CONSIDERATIONS ON THE BIOREMEDIATION CAPABILITY OF SOME BACTERIAL STRAINS ISOLATED FROM CONTAMINATED AREAS

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Abstract. The paper is a review of the researches and experiments undertaken to assess the capability of some bacterial strains, isolated mainly from contaminated environments, to intervene in bioremediation processes. An unconventional alternative was thus attempted to reduce contaminants in various areas, not just industrial ones, by identifying the source of damage to the area under study, reducing the level of exposure of population and maintaining or restoring ecological balance in the absence of disruptive products. Laboratory research has been focused both on the analysis of soil and water samples taken from affected areas, the isolation, cultivation, testing, preservation and identification of bacterial strains of biotechnological interest, as well as the study of microorganisms under the influence of contamination stress. The paper cites only the list of papers published by the research team of the Department of Microbiology of IBB, involved in bioremediation, the results and observations recorded being a substantial contribution both in the understanding of the mechanisms of action of some physiological groups of bacteria and in demonstrating the applicability of some technologies, recognized as effective and sometimes more affordable, as compared to conventional processes.

Keywords: bioremediation, petroleum hydrocarbons, bioproducts, acidophilic chemolithotrophic bacteria, phytoremediation.

Rezumat. Consideraţii privind capacitatea de bioremediere a unor tulpini bacteriene izolate din zone contaminate. Lucrarea constituie un review al activităţii de cercetare şi experimentare, privind capacitatea unor tulpini bacteriene, izolate cu precădere din medii contaminate, de a interveni în procesele de bioremediere. S-a încercat în felul acesta aplicarea unei alternative neconvenţionale de diminuare a contaminanţilor din variate zone, nu doar industriale, prin identificarea sursei de afectare a zonei luate în studiu, reducerii nivelului de expunere al populaţiei şi menţinerii sau reinstalării echilibrului ecologic, în absenţa produşilor perturbatori. Cercetările de laborator au urmărit atât analiza probelor de sol şi apă, prelevate din zonele afectate, izolarea, cultivarea, testarea, prezervarea şi identificarea unor tulpini bacteriene de interes biotehnologic, dar şi studierea microorganismelor sub acţiunea stresului de contaminare. Lucrarea citează doar lista de lucrări publicate de colectivul de cercetare al Departamentului de Microbiologie al IBB, implicat în problematica bioremedierii, rezultatele şi observaţiile înregistrate constituind o contribuţie substanţială atât în înţelegerea mecanismelor de acţionare a unor grupe fiziologice de bacterii, cât şi în demonstrarea aplicabilităţii unor tehnologii, recunoscute drept eficiente şi uneori mai accesibile, comparativ cu procedeele convenţionale.

Cuvinte cheie: bioremediere, hidrocarburi petroliere, bioproduşi, bacterii chemolitotrofe acidofile, fitoremediere.

INTRODUCTION TO THE APPROACHED BIOREMEDIATION PROBLEM

After a long period of in-depth studies concerning the microbiology of oil, not accidentally generated by the oil crisis in the ‘70s, a group of researchers from the Center for Microbiology of the Institute of Biology of Bucharest (IBB) have approached biotechnological researches of microbial recovery of low-yield deposits of oil, in parallel with understanding and laboratory simulation of the mechanisms of oil release from porous media under the action of micro-organisms.

The areas where the bacterial samples were collected were, besides the oil and sewage water from the extraction wells, batches, tailings and oil reservoirs, sludge deposits from oil park areas or pumping stations, all that consisted of petroleum contamination generated by extraction, processing or transport of oil, but also infiltration of crude oil or its derivatives at the level of surface waters, running or standing, fresh or coastal, up to the level of the aquifer (LAZĂR et al., 1998a; b; c), with water that carries kilometers from the source of contamination, that film with rainbow reflections, called "plume", which betrays the presence of chronic, historical pollution with oil hydrocarbons (BICA et al., 2008).

It was necessary, or only attempted, to test unconventional remediation biotechnologies of the affected areas, "accidentally" contaminated, or as a result of a long exposure, as is the case of railway sleepers and other access ways soaking, in order to start the vast process of decontamination, depollution and rendering in the circuit of a clean environment of vast and varied areas ignored, forgotten or postponed to be improved, with repercussions and implications often extremely difficult to correct.

Isolation of some hydrocarbon-oxidant bacterial strains directly from the contaminated environments (LAZĂR et al., 1994; LAZĂR et al., 1995a; b; c), selection in order to obtain metabolites producing populations similar to detergents (DOBROTĂ et al., 1988; 1994; 1999a, b; 2005; DOBROTĂ & ŞTEFĂNESCU, 2005), the ability of some bacterial inocula to consume, as sole source of carbon and energy, oil hydrocarbons (DOBROTĂ et al., 1997; 2000; DOBROTĂ & ŞTEFĂNESCU, 2005) represented the basis for our laboratory researches and field experiments for testing alternative biotechnologies for cleaning and protection of the environment.

Contaminated environments can also be industrial installations, ducts and closed spaces belonging to the oil industry, clogged with paraffin deposits (LAZĂR et al., 1999a). Thus, the microbial researches, studies and experiments have expanded to the reduction of wax and paraffin deposits within the oil extraction pipelines of the marine oil platforms.

Another aspect addressed by IBB's Department of Microbiology researchers, largely the same who studied the aspects related to oil contamination and pollution, was the problem of mining waste deposits (CISMAŞIU et al., 2015)

and industrial effluents represented by the infiltration and sinking waters from the mining tailings dumps (LAZĂR et al., 1997a; KONTOPOULOS et al., 1998) and from tailing ponds with high content of heavy metals and radioactive ions. The tolerance of some bacterial strains to increased acidity conditions, the bioaccumulation capacity of some metal ions, biosorption and bioprecipitation, demonstrated under laboratory conditions, led to the setting up of treatments based on biofiltration columns that contribute both to the accumulation of metallic ions of interest, and the reduction of the contamination level of the industrial effluents (PERTIŞOR et al., 2003).

In the case of mining tailings dumps, in order to reduce the level of contamination by wind and pluvial dispersion, phytoremediation methods have been designed by covering the deposits with a vegetative layer (LAZĂR et al., 1998b; 2001a) which, by means of the roots, fixes the surface layers, contributing, together with the tolerant microbiota, to the formation of a persistent rhizosphere (LAZĂR et al., 2001a; b; ŞTEFĂNESCU et al., 2008b). The cultures made in the institute's greenhouse, with enriched soil variants and herbaceous, shrub and tree species with high resistance to high concentrations of metal ions, have been valued experiments, as well as extending the wetland type systems of decontamination and concentration of industrial waste from effluents generated by drug factories (LAZĂR et al., 2002b) or leather processing (LAZĂR et al., 2002a; IONESCU et al., 2005; 2010).

All these applicative biotechnological directions involved also advanced microbiological researches, based on molecular techniques for bacterial identification and biochemical characterization of metabolic products implicated in the decontamination processes (CÎRSTEA & ŞTEFĂNESCU, 2012).

At the same time, we tried to improve the tested technologies, by developing applicable patents (IONESCU et al., 2004) and with high adaptive capacity to the conditions enforced by the regulations regarding the pollution prevention and the environmental protection.

STUDY CASES

1. Reducing crude oil contamination

a) Soils contaminated with oil hydrocarbons. As mentioned above, contamination with crude oil and oil-based hydrocarbons includes huge areas of land, and can be expanded horizontally or vertically to the area adjacent to the surface waters, respectively, the shallow groundwater layer. The laboratory bioremediation experiments and field pilot experiments (DOBROTĂ et al., 1997) have tested the ability of some bacterial strains isolated from hydrocarbon-contaminated areas to use oil as the sole source of carbon. Laboratory simulations used both pure bacterial strains and blends or mixed cultures (LAZĂR et al., 1995a; b; c; 1996). In field applications, the test area was soiled in order to increase the contact surface with both the atmospheric air (oxygenation and evaporation) and with the applied bacterial inoculum (DOBROTĂ et al., 2000). The extraction or depositing mud extracted from the batches was preferred over impermeable tars with low permeability and wettability (LAZĂR et al., 1999c; 2002b). At laboratory level, detailed characterizations of hydrocarbon-oxidant bacterial strains (LAZĂR et al., 1994; 1998c) and selections (LAZĂR et al., 1997b) were made, considering the type of crude oil subjected to bioremediation and climatic characteristics, when actions were taken in the field (LAZĂR et al., 1999b). All these researches, related to the involvement of bacterial strains in specific biotechnologies for the remediation of oil hydrocarbon type contaminants in various areas, are widely presented in the bibliographic list (VOICU et al., 1997b; PETRIŞOR et al., 2001; 2002a; ŞTEFĂNESCU & DOBROTĂ, 2005; LAZĂR & ŞTEFĂNESCU, 2006; ŞTEFĂNESCU et al., 2009).

b) Oil hydrocarbons contaminated water and aquifers. The presence of oil contaminants in surface waters, standing or flowing, is generally the result of accidents due either to corrosion of transport pipelines or to discharge or negligence in handling. The study cases ensured sampling of water from the sea (ŞTEFĂNESCU et al., 1999) and from lakes, swamps and from the lower course of the Danube (Sulina arm). At the same time, the oil-contaminated aquifer of the Ploiesti oil extraction area (LAZĂR et al., 1998a; VOICU et al., 2000; ŞTEFĂNESCU et al., 2008b; BICA et al., 2009) was analysed, whereby micro-organisms were isolated and further selected in the laboratory (VOICU et al., 2009b).

At the laboratory level, in collaboration with researchers of the Technical University of Civil Engineering in Bucharest, pilot systems were developed for evaluation, simulation, retention and testing of the remediation capacity of some bacterial strains, using unconventional technologies applied to aquifers affected by historic pollution (BICA et al., 2008).

c) Reduction of paraffin deposits in oil pipelines. Another successful challenge was the laboratory testing of the capacity to reduce the paraffin deposits (VOICU et al., 1997c; 2002) and the use of hydrocarbon-oxidant bacterial consortia for prevention and for the cleaning of oil pipelines from offshore drilling platforms (LAZĂR et al., 1995a; VOICU et al., 1995a; b; 1997a).

d) Isolation and selection of bio-products with remedial potential. The remedial action of some isolated bacterial consortia isolated from hydrocarbon-contaminated environments on a wide variety of contaminants is due to the direct and/or synergistic activity of some emulsifying/de-emulsifying properties of several metabolites of rhamnolipid type (surfactants). Laboratory studies and researches have shown their ability to break the oil contaminant film, thus increasing the contact surface between the two phases, water/crude oil, which leads to faster metabolization of the carbon source. The obtained results (DOBROTĂ et al., 1988; 1994; VOICU et al., 1994a; ŞTEFĂNESCU et al., 1997; DOBROTĂ et al., 1999a; b; DOBROTĂ & ŞTEFĂNESCU, 2004; 2005; CÎRSTEA & ŞTEFĂNESCU, 2012) confirmed the theory, leading also to the isolation and characterization of these bacterial bioproducts with large biotech applications.

2. Others biotechnicals domains

e) Reduction in the contamination level of industrial effluents. Another issue regarding the reduction of the contamination of the spill water by bacterial technologies was the bacterial degradation of phenols in waste waters (VOICU et al., 1994a; b; LAZĂR et al., 1995b) from the chemical-pharmaceutical and leather industries. Microbial consortia capable of metabolizing a number of organic compounds (LAZĂR et al., 2002a; VOICU et al., 2003a) present in the industrial effluents were used and filtration columns (percolator batteries) were provided, supporting a wide range of porous materials such as sand quartz, montmorillonite, kaolin and shell sand, these being true biofilters, the efficiency of which has been tested in both laboratory and pilot stations, designed by our specialists, in collaboration with those from the National Institute For Chemical - Pharmaceutical Research and Development – ICCF Bucharest (IONESCU et al., 2005). Recognizing the effectiveness of the biotechnological purification processes has led to the award of medals for the patents registered with OSIM (IONESCU et al., 2004; 2010). The opportunity to isolate and select bacterial strains or consortia with bioremediation potential from contaminated waters, creates the framework for testing some alternative and unconventional ways to reduce the level of environmental damage, by self-regulation (natural remediation) without disturbing its biological balance (MACOVEI et al., 2005).

f) Intervention of some groups of acidophilic chemolithotrophic bacteria in biohidrometallurgical processes. Bioremediation applications have also covered areas affected by metal ion contamination from either mining or mobilizing these toxic ions from mining tailings dumps. The study cases were around the villages of Şesei Valley, Roşia Poieni and the Aries basin (PETRIȘOR et al., 1997; LAZĂR et al., 1999a; VOICU et al., 2009a; b; c). Water samples were taken from the tailing ponds of these operations, from which heterotrophic and acidophilic chemolithrophy bacterial strains were isolated, exhibiting biotechnological potential in leaching, bioaccumulation and bacterial bioprecipitation processes (CISMAȘIU et al., 2015). Laboratory tests have demonstrated the ability of these microorganisms to metabolize and fix some metallic ions present in excess in industrial spill waters, thus contributing to the reduction of the heavy metal pollution level (VOICU et al., 1998; PETRIȘOR et al., 2003). An interesting study was the signalling of Black Sea pollution with metal ions, brought by pluvial infiltration streams formed in the mining tailing dumps from Baia and Somova (Tulcea County) (KONTOPOULOS et al., 1998).

g) mining pits protection by phytoremediation. Concerning the same aspects of preventing the release of contaminating particles from industrial storage dumps under the action of precipitations, that generate infiltration waters and winds (wind processes) that erodes and mobilizes dust at great distances from the source of danger, we have tried to cover and plant on the heaps resistant plant species, while generating a rhizospheric layer with a rich microbiotic activity. Previous researches on phytoremediation were carried out under greenhouse conditions, on soil variants with amendments and different concentrations of chemical elements, testing the vegetal species chosen for cultivation until the flowering stage. The mining tailings dumps at Baia and the phosphogips tailings dump from Năvodari were taken into consideration. The microbiological researches focused on characterization of the microbial activity from the rhizosphere of the vegetal layer installed on the heap surface (LAZĂR et al., 2001a; c; ȘTEFĂNESCU et al., 2001) and on monitoring the development of vegetal carpet (LAZĂR et al., 1998b; 2001b; PETRIȘOR et al., 2002b).

CONCLUSIONS

Presentation of the various fields of research that have been approached give an idea on the many potential applications of microbial technologies for the remediation of environments affected by the presence of anthropogenic contaminants.

The paper, based on a several decades' work of the research team, is a synthesis of the bioremediation researches concerning the testing and implementing of widely applicable biotechnologies (VOICU et al., 2003a; b; ȘTEFĂNESCU et al., 2008a; ȘTEFĂNESCU & CÎRSTEA, 2010; 2011).

We preferred to isolate the bacterial strains directly from affected areas of (VOICU et al., 2009c), which are adapted to the local conditions, precisely because the potential application of the decontamination technologies does not destabilize the natural balance of the areas under study.

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