~ Contaminated Sites 2016~

# MANAGEMENT OF CRUDE OIL AND HEAVY METAL CONTAMINATED LAND IN RUSSIA USING A RISK ASSESSMENT APPROACH

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# Introduction

- Crude oil extraction and transport are often accompanied by soil contamination
- Land contamination negatively impacts economical and social developments, threats human health and natural biodiversity
- Bioremediation has a great potential to restore polluted environments by using biodegradative activities of microorganisms
- A risk based approach to the management and bioremediation of a crude oil contaminated site is proposed

# Oil industry



- The Urals is the second largest oil-production area in Russia.
- A quarter of the industry of Perm region is oil and gas.
- There are 222 oilfields in Perm region, and unexplored oil resources estimate about 600 million tones.

# Usinsk catastrophe, 1994

The worst accidental spill on land

130 000 tones of crude oil released from a ruptured pipeline

# Waste oil pits

- A leftover from oil exploration and production on land
- Over the years light fractions evaporate, and the pits contain viscous and debris laden asphalt-like oil
- Oil wastes are harmful due (i) volatile hydrocarbon emission; (ii) penetration into soil and groundwater
- There are 60 crude oil waste storage pits in Perm region

# Contaminated site management scheme

**Site evaluation & analyses**

- Topography
- Hydro(geo)logy
- Climate
- **Humans & biota**

- Soil agrochemistry
- **Oil** composition & concentrations
- Heavy metals
- Oil-degrading **bacteria**

**Risk assessment**

Hazard indices - Carcinogenic risk - Ground/surface water contamination risk - **Cleanup levels**

**Feasibility study**

- **Biodegradation limitations** (lack of electron acceptor & nutrients, low temperature, recalcitrant substances)
- Bioremediation time (**bioaugmentation & biosurfactants**)

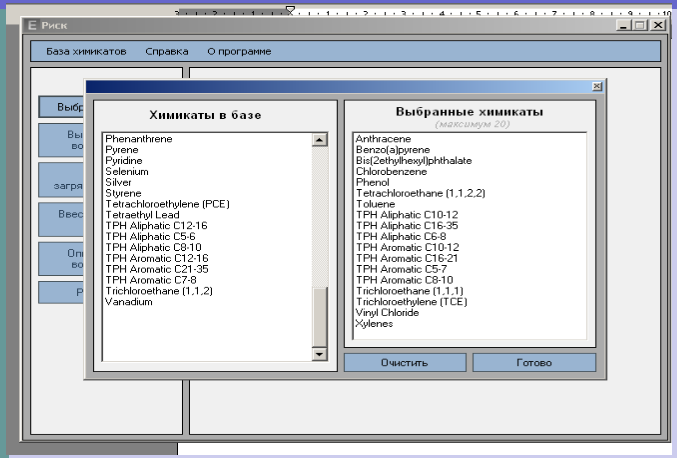
**On-site bioremediation**

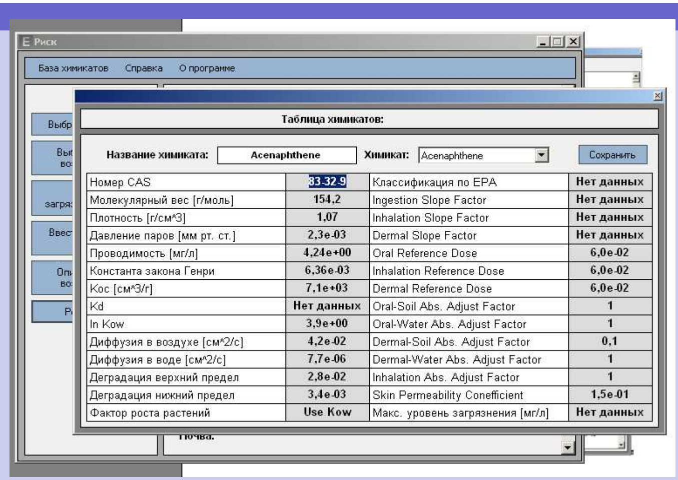
Soil-slurry **bioreactor** / **Biopile** system

**Site monitoring & closure**

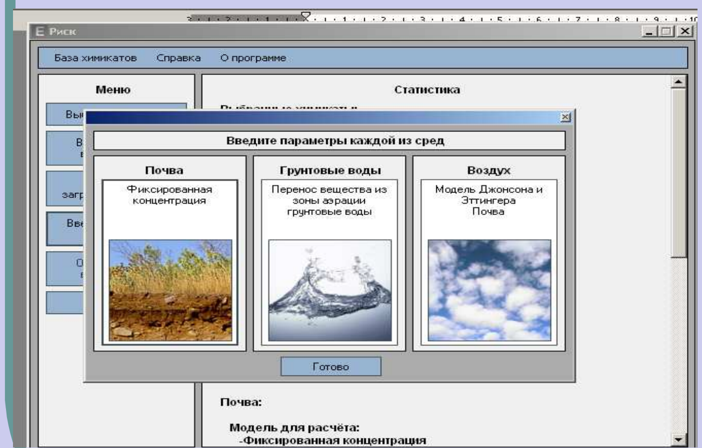
- Soil **phytotoxicity** testing
- Periodic soil sampling from biopiles
- Ground / surface water & air **monitoring**

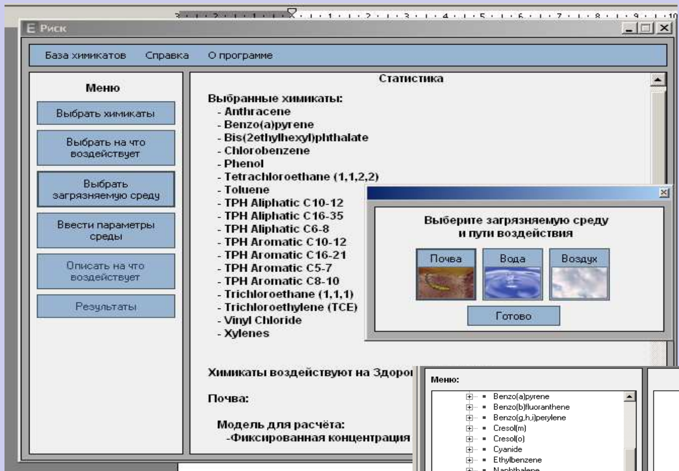
# **Risk assessment** (ecological and human health) from oil contamination

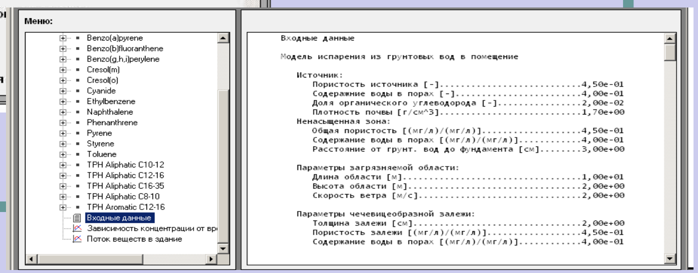




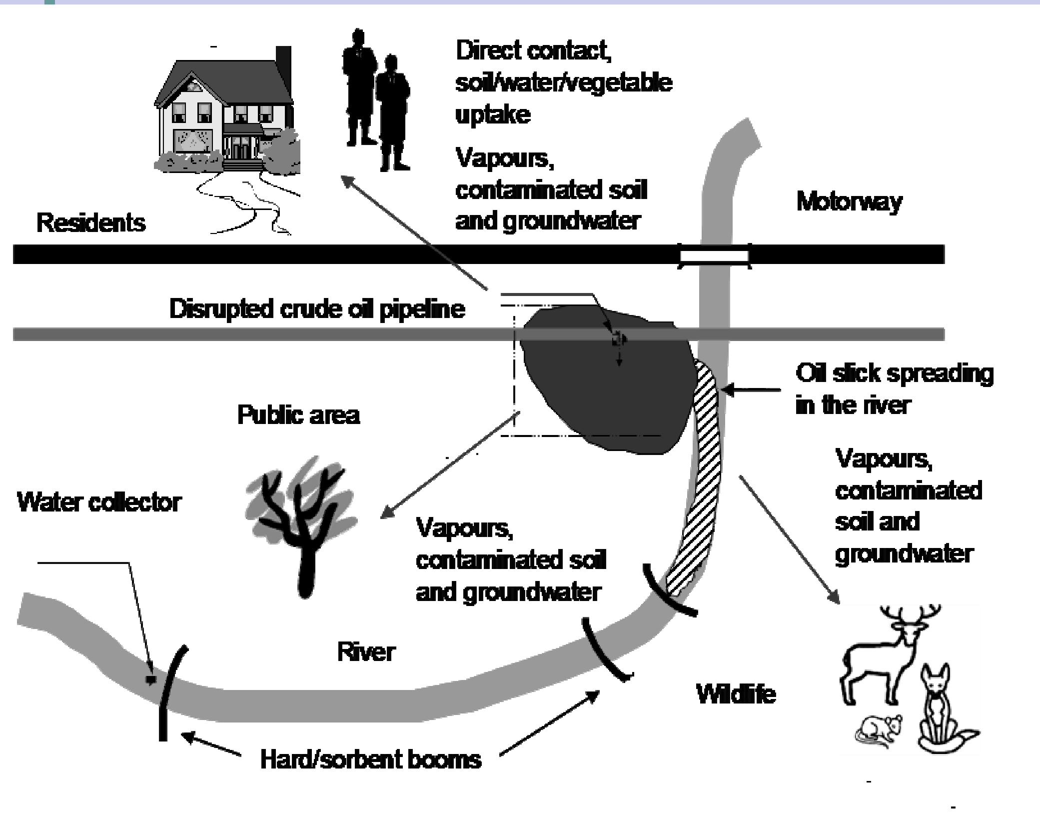



Registration Certificate of PC Software No. 2011611923. Ecological Risk Assessment of Hydrocarbon Contamination Impacts. Registered in the Federal Register of PC software of 02.03.2011.

# Risk assessment model for crude oil spillage from a disrupted pipeline




Kuyukina *et al.* (2012). *In:* Environmental Contamination / Ed. J. K. Srivastava. InTech, 2012. P. 177-198.

**Step 1.** Choose PH of concern from the database

**Step 2.** Select contaminated media, F&T model and exposure pathways

**Step 3.** Determine receptor point concentrations

**Step 4.** Describe the receptors

**Step 5.** Results (Risk calculation)

# Risk assessment data

| PHs | Concentration in soil (mg/kg) |
|---|---|
| Acenaphthene | 2.1 |
| Acenaphthylene | 0.3 |
| Antracene | 2.1 |
| Benz(a)anthracene | 8.7 |
| Benzo(a)pyrene | 14.1 |
| Benzo(b)fluoranthene | 15.6 |
| Benzo(g,h,i)perylene | 14.4 |
| Benzo(k)fluoranthene | 17.1 |
| Chrysene | 10.5 |
| Dibenz(a,h)anthracene | 2.4 |
| Fluoranthene | 17.4 |
| Fluorene | 0.6 |
| Naphthalene | 12.3 |
| Pyrene | 18.3 |
| TPH Aliphatic C10-12 | 1890 |
| TPH Aliphatic C16-35 | 126900 |
| **Total** | **$1.3 \times 10^5$** |

| PHs | Carcinogenic risk | Hazard index |
|---|---|---|
| Acenaphthene | 0 | 1.2e-04 |
| Acenaphthylene | 0 | 0 |
| Antracene | 0 | 2.5E-05 |
| Benz(a)anthracene | 8e-06 | 0 |
| Benzo(a)pyrene | 1.3e-04 | 0 |
| Benzo(b)fluoranthene | 1.4e-05 | 0 |
| Benzo(g,h,i)perylene | 0 | 0 |
| Benzo(k)fluoranthene | 1.6e-06 | 0 |
| Chrysene | 9.7e-08 | 0 |
| Dibenz(a,h)anthracene | 2.2e-05 | 0 |
| Fluoranthene | 0 | 1.5e-03 |
| Fluorene | 0 | 5.3e-05 |
| Naphthalene | 0 | 2.2e-03 |
| Pyrene | 0 | 2.2e-03 |
| TPH Aliphatic C10-12 | 0 | 1.7e-01 |
| TPH Aliphatic C16-35 | 0 | 2.2e-01 |
| **Total** | **$1.8 \times 10^{-4}$** | **$4.0 \times 10^{-1}$** |

# Summary of clean-up levels

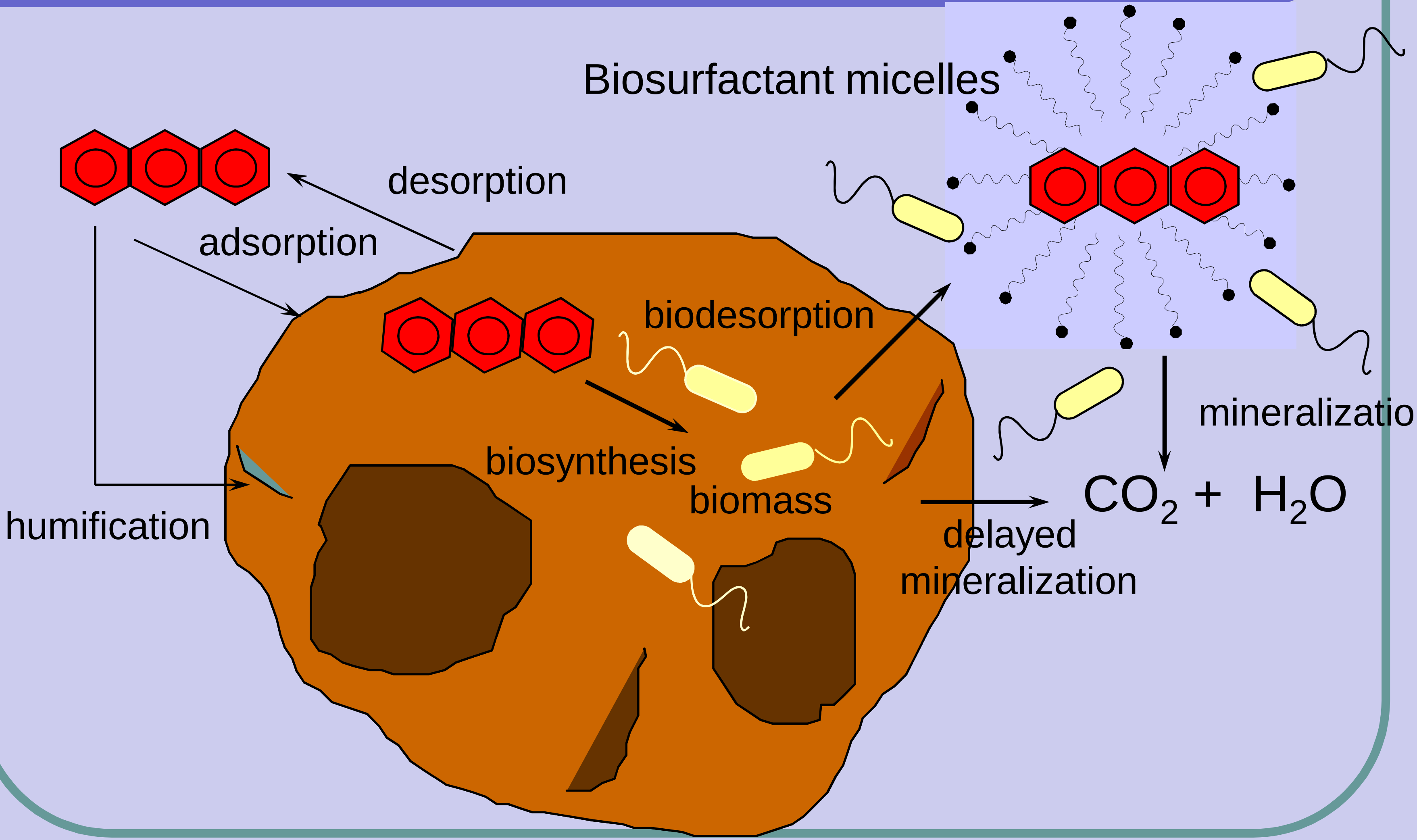
| Clean-up Levels in Surface Soil | CLSSs [mg/kg] |
|---|---|
| Acenaphthene | 1.5E+03 |
| Acenaphthylene | 1.0E+03 |
| Anthracene | 7.6E+02 |
| Benz(a)anthracene | 8.1E+02 |
| Benzo(a)pyrene | 8.1E+00 |
| Benzo(b)fluoranthene | 8.1E+01 |
| Benzo(g,h,i)perylene | 1.0E+04 |
| Benzo(k)fluoranthene | 8.1E+01 |
| Chrysene | 8.1E+02 |
| Dibenz(a,h)anthracene | 8.1E+00 |
| Fluoranthene | 1.0E+03 |
| Fluorene | 1.0E+03 |
| Indeno(1,2,3CD)pyrene | 7.1E+00 |
| Methyl napthalene (2) | 1.0E+03 |
| Naphthalene | 5.1E+02 |
| Phenanthrene | 1.0E+04 |
| Pyrene | 7.6E+02 |
| TPH Aliphatic C10-12 | 2.5E+04 |
| TPH Aliphatic C16-35 | 1.0E+04 |
| **TPH (total)** | **1.3E+04** |

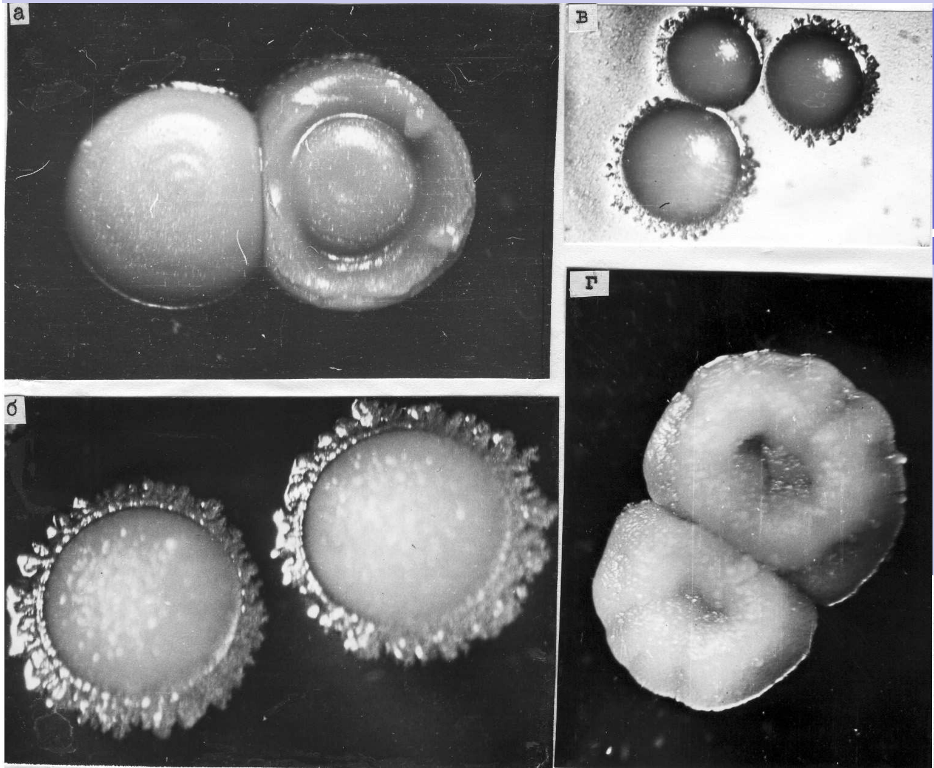
**"Is the contamination appropriate for bioremediation?"**

**Feasibility study**

- ❖ Slurry bioreactor operating during cold seasons.
- ❖ Bioaugmentation with immobilized cultures of hydrocarbon-oxidizing bacteria.
- ❖ Biosurfactant addition.

# Fate of hydrocarbon pollutants in soil

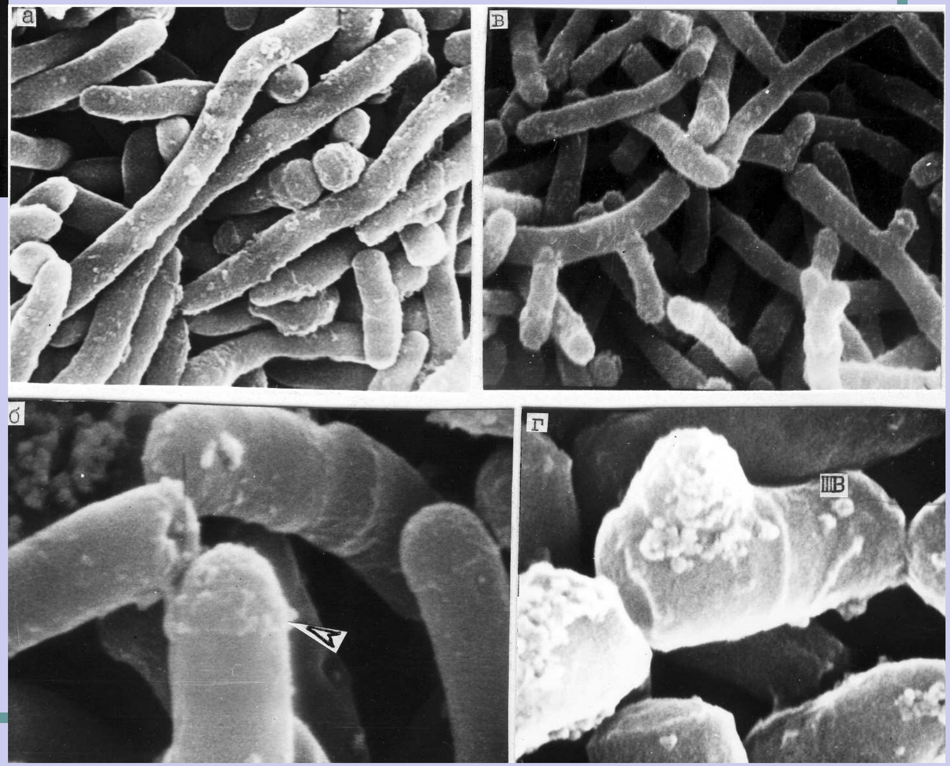




# The IEGM Collection of Alkanotrophic Microorganisms

The **2000** non-pathogenic and aerobic bacterial cultures isolated from **contrasting** climatic regions.

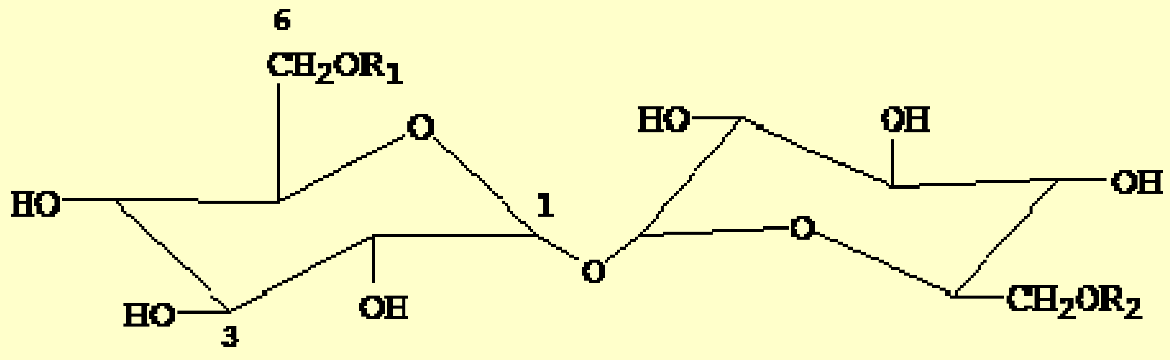
**www.iegm.ru/iegmcol/index.html**



- 86 species of 19 bacterial genera
- Actinobacteria of the genus ***Rhodococcus*** comprise the major portion of the Collection
- Strains – **biodestructors** of organic pollutants, **producers** of amino acids, vitamins and **biogenic surfactants**

**WDCM # 768 http://www.wfcc.info/datacenter.html**

# Structure of *Rhodococcus* biosurfactant




**General scheme of Trehalose Lipid complex**

**TL1, TL2 and TL3 – structural components**

**TL1**

$$R_1/R_2 = -C(=O)-\overset{\alpha}{CH}[(CH_2)_n-CH_3]-\overset{\beta}{CH}(OH)-(CH_2)_m-CH_3$$

m + n = 29 – 41 (centered at 35)

**TL2**

$$R_1/R_2 = -C(=O)-(CH_2)_m-CH_3$$

m = 13 – 15 (i.e. probably 14 + 12 and 14 + 16 with main component 14 + 14)

**TL3**

$$R_2 = H$$

$$R_1 = -C(=O)-(CH_2)_m-CH_3$$

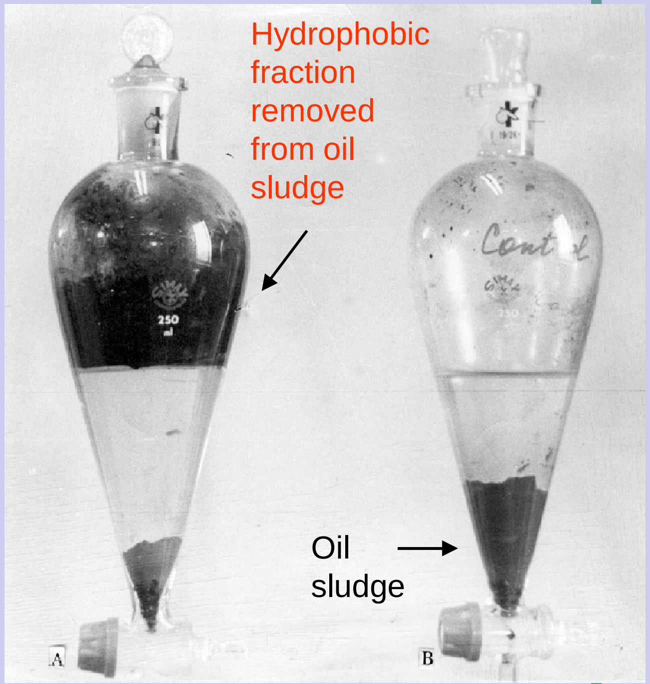
m = 10 – 14 (main component 12)

**Kuyukina *et al.* (2001). *J. Microbiol. Methods* 46: 149-156**

**Philp *et al*. (2002). *Appl. Microbiol. Biotechnol.* 59: 318–324**

# Oil removal from soil using *Rhodococcus* biosurfactants

| *Rhodococcus* species | Oil removed, % I | II | III | IV |
|---|---|---|---|---|
| *R. erythropolis* | 96 | 77 | 70 | 63 |
| *R. opacus* | 87 | 77 | 22 | 10 |
| ***R. ruber*** | **98** | **98** | **87** | **50** |
| Control (water) | 31 | 20 | 5 | 2 |

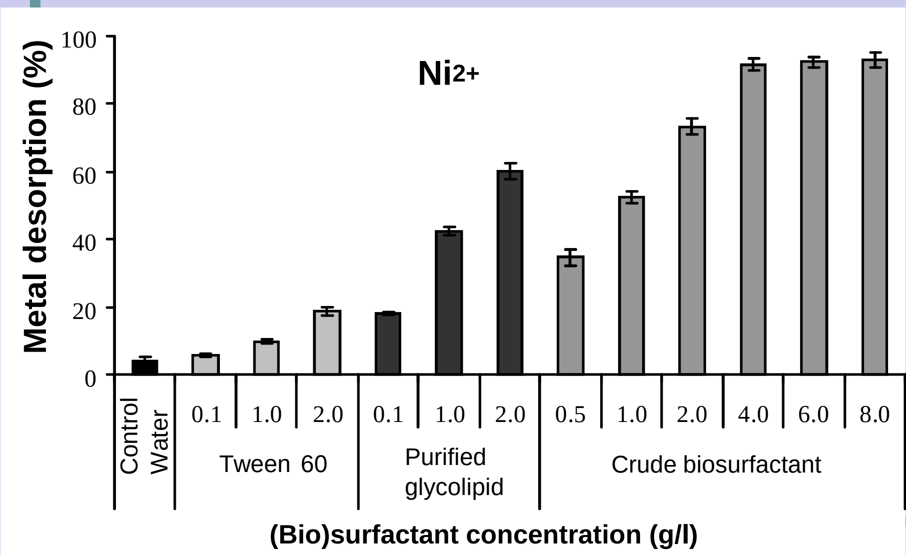


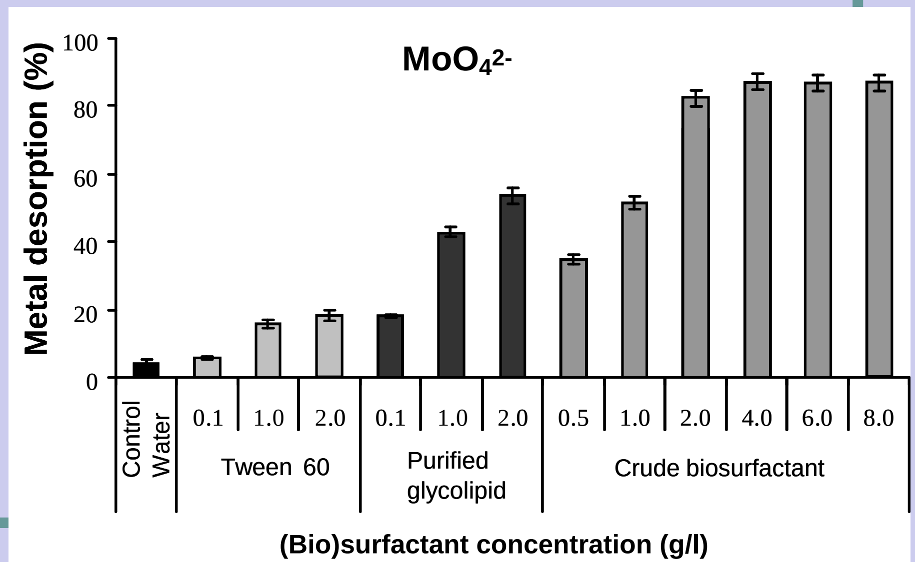

Oils have increasing asphaltenes and high molecular weight paraffins

**Ivshina *et al*. (1998). *World J. Microbiol. Biotechnol.* 14: 711-717**
**Kuyukina *et al.* (2005). *Environ. Int.* 31: 155-161**

# Heavy metal removal (%) from soil

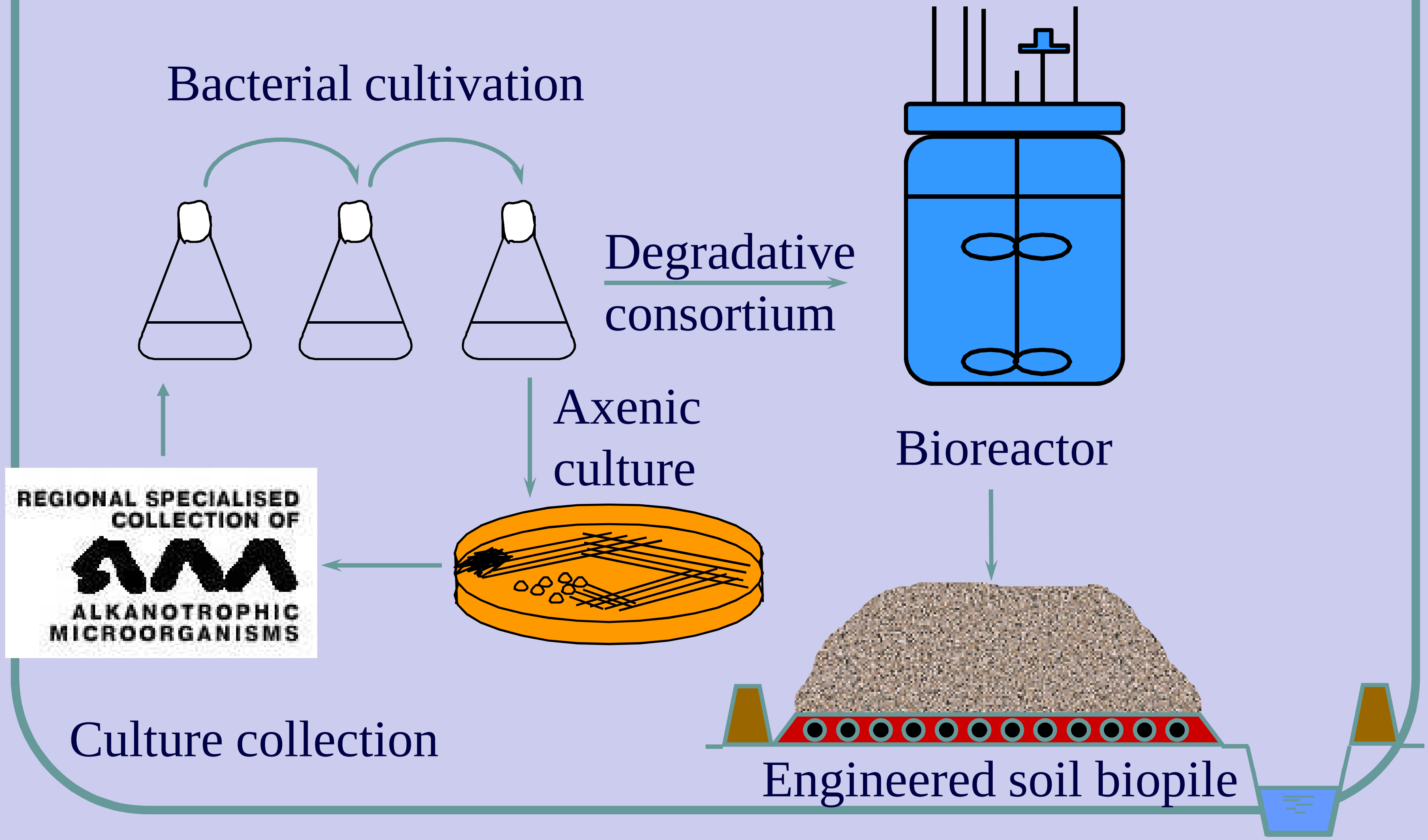
| Heavy metals | *Rhodococcus* biosurfactant | | Tween 60 | Control (water) |
|---|---|---|---|---|
| | Crude | Purified | | |
| $Cd^{2+}$ | **82.3** | 48.1 | 16.5 | 2.3 |
| $CrO_4^{2-}$ | **87.1** | 58.0 | 19.3 | 3.9 |
| $MoO_4^{2-}$ | **88.3** | 54.6 | 19.7 | 6.3 |
| $Ni^{2+}$ | **92.5** | 66.7 | 21.1 | 4.8 |
| $Pb^{2+}$ | **68.7** | 42.3 | 15.1 | 1.8 |




**Kuyukina *et al.* (2010). *Russian J. Biomechanics.* 14, 4 (50), 34-40; Ivshina et al., (2013). *Ecology. 44, 123-130.***

# Bioaugmentation



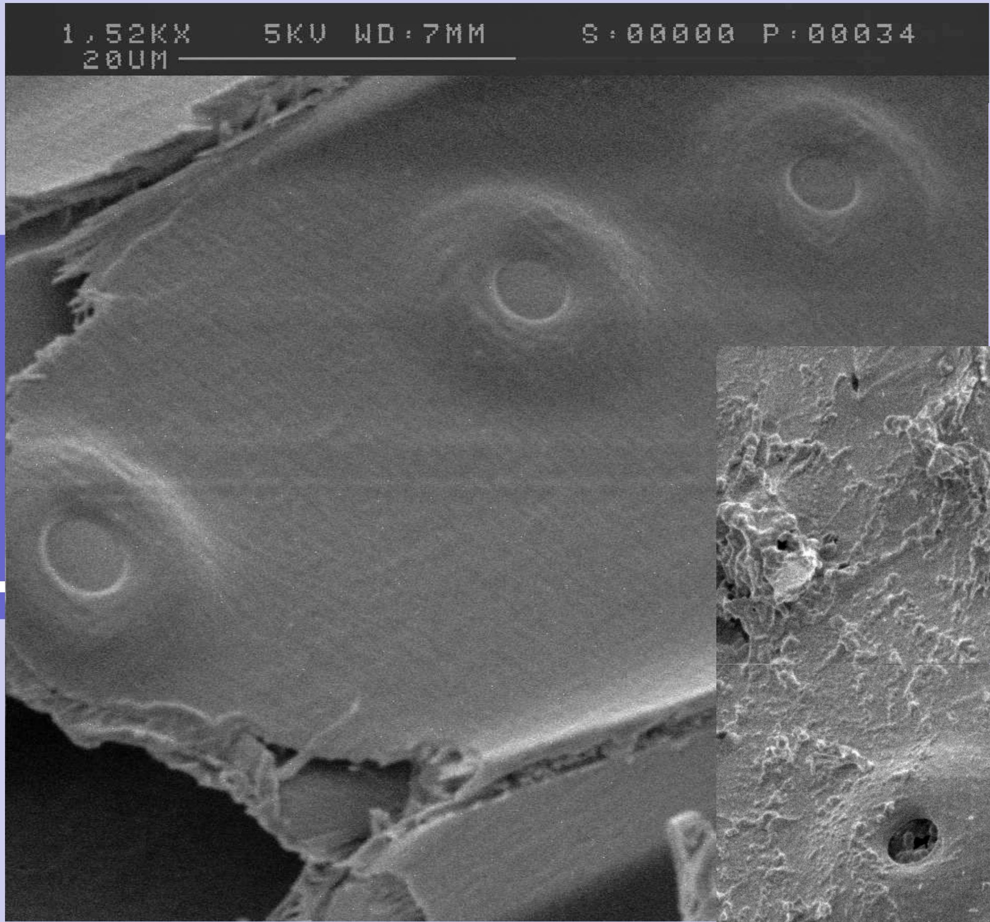
# Oil absorbing capacity of agriculture wastes

| **Type of waste** | **Oil sorption capacity (%, wt.)** | **Availability in Urals Region** |
|---|---|---|
| Cotton waste | 600 - 3000 | No |
| Hemp fibre | 1000 - 1300 | No |
| Corncobs | 500 - 700 | No |
| **Sunflower husks** | 600 - 800 | **Yes** |
| **Sawdust** | 450 - 850 | **Yes** |
| **Poultry feathers** | 500 - 900 | **Yes** |

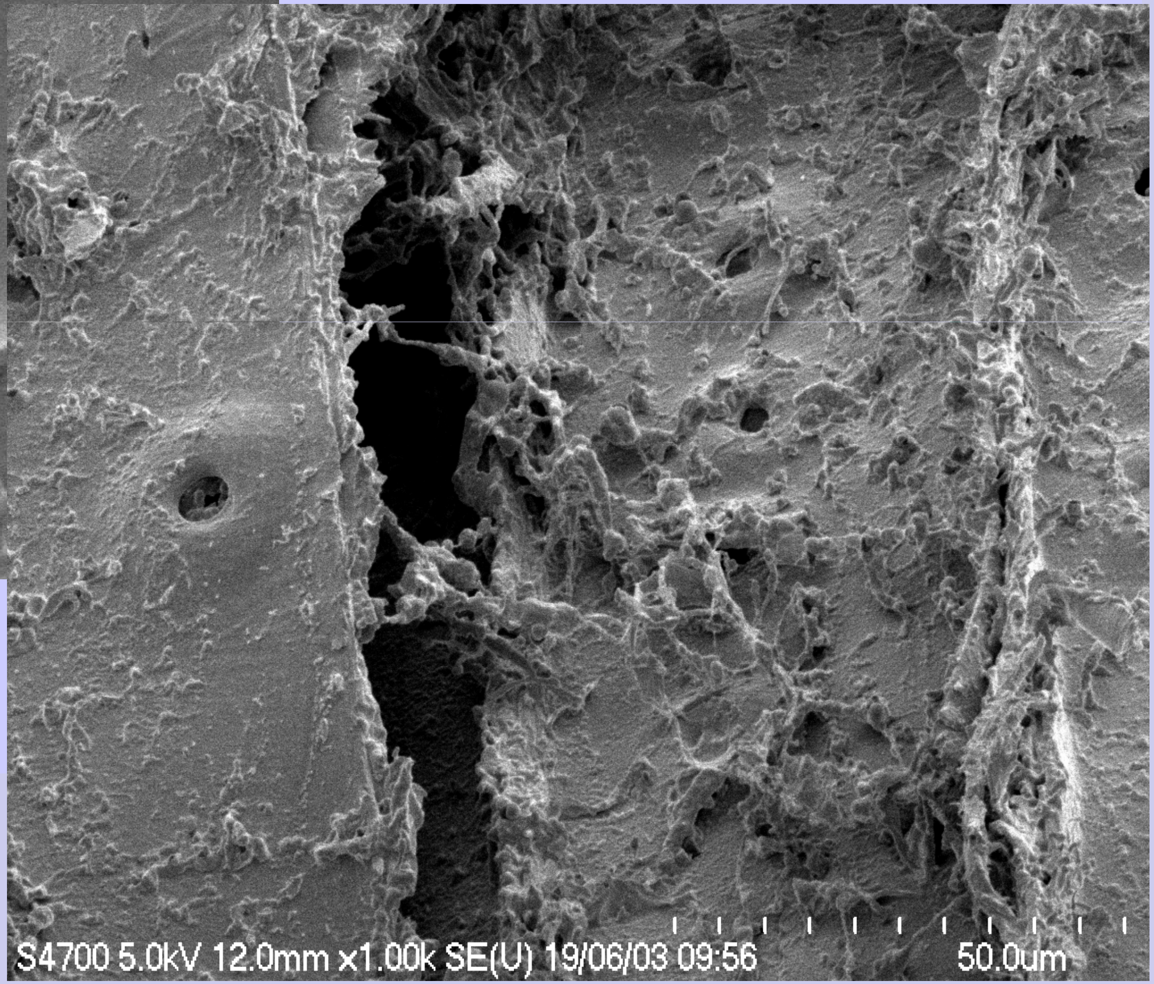
# Immobilisation of *Rhodococcus* cells on different matrices

| Immobilisation matrix | | Water-absorbing capacity, g $H_2O$/g | Bacterial adsorption, mg of dried cells/g | Hexadecane degradation rate, mg/g·h |
|---|---|---|---|---|
| **Base material** | **Treated with hydrophobizing agent** | | | |
| **Sunflower husks** | None | 2.03 ± 0.18 | 9.0 ± 3.0 | 53.0 ± 4.0 |
| | **Linseed oil varnish (“Olifa”)** (1:2, v/v) | 1.52 ± 0.08 | 2.0 ± 0.5 | 42.0 ± 6.0 |
| **Sawdust** | None | 2.55 ± 0.15 | 39.0 ± 5.0 | 71.0 ± 7.0 |
| | “Olifa” (1:2, v/v) | 0.39 ± 0.02 | 15.5 ± 1.5 | 46.0 ± 6.0 |
| | **“Olifa” (1:0.1, v/v)** | **1.24 ± 0.09** | **46.5 ± 1.0** | **107.0 ± 9.0** |
| | Si-organic emulsion | 1.93 ± 0.10 | 46.0 ± 3.0 | 65.0 ± 2.5 |
| | **Biosurfactant** | **1.54 ± 0.05** | **40.0 ± 4.5** | **72.0 ± 4.5** |
| | *n*-Hexadecane vapour | 1.68 ± 0.12 | 41.0 ± 4.0 | 42.5 ± 5.0 |
| **Poultry feathers** | None | 1.65 ± 0.10 | 6,0±1,0 | 43.0 ± 7.0 |
| | “Olifa” (1:0.1, v/v) | 1.48 ± 0.12 | 56.0 ± 6.5 | 61.0 ± 4.0 |
| | **Si-organic emulsion** | **1.60 ± 0.04** | **69.0 ± 5.6** | **83.0 ± 8.0** |

**RU Patent 2298033; Podorozhko et al. (2008) *Biores. Technol*. 99:2001-2008;**
**Kuyukina et al. (2009) *Int. Biodeter. Biodegrad*. 63:427–432**



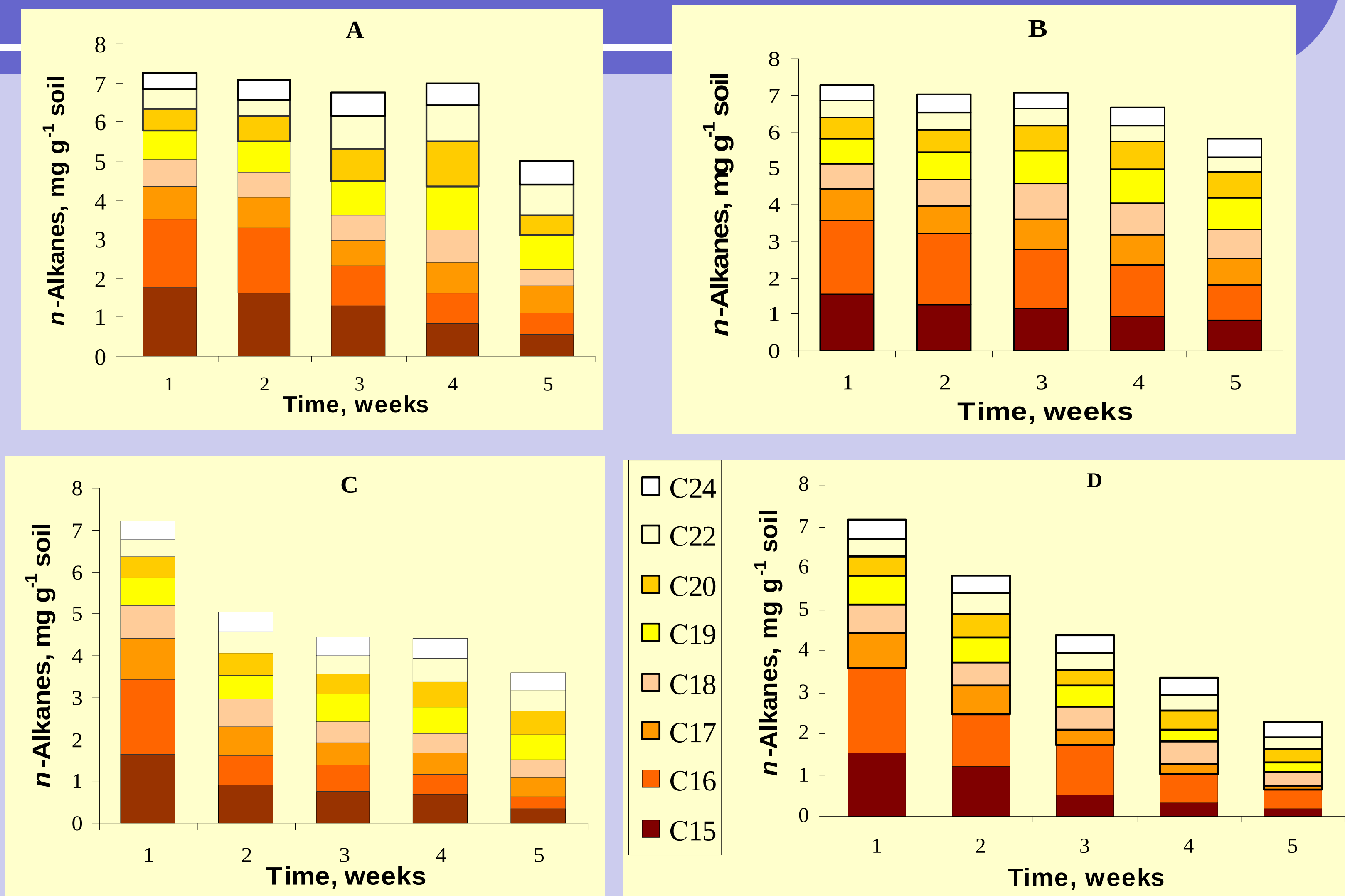

Electron micrograph
of unmodified sawdust
x 1000




Hydrophobized sawdust with
immobilised *Rhodococcus* cells
x 1000

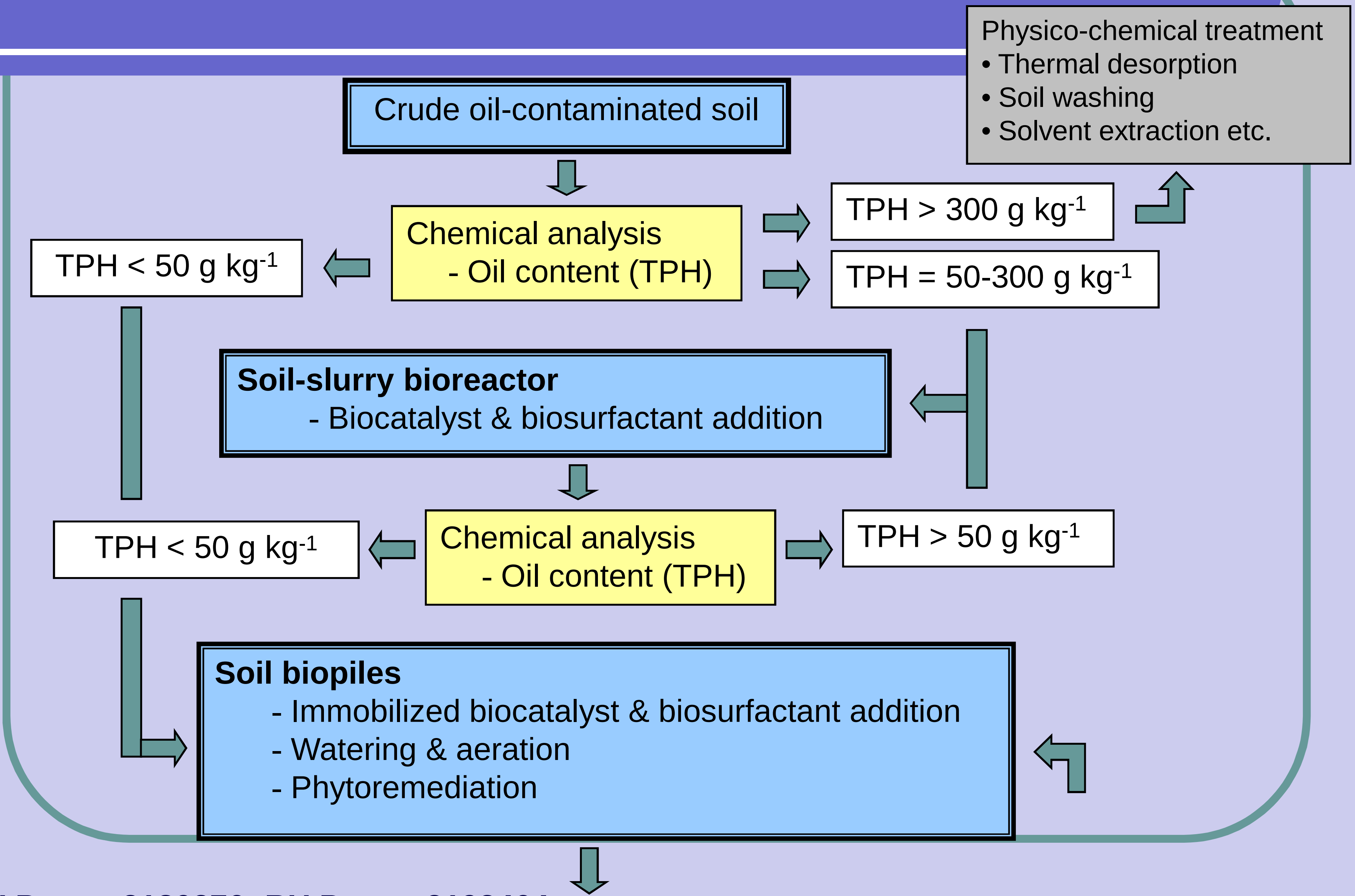
**RU Patent 2298033**

# Dynamics of oil biodegradation in laboratory piles



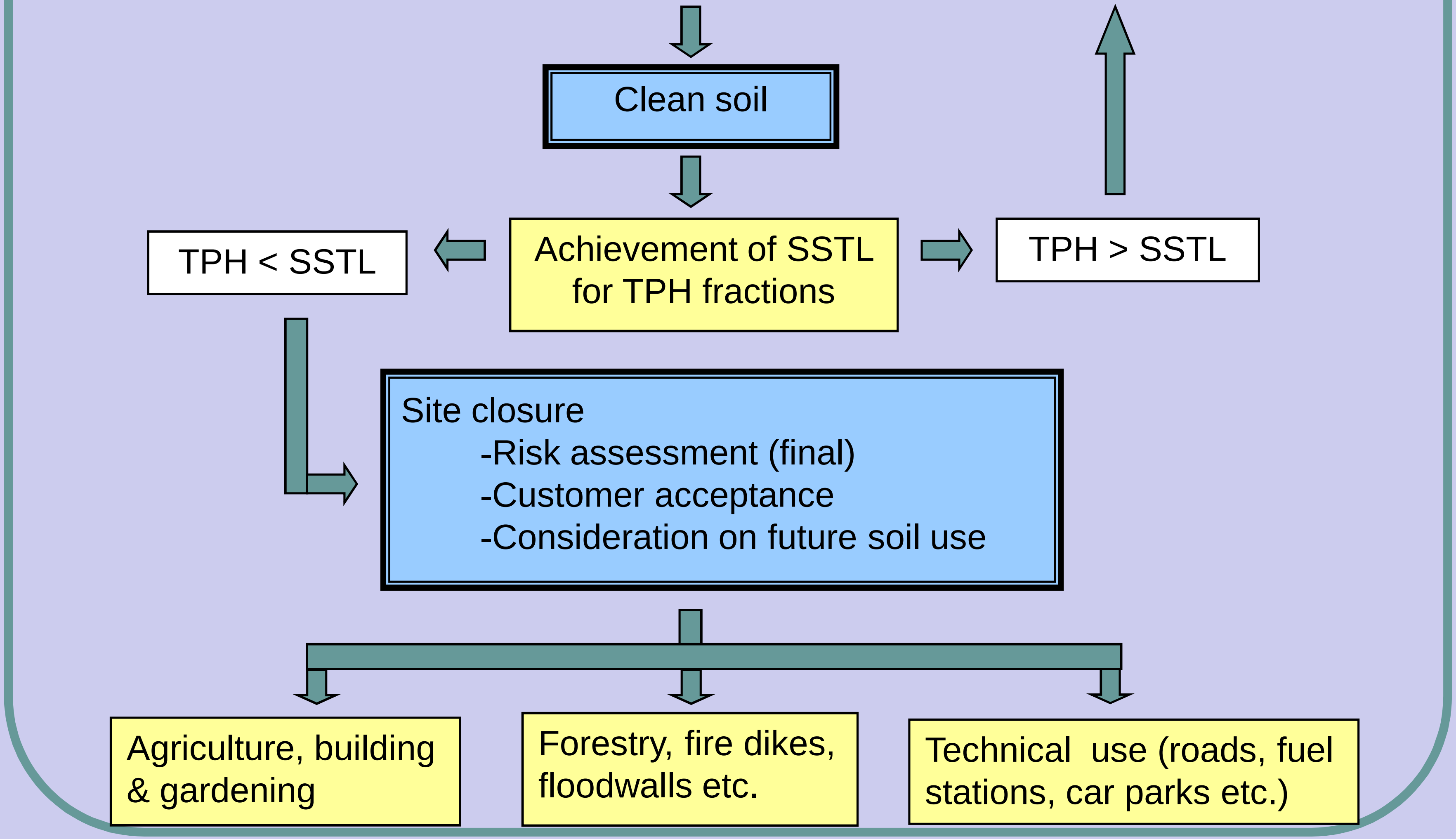

**Soil systems: A – control (no additions); B – non-inoculated sawdust; C – immobilized *Rhodococcus* cells; D –immobilized *Rhodococcus* + biosurfactant**

# Bioremediation scheme for oil-contaminated soil




**RU Patent 2180276; RU Patent 2193464**

# Bioremediation scheme for oil-contaminated soil




**RU Patent 2180276; RU Patent 2193464**

# Why slurry bioreactor ?

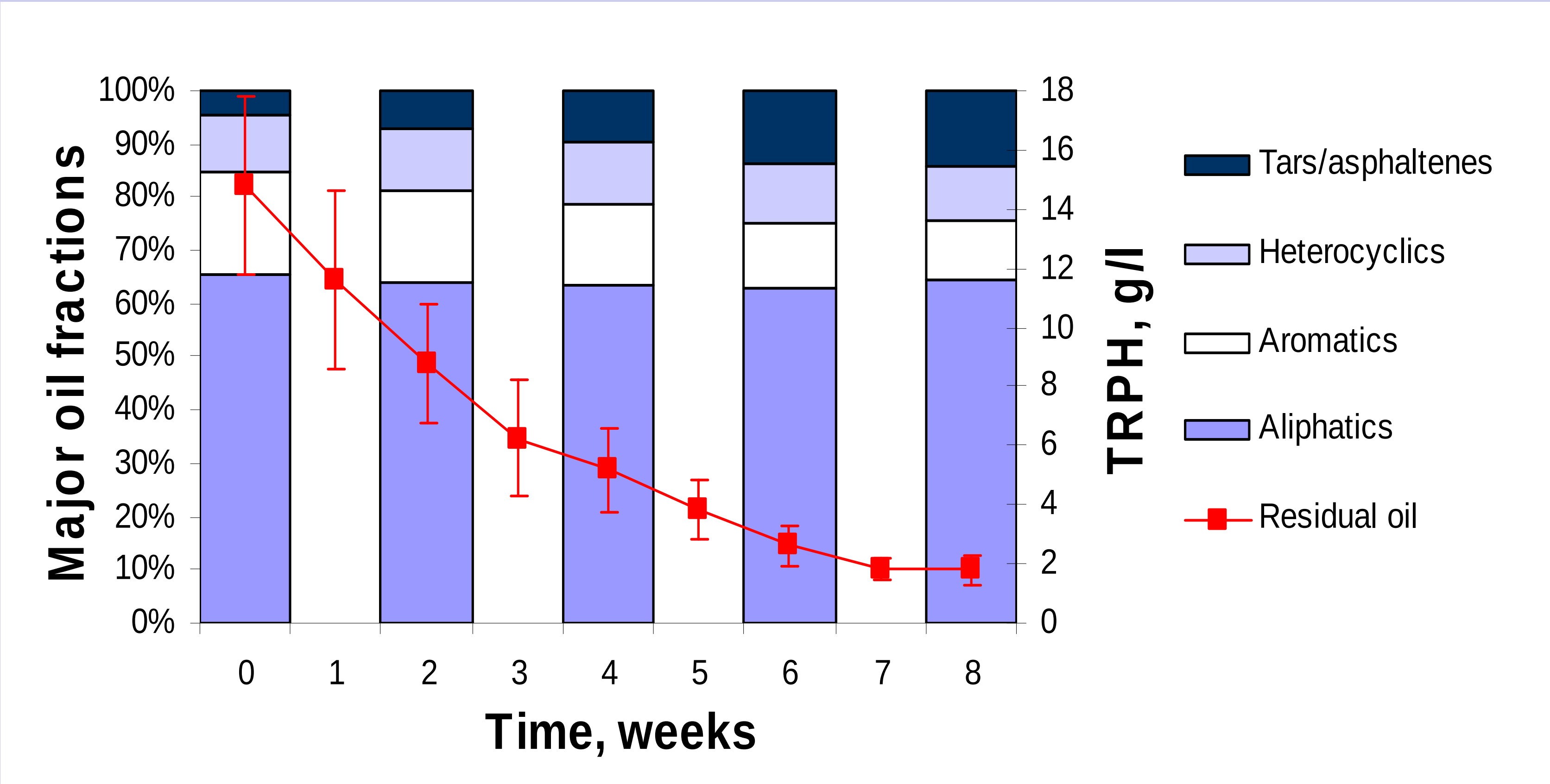
- Facilitates growth of hydrocarbon-oxidizing bacteria
- High contact area between oil degraders and pollutant
- Control of operating parameters ($T^o$, pH, $O_2$, biomass)
- Operation under cold conditions
- Reduction of treatment time and biocatalyst application rate

# Slurry bioreactor

- Work volume – 30 $m^3$.
- Work regime – periodic.
- Solid phase – 30-40 %.
- Air supply – 50 liter/min.
- Mixing rate – 50 rpm.
- Biocatalyst (2 kg/$m^3$) – weekly.

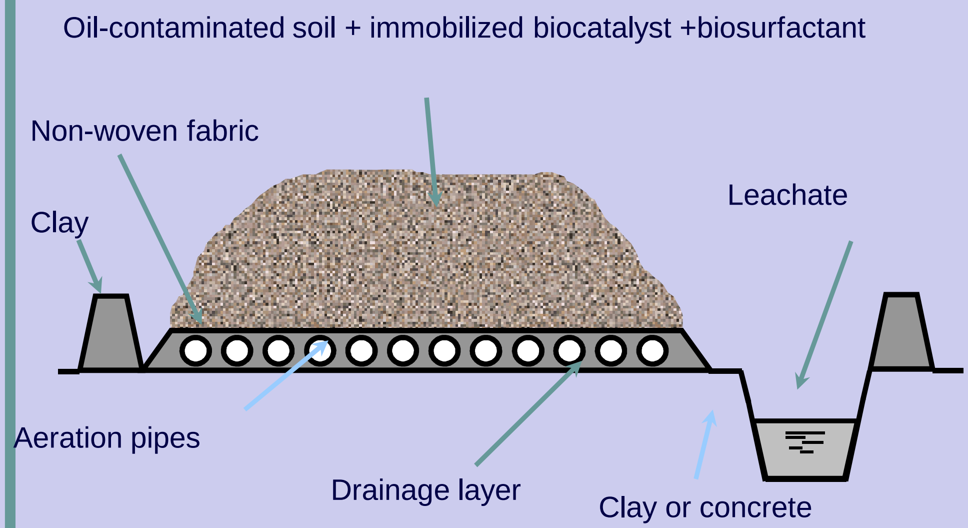
Kuyukina et al (2003) *Soil Sediment Contamin*. 12:85–99; Kuyukina et al. (2009) *Int. Biodeter. Biodegrad*. 63:427–432

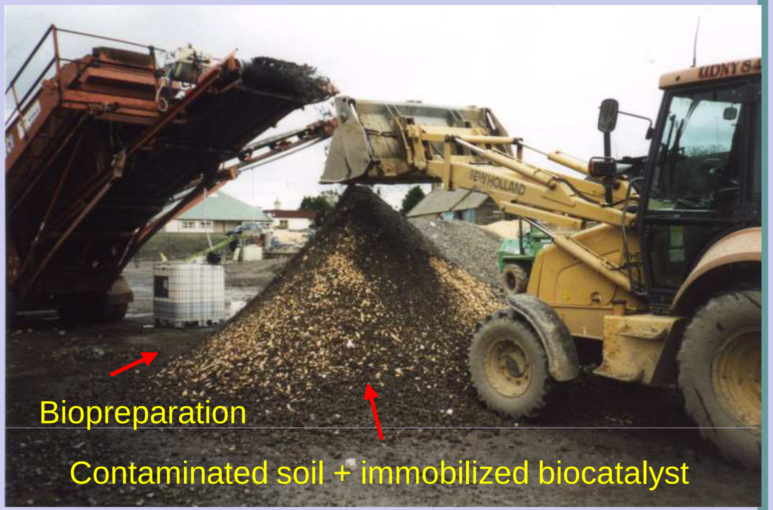
# Oil degradation dynamics in slurry bioreactor



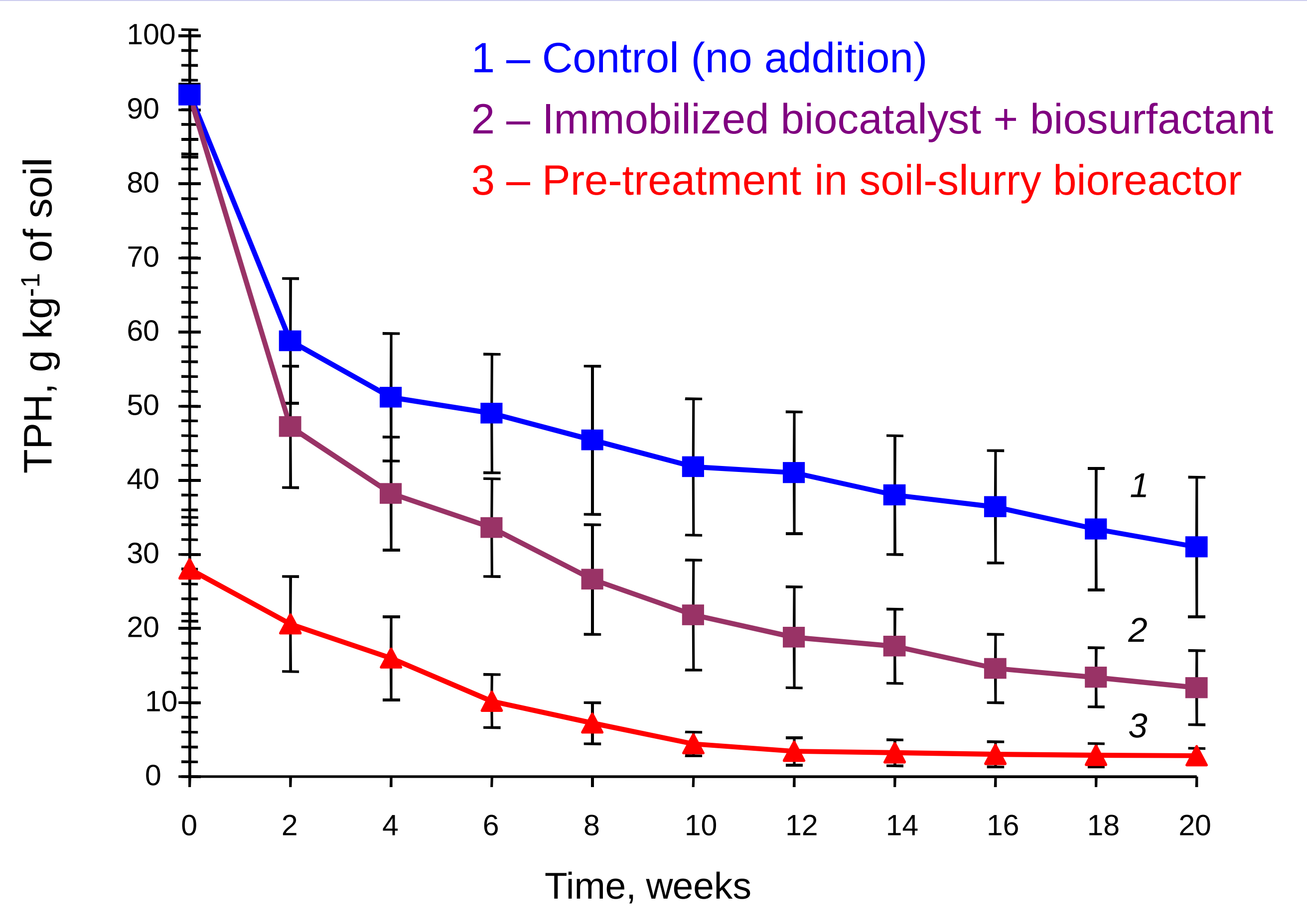

Note. The samples were taken from a liquid phase of bioreactor

# Field biopile system

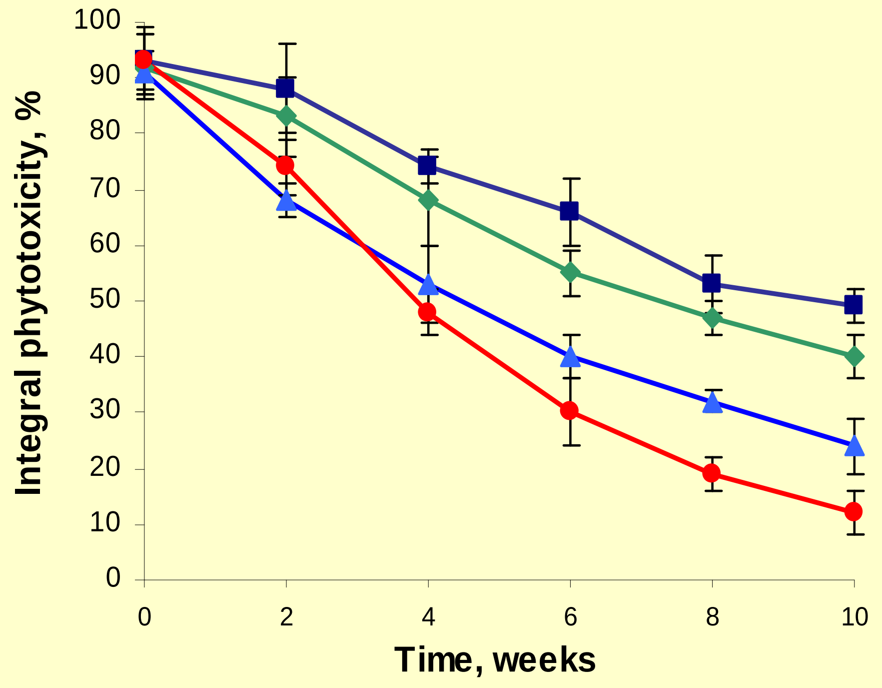


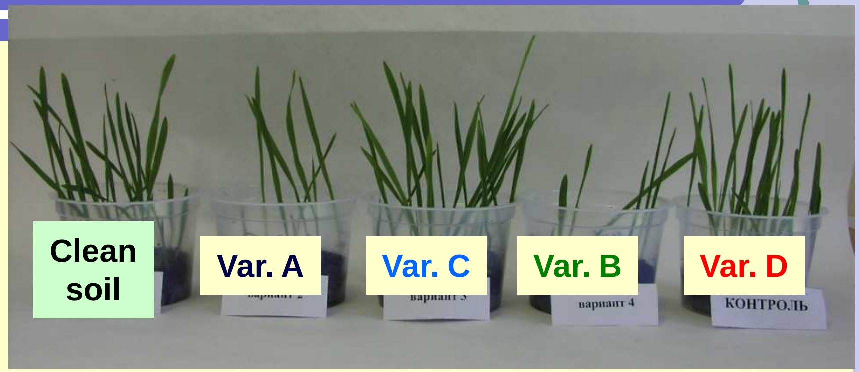




# Oil contamination decrease in field biopiles

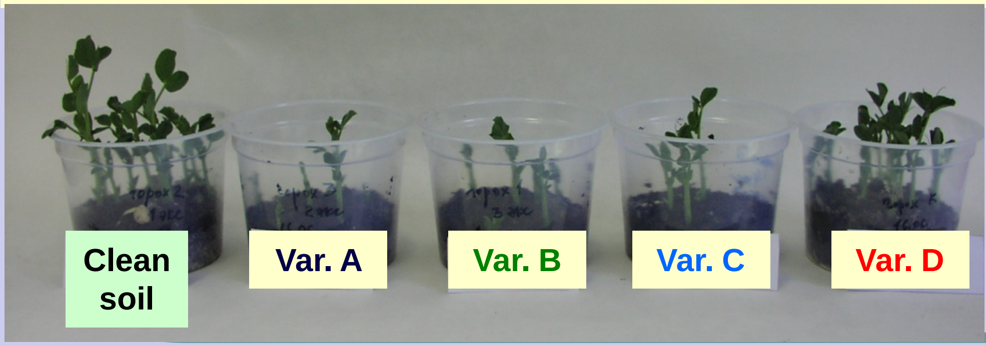


# Phytotoxicity results

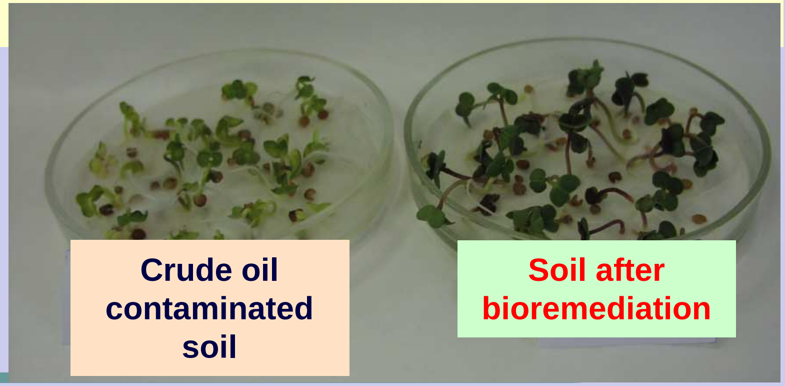



Soil phytotoxicity testing using oats




Soil phytotoxicity testing using peas




Water-soluble fraction phytotoxicity testing using radish seed germination

# Ecotechnology developments are protected by RU patents

- **RU Patent 2180276.** An oleophilic preparation for oil-contaminated soil treatment. 10.03.2002.
- **RU Patent 2193464.** Bioremediation method for soils contaminated with oil or oil products. 27.11.2002.
- **RU Patent 2216525.** Microbiological treatment of industrial wastes contaminated with heavy metals, including zinc, cadmium and lead. 20.11.2004.
- **RU Patent 2298033.** Composition for production of carrier for immobilized hydrocarbon-cleaving microorganisms, and method for carrier production. 27.04.2007.
- **RU Patent 2475542.** A method and facility for determining the efficacy of adsorptive immobilization of microorganisms and monitoring of the functional activities of immobilized microbial cell-based biocatalysts. 20.02.2013.
- **Software registration certificate 2011611923.** Assessment of ecological risk from hydrocarbon contamination. 02.03.2011.
- **Software registration certificate 2011617650.** Calculation of soil-washing processes for oil- and heavy metal-contaminated soil using a Rhodococcus biosurfactant. 30.09.2011.
- **Software registration certificate 2012616511.** Module system for calculation of hydrocarbon contamination impact on human health. 24.09.2012.

# Priroda-Perm, Plc. is a strategic partner of Perm University

### Activity fields

1. Processing and utilization of solid/liquid oily wastes.
2. Treatment and utilization of drilling mud cuttings.
3. Utilization of paraffin sediments, contaminated materials, wastewaters.
4. Emergency response to oil spills.
5. Oil storage tank cleanout.
6. Oil contaminated soil remediation.
7. Expert examination of production safety.



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# Processing and treatment of oil-contaminated soil (OCS) using a bioremediation technique

Unloading of OCS from oil waste storage pit using special-purpose machinery

Zone of liquid waste accumulation

Development of a technological site

Unloading of OCS to the technological site

# Cleanup of the oil waste storage pit


# Conclusion

- Risk based approach to the management and bioremediation of a crude oil contaminated site is applied.
- Bioremediation techniques such as soil-slurry bioreactors, augmentation with immobilized cultures of hydrocarbon-oxidizing bacteria and biosurfactant addition were proven to be efficient in the clean-up of oil-contaminated soil in cold climate conditions.
- In a pilot scale field trial, heavily contaminated soil was cleaned-up to within risk assessment standards.
- Eco-biotechnology developed is commercialized with the Priroda-Perm company.

**Acknowledgements**
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# Our team

## PGNIU/IEGM group

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# Thank you for your attention !

Questions ??