**SUPPLEMENTARY MATERIAL – ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ**

**Development of a microbial consortium for bioremediation of oil-contaminated soils of the Middle Ob region**

**Разработка микробного консорциума для биоремедиации нефтезагрязненных почв Среднего Приобья**

A. A. Vetrova, S. Ya. Trofimov, R. R. Kinzhaev, N. A. Avetov, A.V. Arzamazova, I. F. Puntus, O. I. Sazonova, S. L. Sokolov, R. A. Streletskii, K. V. Petrikov, Ya. A. Delegan, V. A. Samoylenko, A. E. Filonov

**А. А. Ветрова, С. Я. Трофимов, Р. Р. Кинжаев, Н. А. Аветов, А. В. Арзамазова, И. Ф. Пунтус, О. И. Сазонова, С. Л. Соколов, Р. А. Стрелецкий, К. В. Петриков, Я. А. Делеган, В. А. Самойленко, А. Е. Филонов**

**Eurasian Soil Science.**

**Почвоведение.**

**Table S1.** The ability of microorganisms to degrade oil in the temperature range 6-37℃ and at acidic pH values 4-5.

**Таблица S1.** Способность микроорганизмов деградировать нефть в температурном диапазоне 6-37℃ и значении pH 4-5.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain |  6ºС | 24ºС | 30ºС | 37ºС |
| рН 4 | рН 5 | рН 4 | рН 5 | рН 4 | рН 5 | рН 4 | рН 5 |
| 24-21 | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** |
| 24р-51 | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** |
| 24р-71 | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** |
| 24р-83 | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** |
| 24р-61 |  |  |  | **±** |  | **+** | **±** |  |
| 24-41 | **+** | **+** |  | **+** | **+** | **±** |  | **±** |
| 24-44 | **±** | **+** | **±** | **+** | **±** | **+** | **±** | **+** |
| 7р-72 | **+** | **+** |  | **+** | **+** | **+** | **+** | **+** |
| 7-43 |  | **±** |  | **±** | **+** | **+** | **+** | **+** |
| 7-41 |  | **+** | **+** | **±** |  | **±** |  | **±** |
| 7р-62 | **+** | **+** |  | **±** |  | **+** | **±** |  |
| 7-31 | **+** | **+** |  | **+** | **+** | **±** | **±** | **±** |
| 7р-51 |  |  |  | **±** |  |  |  |  |
| 7р-81 |  | **+** |  | **±** |  |  |  |  |

+ - good growth, ± - poor growth

+ - хороший рост, ± - слабый рост

**Table S2.** The ability of microorganisms to degrade oil at a temperature of 24 ℃ and 3% sodium chloride.

**Таблица S2.** Способность микроорганизмов деградировать нефть при температуре 24 ℃ и 3%-ном хлориде натрия.

|  |  |
| --- | --- |
| Strain | Results of visual growth assessment |
| 24-21 | 3 |
| 24р-51 | 3 |
| 24р-71 | 3 |
| 24р-83 | 2 |
| 24р-61 | 1 |
| 24-41 | 0.5 |
| 24-44 | 3 |
| 7р-72 | 1 |
| 7-43 | 3 |
| 7-41 | 3 |
| 7р-62 | 1 |
| 7-31 | 2 |
| 7р-51 | 0.5 |
| 7р-81 | 1 |

3 – very good growth, 2 – good growth, 1 – growth, 0.5 - poor growth

3 – очень хороший рост, 2 – хороший рост, 1 – средний рост, 0.5 – слабый рост

**Table S3.** Data of PCR analysis of key genes of catabolism of aliphatic and aromatic hydrocarbons and size of detected plasmids.

**Таблица S3.** Результаты ПЦР-анализа ключевых генов катаболизма алифатических и ароматических углеводородов и размер обнаруженных плазмид.

|  |  |  |
| --- | --- | --- |
| Strain | Plasmid(Kb) | The catabolic gene |
| *nahAc* | *nahG* | *nahH* | *phnAc* | *nagG* | *alkB* |
| *Rhodococcus* *erythropolis* 24-44 | ND\* | - | - | - | - | - | - |
| *Candida fluviatilis* 24-21 | ND | - | - | - | - | - | - |
| *Candida fluviatilis* 24р-51 | ND | - | - | - | - | - | - |
| *Candida fluviatilis* 24р-71 | ND | - | - | - | - | - | - |
| *Candida fluviatilis* 24р-83 | ND | - | - | - | - | - | - |
| *Kocuria rosea* 24р-61 | ND | - | - | - | - | - | - |
| *Raoutella planticola* 24-41 | ND | - | - | - | - | - | - |
| *Candida fluviatilis* 7р-72 | ND | - | - | - | - | - | - |
| *Acinetobacter calcoaceticus* 7-43 | ND | - | - | - | - | - | - |
| *Pseudomonas* *fluorescens* 7-41 | ~210 | + | + | + | - | - | + |
| *Pseudomonas* *veronii* 7р-62 | ~170 | + | + | + | - | - | + |
| *Pseudomonas* *extremaustralis* 7-31 | ~150 | + | + | + | - | - | + |
| *Pseudomonas* *putida* 7р-51 | ~100 | + | + | + | - | - | - |
| *Pseudomonas* *fluorescens* 7р-81 | ~250 | + | + | + | - | - | + |

ND - not detected

ND - плазмиды не обнаружены

**Table S4.** Surface-active properties of microorganisms when growing on various substrates.

**Таблица S4.** Поверхностное натяжение культуральной среды при выращивании микроорганизмов на различных субстратах.

|  |  |  |
| --- | --- | --- |
| Strain | Substrate | Surface tension, mN/m |
| 24-21 | diesel fuel | 52.0±2.8 |
| 24р-51 | diesel fuel | 60.0±1.0 |
| 24р-71 | diesel fuel | 60.3±0.6 |
| 24р-83 | diesel fuel | 61.3±0.6 |
| 24р-61 | diesel fuel | 57.0±1.0 |
| 24-41 | diesel fuel | 56.3±1.2 |
| 24-44 | diesel fuel | **29.7±0.6\*** |
| 7р-72 | diesel fuel | 55.0±1.4 |
| 7-43 | diesel fuel | 63.0±0.1 |
| 7-41 | Naphthalene | 49.5±1.7 |
| 7р-62 | Naphthalene | 52.8±2.8 |
| 7-31 | Naphthalene | 55.0±1.0 |
| 7р-51 | Naphthalene | 54.7±2.5 |
| 7р-81 | Naphthalene | 51.7±0.6 |

\* The best surface tension coefficient

\* Наилучший коэффициент поверхностного натяжения

**Table S5.** Residual content of polycyclic aromatic hydrocarbons in model systems with mineral medium and 2% crude oil.

**Таблица S5.** Остаточное содержание полициклических ароматических углеводородов в модельных системах с минеральной средой и 2% сырой нефти.

|  |  |
| --- | --- |
| Defined indicator | The amount of hydrocarbon in model systems, µg/dm3 |
| Control without microorganisms | with microorganisms |
| 24-44 | 24р-51 | 7-31 | 7-43 |
| naphthalene  | 495±124 | 350±88 | **266±66** | **172±43** | **185±46** |
| acenaften | 160±48 | 120±36 | 150±45 | 130±39 | 110±33 |
| fluorene | 170±48 | 110±31 | 160±45 | 130±36 | 110±31 |
| Phenanthrene | 2021±566 | 1582±443 | 2102±589 | 1695±474 | 1489±417 |
| Anthracene | 15.2±4.5 | 10.9±3.3 | 13.1±3.9 | **8.56±2.57** | 15.0±4.5 |
| Fluoranten | 90±27.0 | 75.6±22.7 | 82.9±24.9 | **61.9±18.6** | **54.8±16.4** |
| peren | 254±69 | 229±62 | 269±73 | 254±69 | 1421±384 |
| Benz(a)anthracene | 649±188 | 531±154 | 692±201 | 571±166 | 499±145 |
| Chrysene | 711±206 | 581±168 | 766±222 | 635±184 | 547±159 |
| Benzo(b)fluoranthene | 811±243 | 623±187 | 846±254 | 714±214 | 619±186 |
| Benzo(K)fluoranten | 61.3±15.3 | 49.4±12.4 | 67.1±16.8 | 57.7±14.4 | 48.6±12.2 |
| Benz(a)pyrene | 83.6±20.1 | 71,.9±17.3 | 81.2±19.5 | **56.9±13.6** | 73.7±17.7 |
| Dibenz(a, h)anthracene | 1069±438 | 880±361 | **478±196** | **455±187** | **359±147** |
| Benzo(g,h, i)perylene | 50.7±20.8 | 44.7±18.3 | 37.2±15.3 | **5.92±2.43** | 48.8±20.0 |
| The sum of PAH | 6642±996 | 5258±789 | 6011±902 | **4971±746** | 5556±833 |

The lowest indicators are highlighted in yellow.

Самые низкие показатели выделены полужирным шрифтом.

**Table S6.** Residual content of alkanes in model systems with mineral medium and 2% crude oil

**Таблица S6.** Остаточное содержание алканов в модельных системах с минеральной средой и 2% сырой нефти.

|  |  |
| --- | --- |
| Defined indicator | The amount of hydrocarbon in model systems, µg/dm3 |
| Control without microorganisms | with microorganisms |
| 24-44 | 7-31 | 24р-51 | 7-43 |
| С12Н26 | <0.02 | **<0.02** | **<0.02** | **<0.02** | **<0.02** |
| С13Н28 | 8.3±1.2 | **<0.02** | **<0.02** | **<0.02** | **0.60±0.09** |
| С14Н30 | 16±2.3 | **0.22±0.03** | **0.08** | **0.23±0.03** | 2.6±0.39 |
| С15Н32 | 40±6.0 | **0.95±0.14** | **1.9±0.29** | **1.8±0.28** | 8.8±1.3 |
| С16Н34 | 43±6.4 | **1.7±0.26** | 9.7±1.5 | 4.3±0.65 | 8.2±1.2 |
| С17Н36 | 42±6.3 | **2.2±0.34** | 17±2.6 | 6.3±0.94 | 8.2±1.2 |
| pristane | 18±2.6 | 6.7±1.0 | 7.2±1.1 | 4.92±0.74 | 15±2.2 |
| С18Н38 | 39±5.9 | **1.4±0.21** | 21±3.1 | 6.5±0.98 | 7.4±1.1 |
| fitan | 28±4.1 | 13±1.9 | 15±2.2 | 9.5±1.4 | 23±3.4 |
| С19Н40 | 38±5.7 | **2.0±0.30** | 23±3.4 | 6.7±1.0 | 8.0±1.2 |
| С20Н42 | 37±5,6 | **0.02** | 24±3.6 | 6.7±0.99 | 6.9±1.0 |
| The sum of n-alkanesC12-C20(medium boiling fraction) | 309.3 | **28.15** | 118.88 | **46.95** | 88.7 |
| С21Н44 | 34±5.0 | **2.0±0.30** | 22±3.3 | 5.7±0.86 | 5.3±0.80 |
| С22Н46 | 31±4.7 | **<0.02** | 21±3.2 | 5.6±0.83 | 4.3±0.64 |
| С23Н48 | 34±5.0 | **0.03** | 22±3.2 | 5.3±0.80 | **2.7±0.41** |
| С24Н50 | 32±4.7 | **1.8±0.27** | 21±3.2 | 4.9±0.74 | **1.9±0.29** |
| С25Н52 | 28±4.2 | **2.6±0.39** | 19±2.8 | 5.1±0.77 | **<0.02** |
| С26Н54 | 27±4.1 | **0.17±0.03** | 17±2.5 | **3.9±0.58** | **1.9±0.28** |
| С27Н56 | 14±2.1 | **0.22±0.03** | 10±1.5 | **2.1±0.31** | **<0.02** |
| С28Н58 | 13±1.9 | **0.94±0.14** | 9.6±1.4 | **1.8±0.27** | **0.09** |
| С29Н60 | 11±1.7 | **<0.02** | 8.0±1.2 | **1.6±0.24** | **<0.02** |
| С30Н62 | 9.9±1.5 | **0.03** | 6.7±0.99 | **1.9±0.29** | **0.18±0.03** |
| С31Н64 | 6.9±1.0 | **0.04** | 5.3±0.80 | **1.4±0.22** | **0.42±0.06** |
| С32Н66 | 3.8±0.57 | **0.07** | 4.0±0.60 | 1.3±0.20 | **0.29±0.04** |
| С33Н68 | <0.02 | 0.08 | 3.1±0.46 | 1.2±0.18 | 0.08 |
| С34Н70 | 3.9±0.58 | **0.06** | **0.72±0.11** | **0.11** | **0.15±0.02** |
| С35Н72 | <0.02 | <0.02 | 1.6±0.24 | <0.02 | <0.02 |
| С36Н74 | <0.02 | <0.02 | 0.99±0.15 | <0.02 | <0.02 |
| The sum of n-alkanesC21-C36(high boiling fraction) | 248.5 | 8.04 | 171.99 | 41.91 | 17.31 |

The lowest indicators are highlighted in yellow.

Самые низкие показатели выделены полужирным шрифтом.