group on an aromatic ring (Figure 2, a).

Biochemical conversion shown in Figure 2 (a) is supported by 2-formylbenzoate dehydrogenase enzyme belonging to the class of Oxidoreductases that can influence the aldehyde or oxo group of donors, with acceptors NAD^+ or NADP^+ . This enzyme is produced by the following strains of bacteria: *Mycobacterium* sp. MCS, *Mycobacterium* sp. KMS, *Mycobacterium* sp. JLS, *Mycolicibacterium vanbaalenii*, *Mycolicibacterium gilvum* PYR-GCK, *Mycolicibacterium gilvum* Spy1, *Mycolicibacterium rhodesiae*, *Pseudarthrobacter phenanthrenivorans*.

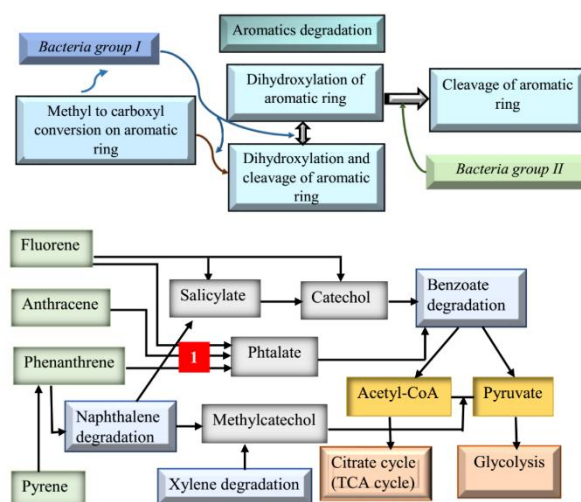


Figure 2 – Flowchart of aromatic compounds degradation with different pathways modules (a), simplified scheme of Polycyclic aromatic hydrocarbon degradation (b)

Such a group of petroleum hydrocarbons as PAHs is the most stable in relation to microbiological degradation. References pathways analysis of some chemicals, particularly fluorene, anthracene, phenanthrene, pyrene, toluene, and xylene, indicate Polycyclic aromatic hydrocarbon degradation transition into Benzoate degradation (Figure 2, b). Moreover, the first four PAH from the above-mentioned list has interconnected pathways. In this case, phthalate acts as a link, the metabolic pathway of transformation that also closes to Benzoate degradation.

A pathways analysis of the degradation of the aromatic compounds indicated that main degradation modules include Toluene and Benzoate degradation, Catechol ortho-cleavage and Catechol meta-cleavage, Xylene, Naphthalene, Phthalate degradation (Figure 2, b). The flowchart for the transformation of 2-Carboxybenzaldehyde during the bacterial biodestruction, indicated by the number 1 in the Polycyclic aromatic hydrocarbon degradation (see Figure 2, b), is shown in Figure 3.

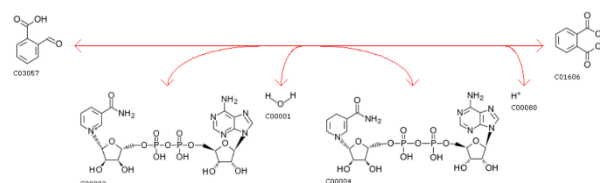


Figure 3 – 2-formylbenzoate (2-Carboxybenzaldehyde) (C03057) to o-phthalic acid (Phthalate) (C01606) conversion scheme: C00003 – NAD^+ , C00001 – H_2O , C00004 – NADH , C00080 – H^+

Based on the study of metabolic schemes for the transformation of aromatic hydrocarbons, many different pathways for their destruction have been determined, as well as connections with other chains, in relation with which several enzyme systems can participate in the transformation of one substance.

The oxidation process of benzoate is provided by benzoate, benzoic enzymes, as well as dioxygenase, hydroxylase, and oxidoreductase enzymes classes acting on paired donors to reduce molecular oxygen. On the basis of the study of cultural and morphological signs, markers of resistance related to the genomic fingerprints method, scientists managed to find out the fate of microorganisms capable of oil degradation and prove their sustainability. Microorganisms-destroyers of *Rhodococcus* and *Pseudomonas* strains are selective for individual oil products. It is necessary to form an influential association of microorganisms-destroyers, a biological product for cleaning the soil from oil pollution [25, 26].

4.2 Modeling of oil-degradable bacteria consortium capable for bioaugmentation

The essential principle of bacteria consortium successful functioning is based on the synergetic effect in the relationship between different ecological trophic groups of microorganisms because one's metabolites have to be a carbon source for others [27, 28]. Analysis of degradation modules of main aromatic compounds shows intermediate and final products and the necessary enzymatic systems to accelerate these transformations (Figure 4).

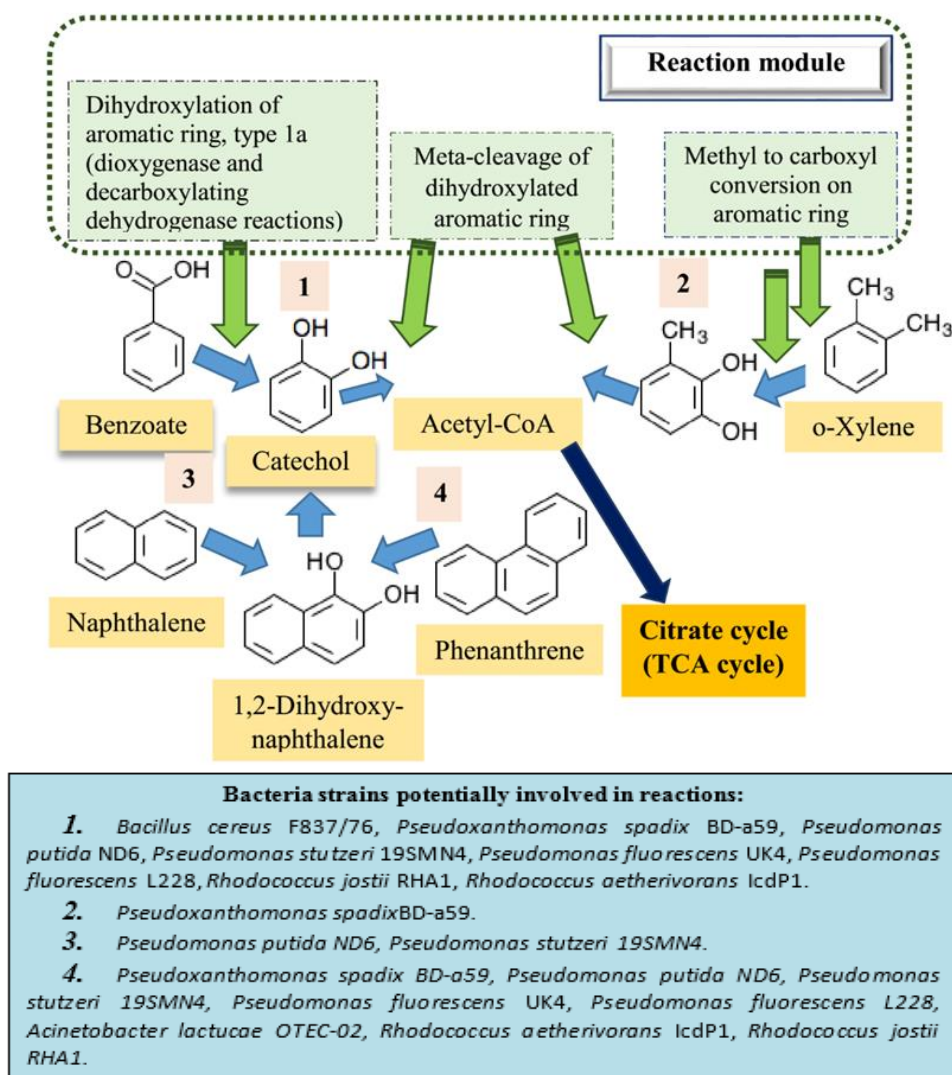


Figure 4 – Reaction modulus for degradation of aromatic compounds

Legend: Green boxes are different reaction types of aromatic compounds destruction; green arrows demonstrate conversions for appropriate reactions; blue arrows indicate a such conversion one aromatic compounds into others. Numbers signify certain pathways: 1 – benzoate=> catechol; 2 – o-xylene => 3-methylcatechol; 3 – naphthalene => 1,2-dihydroxynaphthalene; 4 – phenanthrene => 1,2-dihydroxynaphthalene.

The first conversion belongs to Benzoate degradation due to the reaction of dihydroxylation of the aromatic ring

involving enzymes benzene 1,2-dioxygenase and dihydroxycyclohexadiene carboxylate dehydrogenase

from the class of Oxidoreductases. Enzyme catechol 1,2-dioxygenase (class Oxidoreductases) effected on sole donors with inclusion of oxygenases (molecular oxygen) starts the meta-cleavage pathway of catechol degradation. Naphthalene 1,2-dioxygenase is related to the ring-hydroxylating dioxygenase (RHD) that significantly impacts the destruction of aromatic substances, including PAHs. Aryl alcohol dehydrogenase includes enzymes with a broad specificity for alcohols with an aromatic or cyclohex-1-ene ring. At the same time, this enzyme has little or no activity against short-chain aliphatic alcohols (https://www.genome.jp/dbgetbin/www_bget?K00055+1.1.1.90+R05348).

Alcaligenes sp. strain PPH degrades phenanthrene via 1-hydroxy-2-naphthoic acid hydroxylase, salicylic acid, and catechol [29]. *Pseudoxanthomonas spadix* BD-a59 must be involved in the degradation of all six BTEX (benzene, toluene, ethylbenzene, and o-, m-, and p-xylene) compounds. It is isolated from gasoline-contaminated sediment. *Pseudoxanthomonas spadix* BD-a59 complete

genome information was successfully obtained using simulating tool IslandViewer 4 (http://www.pathogenomics.sfu.ca/islandviewer/accession/NC_016147.2/) it has to be potentially involved in mostly PAH degradation reactions. In this relation, *Pseudoxanthomonas spadix* BD-a59 was determined as the dominant species in the consortium model.

The complete set of enzymatic systems involved in PAH biodegradation has to be provided by including bacteria strains into consortium: *Pseudoxanthomonas spadix* BD-a59, *Pseudomonas putida* ND6, *Pseudomonas stutzeri* 19SMN4, *Pseudomonas fluorescens* UK4, *Pseudomonas fluorescens* L228, *Acinetobacter lactucae* OTEC-02, *Rhodococcus aetherivorans* IcdP1, *Rhodococcus jostii* RHA1.

Figure 5 shows a bacterial consortium that was created for the degradation of aromatic hydrocarbons, based on data on metabolism, diversity, the presence of certain enzymes, and the main transformation pathways.

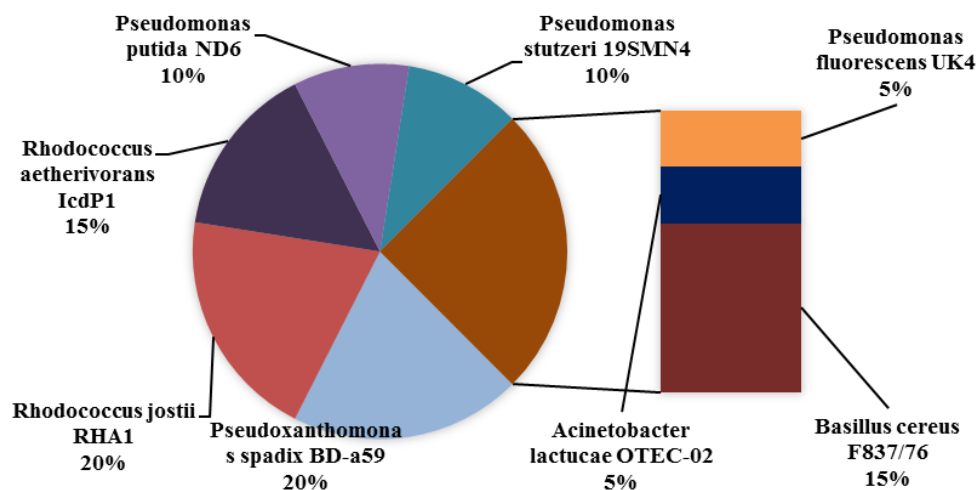


Figure 5 – The diagram of different bacteria related to the bio destruction of aromatic hydrocarbons

The created consortium includes bacteria strains in a different ratio based on the bacteria group is 100 %. The difference in the mass ratio between the strains used for this model is due to differences in the necessary enzymes. The creation of such a consortium became possible based on data on the enzymes required to decompose aromatic hydrocarbons, mainly oxidoreductase and oxygenase. Bioinformatic databases made it possible to select such bacterial strains that are characterized by synergy, that is, an increase in the overall effect regarding the use of carbon in oil products as an energy source for bacteria such as *Pseudoxanthomonas* and *Pseudomonas* strains. In this case, the intermediate decay products are a substrate for other bacteria (*Acinetobacter* and *Rhodococcus* strains) from the consortium.

For instance, *Pseudoxanthomonas spadix* BD-a59 and *Rhodococcus aetherivorans* IcdP1 are more capable of cyclic hydrocarbon destruction. *Rhodococcus aetherivorans* IcdP1 was retrieved from polluted soil of the abandoned Beijing Coking Plant in China” (doi:10.13145/bacdiv11035.20190402.4). “The genome

highlights important features for bioaugmentation, including the genes involved in the degradation of methyl-ethyl-ketone” [30].

Rhodococcus jostii RHA1 has been reported to be soil actinomycete, capable of decomposition of polychlorinated biphenyls in soil and includes a wide range of compounds in metabolism (doi:10.13145/bacdiv11034.20190402.4).

Under PAH pollution, PAH-catabolic genes, particularly aromatic hydroxylating dioxygenase gene fragments in both α - and γ -Proteobacteria were close to nahAc and phnAc genes, whereas the predominantly actinobacteria were isolated in the study area.

It was reported [31] that *Acinetobacter*, *Achromobacter*, *Bacillus*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Pseudomonas*, and *Staphylococcus* consortium application contributed a 62.08 % decrease in the concentration of total petroleum hydrocarbons. Moreover, such result was recorded at 42 day of the experiment.

Okafor et al. [32] have determined that bacterial strains such as *Pseudomonas*, *Bacillus*, *Klebsiella*, and *Enterobacter* could produce biosurfactants for PAH degradation (benzene and naphthalene) while a consortium of *Pseudomonas*, *Bacillus*, *Lysinibacillus*, and *Enterobacter* had the highest rate of oil degradation.

Jahir et al. [33] postulated that *Bacillus* spp. has resistant endospores to be more tolerant to high levels of hydrocarbons in soil that has been proved according to the research [34]. High biodegradation capacity at the level above 93 % of crude oil was recorded for the bacterial consortium of *Bacillus cereus* and *Pseudomonas putida* bacteria [35].

Jussila et al. [36] reported *Pseudomonas aeruginosa* JI104 must be an effective utilizing and catechol-positive bacteria having the xylE gene participated in the destruction of aromatic hydrocarbons (xylE, BTEX destruction).

Pseudomonas strains, as well as the xylE gene was determined in other *Proteobacteria* isolates, such as *Acinetobacter*, *Achromobacter*, *Enterobacter*, and *Stenotrophomonas*. The GC-MS analysis showed that a consortium of such bacteria *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Cronobacter*, *Stenotrophomonas*, *Achromobacter*, *Ochrobactrum*, *Paenibacillus*, *Bacillus*, *Microbacterium*, *Curtobacterium*, and *Sphingobacterium* was able to reduce the crude oil content of the liquid culture by 40.5 % after 10 days [37].

Another author also reported a consortium of bacteria strains such as *Bacillus cereus* BL01, *Pseudomonas stutzeri* BL02, *Acinetobacter* sp. BL03, *Bacillus* sp. BL04 provided removal efficiency at the level of 46 %, while the consortium mentioned above application supplemented by *Azotobacter vinelandii* AV01 producing surfactant increase this indicator up to 85 % [38]. Consortium of *Bacillus aerius* B2, *Pseudomonas stutzeri* B3, *Ochrobactrum intermedium* B4, *Micrococcus lylae* B5, and *Acinetobacter calcoaceticus* B9 could increase the rate of oil degradation by 88 % [39].

Strains belonging to the *Bacillus* species show mixed resistance to antibiotics and heavy metals. The study [26] results proclaimed that isolates *Aneurini bacillus migulanusstra* in KTPP was the most effective in degrading the wide range of petroleum hydrocarbons. *Bacillus cereus* strain BSP showed multiple resistance against heavy metals and presented a high ability to degrade the selected hydrocarbons.

Thus, the bacteria included in the proposed consortium have a high ability to degrade hydrocarbons, which the studies of other scientists have reliably confirmed.

5 Conclusions

A scheme for the degradation of aromatic compounds has been developed, shown with combination schemes of reaction modules for the biodestruction of aromatic

compounds. The investigated reactions consist of three basic ring dihydroxylation modulus, followed by meta- or ortho-cleavage modulus and co-dihydroxylation and aromatic ring cleavage, together with a pretreatment modulus to convert a methyl group to a carboxyl group on the aromatic ring.

A pathways analysis of the degradation of the aromatic compounds indicated that main degradation modulus includes Toluene and Benzoate destruction, Catechol ortho-cleavage and Catechol meta-cleavage, Xylene, Naphthalene, Phthalate destruction. Modelling of oil-degradable bacteria consortium capable for bioaugmentation was carried out based on reaction modulus for degradation of aromatic compounds, exploration of different enzymatic systems that catalyse certain reactions, and definition of bacteria capable of producing appropriate enzymes. Identification of bacteria complete genome using IslandViewer 4 allowed to create of a consortium of oil-destructive bacteria consisting of such strains: *Pseudoxanthomonas spadix* BD-a59, *Rhodococcus jostii* RHA1, *Rhodococcus aetherivorans* IcdP1, *Pseudomonas putida* ND6, *Pseudomonas stutzeri* 19SMN4, *Pseudomonas fluorescens* UK4, *Acinetobacter lactucae* OTEC-02, *Bacillus cereus* F837/76. The ratio between the mentioned strains of microorganisms in the consortium was set at 20 % : 20 % : 15 % : 10 % : 10 % : 5 % : 5 % : 15 %.

The scientific novelty of this study is the determination of the necessary bacteria in the consortium, which are capable of decomposing polycyclic aromatic hydrocarbons in the course of their vital activity. This approach makes it possible to effectively use the bioaugmentation method on oil-contaminated soils due to accidental oil spills, which often occur and require immediate application of measures to eliminate and clean up the soil.

For the future study, investigation of the native overall microbial community, regarding the impact of oil contamination and bioremediation related to the decontamination efficiency is needed.

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