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Geoelectrical Observation of *Yarrowia lipolytica* Bioremediation of Petrol-Contaminated Soil

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Abstract

Our investigation was localized in the area of fuel base A of the former Soviet firing ground Borne Sulinowo, where petrol contamination of soils had been observed.

The suspenssion of yeast strain *Yarrowia lipolytica* A–101 was introduced into twelve one-metre deep bore-holes. The process of bioremediation was controlled using electromagnetic and resistivity methods, and microbial enumeration was done on Hy–Check slides. The measurements were carried out along the same profile in spring 2003, autumn 2003 and spring 2004. The significant increase of electrical conductivity of soils was observed in the vicinity of bore-holes with introduced yeast. The study showed the ability of *Yarrowia lipolytica* yeast to remedy petrol contaminated soils and the efficiency of applied geoelectrical methods to this process monitoring.

Keywords: *Yarrowia lipolytica,* soil contamination, electrical conductivity, resistivity

Introduction

The environment consists of biotic and abiotic elements which are linked together forming a fragile ecological web. Superimposed on this natural fragility, human activities can lead to serious chemical and mechanical changes. Among other pollutants, contamination from petrol has provoked a durable awareness. Oil contamination of coasts of the Arabian Gulf is one example [1]. One of the methods of soil remediation is bioremediation, which involves treatment of contaminated soil with microorganisms able to metabolise polluting chemicals [2]. Such treatment could be done *in situ* or *ex situ* [3]. In the *ex situ* protocol the monitoring of such decontamination is easy to control, not only for the measure of the concentration of undesirable chemicals but also for microbial monitoring. One of the main problems ocurring in the *in situ* microbial bioremediation is the difficulty measuring not only the growth of microorganisms, but also their effect on a particular microenvironment. The impact of the introduced microbial biomass on a particular microecosystem comprised the influence on plants and animal development and caused many possible interactions between endogenous microorganisms, very often favouring their growth.

The second main difficulty is the measure of such decontamination *in situ*. Some of the applied methods are based on measurements of physical properties of soils, like electrical conductivity and resistivity. Electrical specific resistivity of hydrocarbons is high, varying from 10^6-10^9 Ωm [4, 5]. Consequently, oil contamination of soils causes geophysical anomalies related to changes of physical soils properties, specially a significant increase of electrical resistivity and appropriate de-

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crease of electrical conductivity were observed. It is assumed that an increase of electrical conductivity (decrease of electrical resistivity) indicates that bioremedation process proceeds and oil contamination starts to decay. Thus, the application of geophysical methods may be advantageous in the control of soil decontamination [6, 7, 8]. Time and space variations of the measured physical properties give information on the process of soil bioremediation.

However, the main and attended result of microbial bioremediation is soil decontamination, a pilot study on soil decontamination *in situ* was the subject of this study. Along a one-hundred-metre profile at a location where oil contamination had been found by previous electromagnetic and resistivity investigations [9, 10], twelve bore-holes in a part of contaminated soil of Base A in Borne Sulinowo [11] were inoculated with non-conventional yeast *Yarrowia lipolytica* suspension. This yeast species, recognized as a GRAS organism, non pathogenic, is able to assimilate alkanes, alkenes, and oils to degrade pesticides and is a citric acid producer [12, 13]. The level of contamination was monitored *in situ* by geoelectrical method. Two geoelectrical methods have been applied in this bioremediation project. The first and basic one is the electromagnetic induction method and the second is the resistivity imaging method. Microbial development was controlled by Hy–Check enumeration of the suspensions of soil samples taken from twelve 1-metre deep bore-holes.

Material and Methods

The studied area was localized in fuel base A of the former Soviet firing ground Borne Sulinowo. The investigation was performed several metres from a partly excavated underground fuel container.

Microbiological Material

Yeast strain *Yarrowia lipolytica* A–101 purchased from the Department of Biotechnology and Food Microbiology of Agriculture University of Wrocław was during 48h propagated from 10% inoculum in 5 L Biostat bioreactor, at 28°C with the control of pH (5.5) and continuous steering (500rpm). YPG medium was used for seed and bioreactor culture. The concentration of yeast at the end of propagation was 3.27 x 10^9 cells/ml. The post–culture liquid–yeast with residual medium was diluted ten-fold and applied on contaminated soil, three litres into each of twelve 1m-deep bore–holes.

A set of 12 bore–holes was created on the earlier-monitored territory of Base A of Borne Sulinowo. Yeast suspension was introduced into bore-hole numbers 1-10 and the same volume of water was introduced into bore-holes 11 and 12. Samples of 7g of soil from all 12 bore-holes were taken before and after inoculation of yeast on days 1, 2, 3, 4 and 7 in aseparate plastic bag, mixed with 50ml of commercial drinking water. Hy–Check (Difco) slides were immersed for few seconds in that mixture and incubated at 37°C, during 2-7 days for bacterial and yeasts colony number estimation. The number of cells (bacterial, yeast or fungies) is reported to 1g of soil.

One year later soil yeast enumeration, for the same 12 bore–holes and 2 control sites, was done on oxytetracycline-agar plate (total number of yeast), on hexadecan – MMT–agar (for hexadecan assimilating yeasts) and on CA – production – agar – medium – with bromophenol red (citric acide producing strain). The growth on hexadecan and citric acid secretion were the two main characteristics of the *Yarrowia lipolytica* A–101 strain.

Geoelectrical Methods

Two geoelectrical methods were applied: the electromagnetic induction method and the resistivity imaging method. The measured physical properties were, respectively: electrical conductivity [mS/m] and electrical resistivity [Ωm]. The measurements were carried out along one-hundred-metre profiles (Fig. 1) in spring and autumn 2003 and in spring 2004 using conductometer EM31–MK2 (Geonics, Canada). The field measurements were carried out with horizontal orientation of measuring dipole and automatic recording every 0.5s along the chosen profile. The applied method allowed us to obtain apparent conductivity values to a depth of approximately 6 metres under the surface for points distant about 0.3m along the measured profile. The analysis of changes of the apparent conductivity versus profile length was performed by Oasis Montaj software.

Along the same profile resistivity measurements with Lund Imaging System were carried out in autumn 2003 according to two measuring protocols: Schlumberger L and Schlumberger S with 2.5m electrode spacing. The specific resistivity cross section was performed by Res2D software.

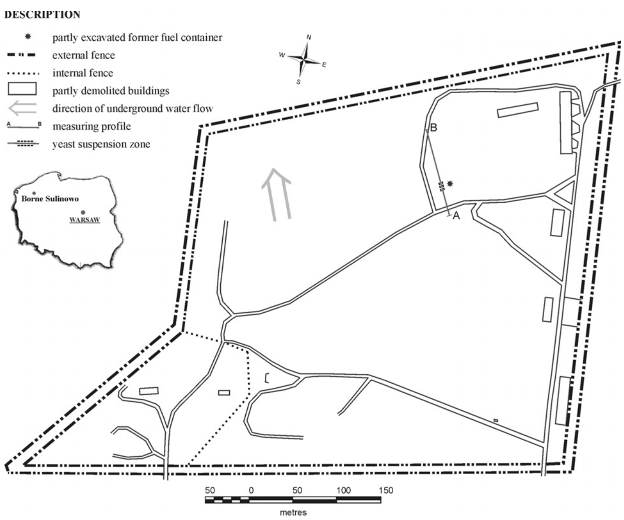

Fig. 1. Scheme of fuel base A – Borne Sulinowo.

Results

The number of bacteria and yeast/fungi in soil sample taken days 1, 2, 3 and 4 from each bore–hole is presented in Fig. 2, and the main values calculated for all analyzed samples are given in Table 1. However, the applied microbial technique did not allow accurate counts of number of cells (only estimation), the comparison of evolution of their number in suscessive days could be taken into consideration as a valuable one.

The increase of the number of microorganismes in soil was observed for samples taken from bore-holes inoculated with yeast in comparison to those inoculated with water. The number of yeast increased: about twice after water inoculation and 65-fold after yeast inoculation. One year later, more microorganisms were observed in the soil samples from inoculated bore–holes. However, putative *Yarrowia lipolytica* strains were detected only in one soil sample (bore–hole 1).

The measurements of conductivity were carried out along one-hundred-metre profiles (Fig. 1) in spring and autumn 2003 and in spring 2004. Diagrams of changes of the apparent conductivity versus profiles length obtained by Oasis Montaj software analysis of data were presented in Fig. 3. The minimum of the curve (minimum peak) indicates the low apparent conductivity (high resistivity) and corresponds to the places with high hydrocarbon content. There are two minimum peaks at 24-32m distance and three minimum peaks at 36-42m distance (Fig. 3 – spring 2003 curve). After introduction of *Yarrowia lipolityca* yeast in spring 2003 a gradual decline of the minimum peaks in autumn 2003 was observed, and the peaks were no longer visible in the spring 2004 curve. This indicates that suspension of yeast decontaminated the soil during one year. Moreover, two-metre dislocations of the peaks were observed in the spring 2004 curve at 40-44m according to the direction of underground water flow (Fig. 3). Thus, we may conclude that the *Yarrowia lipolityca* yeasts migrated in the soil according to the underground water flow.

Along the same profile, resistivity measurements with Lund Imaging System were carried out in autumn 2003 according Schlumberger L and Schlumberger S protocols. The resistivity 2D cross section to a depth of 12m under the surface with 2.5m electrode spacing, obtained by Res2D software specific resistivity data analysis, is shown in Fig. 4. The yeast inoculation zone correlated very well to the electrical resistivity decrease. The resistivity imaging profile repeated in autumn 2004 allowed the estimation of the extent and depth of the decontaminated zone. The decrease of specific resistivity reached the depth of drill hole used for yeast introduction.

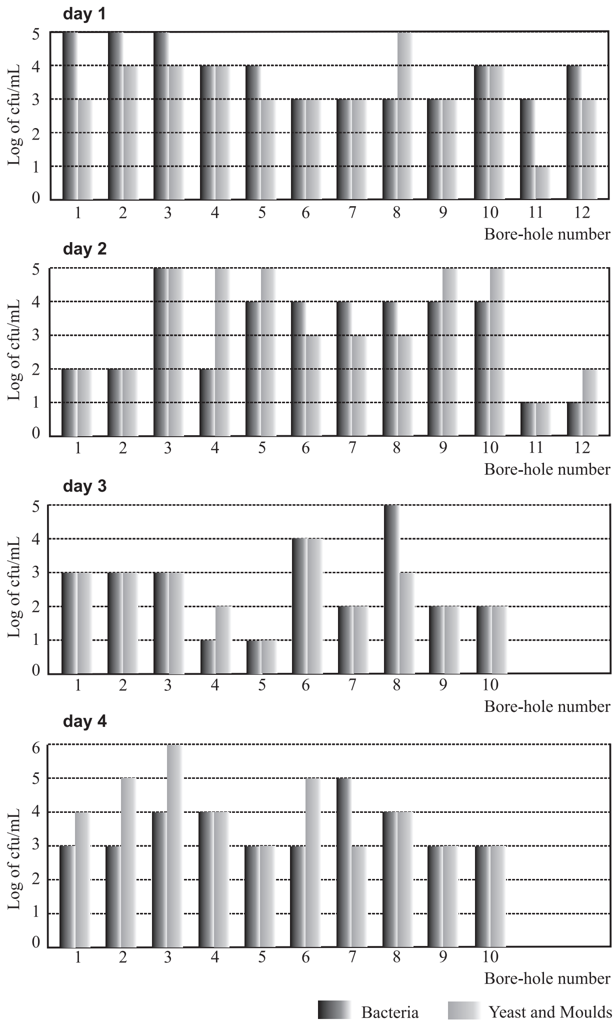

Fig. 2. Time evolution of the number of bacteria, yeast and moulds in soil from bore-holes after yeast inoculation.

Table 1. The main value of bacterial and yeast/fungi enumeration in soil samples calculated from results obtained for each bore–hole. (nd – not determined).

Inoculation (Bore-hole number)	Microbiological enumeration [cfu x10^4/g of soil]	Post treatment time [day]						
		0	1	2	3	4	5	One year later
Yeast (1-10)	Yeast/Fungi	0.16	10.4	35.9	1.0	88.0	0.2	0.32
	Bacteria	nd	23.8	11.5	8.0	9.7	1.4	nd
Water (11-12)	Yeast/Fungi	0.16	0.36	0.04	nd	nd	nd	0.07
	Bacteria	nd	3.9	7.14	nd	nd	nd	nd

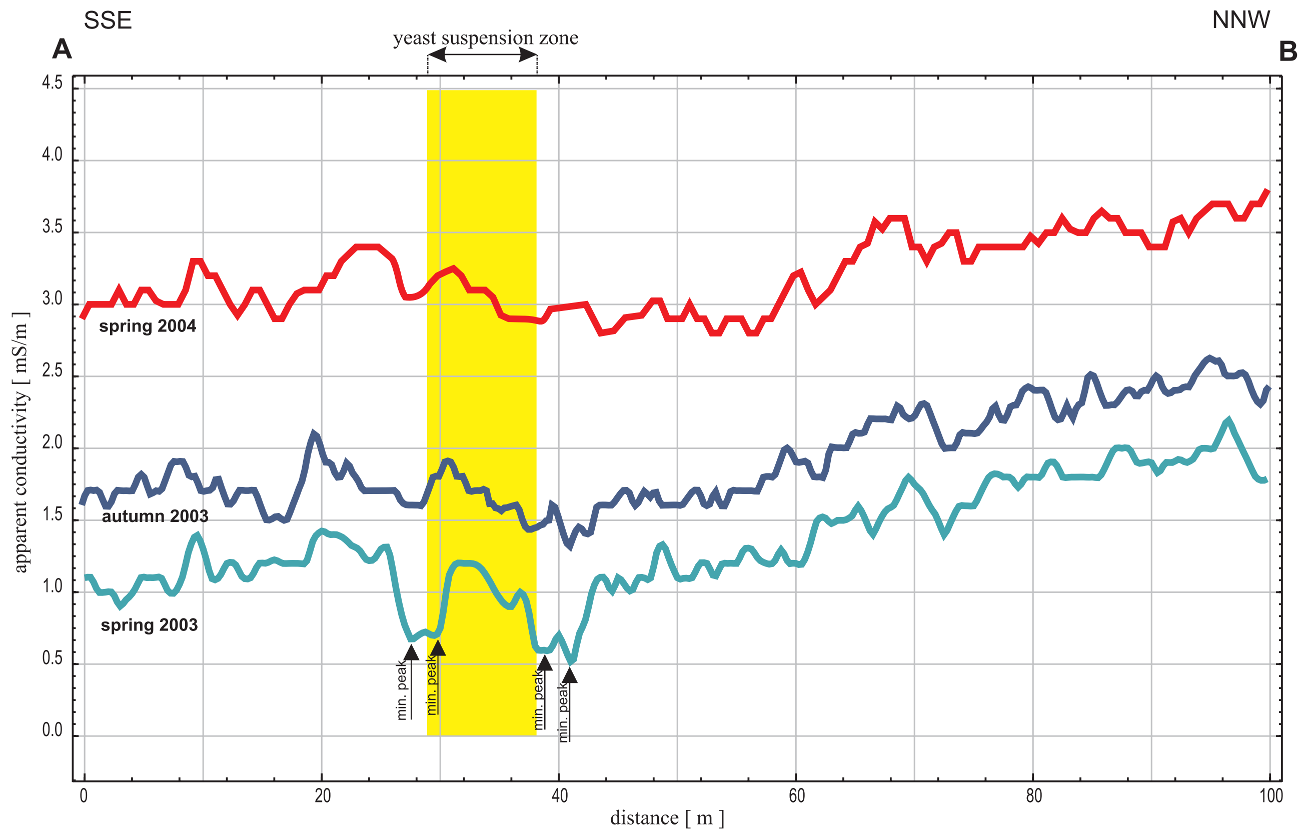

Fig. 3. Changes of apparent electrical conductivity [mS/m] of soil versus A-B profile length.

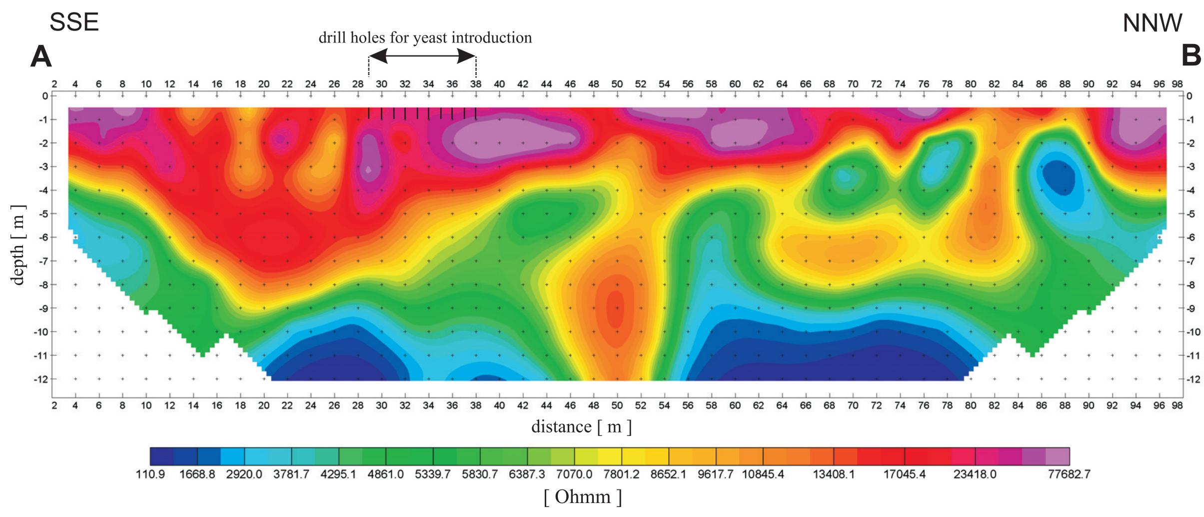

Fig. 4. Inverse model resistivity A-B section (iteration 4 RMS error = 5.8%).

Discussion

The investigation area is covered by glacial deposits: sands interstratified by till. The underground water level is observed at 10-15m depth (below investigated zone). Under the thin 15-20cm sandy soil layer we observed a 1-2m high resistivity hard pan layer. Therefore, the geological structure causes that the studied area is difficult and inconvenient for geoelectrical measurements, especially for resistivity mapping ones. For electromagnetic methods a significant fluctuation of apparent electrical conductivity background according to season and weather conditions was observed. The studied contaminated zone had been localized by previous electromagnetic measurements. Soil samples from 0.5m, 1m, 1.5m, 2m depth contained 390mg/kg D. M. of hydrocarbons of the series C_{12} – C_{35} (unpublished).

The measurements with EM31–MK2 equipment give information on the changes of apparent electrical conductivity of the shallow geological layers. The applied method allowed to obtain apparent conductivity values to the depth of approximately 6m under the surface for points distance about 0.3m along the measured profile. The specific electrical conductivity value depends on humidity of the soil (the higher humidity the higher conductivity background), but a general aspect of conductivity graphs is similar.

The observed (autumn 2003) gradual decline of the minimum peaks of conductivity in the zone of *Yarrowia lipolityca* yeast inoculation proves that the process of decontamination of the soil proceeds, and the lack of those peaks in spring 2004 demonstrated that the suspended number of cells yeast 10^{12} (hole, resulted in decontamination the soil during one year. Decontamination could be enhanced by the environmental microsystem, because yeast suspension caused an increase in the bacterial population but could also influence better plant rhizoids development. The citric acid secreted by *Yarrowia lipolytica* dissolves roaches phosphates into organic forms easily accessible for plants [14, 15].

The presence of yeast only in one bore–hole sample observed in spring 2004 may be due to a dislocation of cells which followed the underground water flow. Two metre dislocation of the conductivity peaks probably was also the result of yeast movement. The immobilisation of microbial cells could prevent their dislocation and favour soil remediation. Immobilized on Ca – alginate gel, *Yarrowia lipolytica* cells secreted citric acid [14] and were applied in mycorrhyzae-forming plant – soil system [16].

The contamination decay was also proved by a decrease of specific electrical resistivity with time. Unfortunately, resistivity measurements were not performed in the period of yeast suspension (spring 2003). The investigators did not expect to apply resistivity methods in the beginning.

In conclusion, the applied geoelectrical methods can be a good tool not only for localization of oil-contaminated zones but also for the controlling and the monitoring of these contaminations' evolution (microbial bioremedation, delocation) and for the estimation of their volume by performing several parallel resistivity 2D cross sections.

References

1. AL-HASSAN R. H., AL-BADER D. A., SORKHOH N. A., RADWAN S. S. Evidence for n–alkane consumption and oxidation by filamentous cyanobacteria from oil – contaminated coasts of the Arabian Gulf. Marine Biology, **130**, 219, **1998**
2. MALICKA M. Biotechnological methods of the remediation of soil contaminated with petrol and other chemical pollutants. Gas, Water and Sanitary Technique, **2**, 40, **1994** (In Polish)
3. ADAMSKI W. Contaminated soil reclamation technologies. Environmental Protection, **1-2** (48-49), 7, **1993** (In Polish).
4. SCHÖN J. H. Physical properties of rocks: fundamentals and principles of petrophysics. Pergamon – Elsevier Science Inc., New York. pp. 393, **1996**
5. MARCAK H. Location of hydrocarbon contaminations using geophysical methods. Proceedings of the Conference: "Geophysics in Engineering and Environmental Protection", pp. 177-190, **2001** (In Polish)
6. ZUBEREK W. M., ŻOGAŁA B., PIERWOŁA J. Application of geoelectrical methods to the detection of polluted soils at shallow depth. Proc. 5th Meeting of the Environmental and Engineering Geophysical Society, DeP7, **1999**
7. GOES B. J. M., MEEKES J. A. C. Evaluating various electrode configurations for detection of DNAPL`S with ERT. Proc. 8th Meeting of the Environmental and Engineering Geophysics, pp. 461-464, **2002**
8. SLOB E., BLOEM E., BREUKELEN B., GROEN K. Two and three – dimensional resistivity surveys in a landfill leachate investigation. Proc. 8th Meeting of the Environmental and Engineering Geophysics, pp. 195-198, **2002**
9. ŻOGAŁA B., PIERWOŁA J., JOCHYMCZYK K. Effect of oil derivatives on electrical properties of soils. Proc. 6th Meeting of the Environmental and Engineering Geophysics P-EL07, **2000**
10. ŻOGAŁA B., ZUBEREK W. M., RUSIN M., WZIENTEK K. Application of geophysical methods in estimation of natural environment conditions. Scientific Papers of the Silesian University of Technology, series Mining (**248**), pp. 229-234, **2001** (In Polish).
11. MADEJSKI C., MADEJSKA E. Report on the contamination of soil, underground and surface waters with oil derivatives and other chemicals and evaluation of ecological damages at the area released by Russian troops of the military camp Borne Sulinowo. Polgeol, Warszawa, **1995** (unpublished) (In Polish).
12. BARTH G., BECKERICH J-M., DOMINGUEZ A., KERSCHER S., OGRYDZIAK D., TITORENKO V., GAILLARIDIN C. Functional genetics of *Yarrowia lipolytica.* Topics in Current Genetic, **2**, 227, **2003**
13. PRZĄDO D., ROBAK M., STEININGER M. Biodegradation of chloroorganic pesticides by *Yarrowia lipolytica*. Chemistry for Agriculture, **5**, 326, **2004**
14. RYMOWICZ W., WOJTATOWICZ M., ROBAK M., JURGIELEWICZ W. The use of immobilized *Yarrowia lipolytica* cells in Ca-alginate for citric acid production. Acta Microbiol. Polon. **42** (2), 63, **1993**
15. KOYAMA H., TAKITA E., KAWAMURA A., HARA T., SHIBATA D. Over expression of mitochondrial citrate synthase gene improves the growth of carrot celle in Al.-phosphate medium. Plant Cell Physiol. **40** (5), 482, **1999**
16. VASSILEV N., VASSILEVA M., AZCON R., MADINA A., Application of free and Ca–alginate–entrapped *Glomus deserticola* and *Yarrowia lipolytica* in soil-plant system. Journal of Biotechnology, **91**, 237, **2001**