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REVIEW

Insights on yeast bioremediation processes

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ORTANSA CSUTAK[1*], ILEANA STOICA[1], RALUCA GHINDEA[2], ANA-MARIA TANASE[1], TATIANA VASSU[1]
[1]*University of Bucharest, Faculty of Biology, Department of Genetics, Aleea Portocalilor 1-3, 060101, Bucharest, Romania*
[2]*University of Bucharest, Faculty of Biology, Center for Research, Consulting and Training In Microbiology, Genetics and Biotechnology, Aleea Portocalilor 1-3, 060101, Bucharest, Romania*
*ortansa@botanic.unibuc.ro; cs_ortansa@yahoo.fr

Abstract

The augmentation of industrial and house wastes and the accidental oil spills raised the problem of their storage and treatment. Bioremediation is an interesting alternative for restauring the ecological equilibrum in polluted environments, based on microbial population dynamics and its ability to consume xenobiotics as carbon source. The yeast species described as having biodegrading abilities belong especially to Pichia, Rhodosporidium, Rhodotorula, Trichosporon and Yarrowia. Successful bioremediation require knowledge on limiting factors such as pollutant composition and nature, microbial community structure, contaminants accessibility, physical state of hydrocarbons, temperature, nutrients and oxygen. Studies on yeasts able to use various petroleum components as sole carbon source, showed that their biodegrability decreases from n-alkanes to high molecular weight aromatic and polar compounds. The alkanes are mainly degraded using the monoterminal oxidation pathway through cytochrome P450 system, and transformed into fatty acids with the same length of the carbon chain. Extensive studies showed that there are more than 80 genes involved in obtaining the alkane specific phenotype. In present, bioremediation technologies may be applied ex situ, in situ or in combination with conventional technologies.

Keywords: bioremediation, yeasts, petroleum, limiting factors, alkanes, oxidation, cytochrome P450.

Introduction

Industrial revolution changed the way of life, increasing the scientific knowledge regarding the human impact on environment and the understanding of the strategies for diminishing any possible damages. Biologic treatment of house wastes has a long history, but studies concerning the possibility of applying this process for degrading organic compounds and industrial products or by-products, are performed only for the last three decades. Bioremediation is an interesting alternative for restauring the ecological equilibrum in polluted environments, involving mainly non-invasive technologies with rather low costs and only rarely the addition of some degradation enhancers may be required. Thus, bioremediation could soon represent the most reliable and least expensive solution for solving various chemical pollution problems [1, 2].

Bioremediation is based on biodegradative processes related to microbial population dynamics in soil or water and its ability to consume xenobiotics as corbon source. Environmental pollution can be caused by: (i) spills during the industrial production process; (ii) disposal of toxic compounds; (iii) excessive treatment of agricultural surfaces. Industrial wastes comprise organic compounds such as alifatic and aromatic hydrocarbons derived from petroleum, charcoal and wood, as well as natural products, halogenated solvents, pesticides, herbicides and explosives.

In present, petroleum (rock oil) is considered to be one of the major pollutants, its heterogenous composition comprising compounds very different in terms of solubility,

molecular weight, toxicity, stereochemistry and biodegrability. The main constituents are alkanes iso-alkanes, cycloalkanes, alkenes, aromatic and condensed aromatic hydrocarbons, phenols and their derivates.

Microorganisms (bacteria and yeasts) are subjects of many bioremediation studies, due to their ability of assimilating hydrocarbons. Until now there have been described at least 100 microbial species belonging to 30 genera from which 22 genera of bacteria and approximately 14 genera of yeasts [3]. The way that bacteria act in the biodegradation processes is relatively well known, while there are still many questions concerning the way yeasts participate in the same processes. The yeast species described in literature as being able to use hydrocarbons as carbon sources belong especially to the genera *Candida* [4, 5], *Clavispora, Debaryomyces, Leucosporidium, Lodderomyces, Metschnikowia, Pichia, Rhodosporidium, Rhodotorula, Sporidiobolus, Sporobolomyces, Stephanoascus, Trichosporon* and *Yarrowia* [6].

Main aspects regarding the biodegrability of petroleum components

Studies on yeasts able to use various petroleum components as sole carbon source, showed that thier biodegrability decreases from n-alkanes > branched alkanes > low molecular weight aromatic hydrocarbons > cycloalkanes > high molecular weight aromatic and polar compounds.

There are four rules for yeasts with xenodegrating abilities: (a) aliphatic compounds are the first to be degraded; (b) alkanes with C_{10}-C_{18} carbon chaines are preferentially assimilated; (c) unsaturated hydrocarbons are transformed with lower rates; (d) branched alkanes are easier degraded than linear ones, but only when the branch is higher than C_9.

The alkanes with long and medium carbon chains are biodegrated in yeast cells through the cytochrome P450 system, and those with less than C_9 require biotine addition to the growth medium. *Yarrowia lipolytica* and *Candida maltosa* are able to use mono-branched alkanes as sole carbon and energy source. These are incorporated in lipids, converted into soluble cellular compounds (proteins, aminoacids), intermediate metabolits (dicarboxylic acids with β-methyl group) and partially oxidated to CO_2.

Cycloalkanes are used only in small rates (5 to 10 %) compared to the n-alkanes, and only when their concentration level is not toxic. Although less is known on their degradation, it seems that it does not involve cytochrome P450.

Phenol and its derivates (resorcinol, chlorophenol, catechol, quinoline, hydroxiquinoline, nitrophenol and dinitrophenols) can be assimilated by *Aureobasidium, Rhodotorula, Candida, Yarrowia* and *Trichosporon* strains through β-ketoadipate pathway. Certain *C. maltosa* cells are able to biodegrade also 2-, 3- and 4-monochlorphenols. Studies on *Trichosporon* strains isolated from heavily oil-polluted soils, revealed their ability to grow on phenol and Diesel [7]**.**

Yeasts cannot grow on polycyclic aromatic hydrocarbons (PAH) but are able to co-oxidize biphenyl, naphtalene and benzopyrene using the monooxigenase cytochrome P450 pathway induced by the presence of n-alkanes. Studies on fungi and yeast (*Candida, Rhodotorula, Trichosporon*) communities from aquatic environments polluted with PAH, especially phenantren, revealed high degradation rates for *Trichosporon penicillatum* [8].

Parameters affecting petroleum hydrocarbon biodegradation

Successful bioremediation processes require deep knowledge on factors that affect the microbial biodegradation of pollutants [9]. Until now, there have been established a number of limiting parameters:

Parameter	Explanation and/or action
Pollutant composition and nature	- petroleum composition varies depending on sources; - is affected by environmental conditions; volatile compounds are wasted, the remaining ones can form non-degradable emulsions
Microorganisms	- xenodegrading microorganisms represent different rates within microbial communities depending on the polluted environment; - preliminary exposure to hydrocarbon can induce specific enzymes production, evolution of new metabolic pathway or obtaining of strains with improved degrading abilities
Contaminants accessibility	- depends on their concentration; - is influenced by oxygen and nutrient presence
Physical state of hydrocarbons	- influences their spreading in nature and bioaccessibility; - solubility is lowered by high concentrations
Temperature	- biodegradation occurs usually at environmental temperatures and sometimes at < 0^{o}C or > 70^{o}C; - the effects depend on the hydrocarbon mixture or the microbial community composition
Nutrients	- iron, phosphorus and carbon sources are essential for microbial growth
Oxygen	- is a limiting factor in the first steps of substrate oxidation

Petroleum biodegradation pathways and genetics in yeast cells

The presence of hydrocarbons, especially alkanes, as sole carbon source trigger a series of biochemical and morphological modifications within yeast cells: changes on the cell surface due to hydrocarbon transport in the cell [10]; induction of cytochrome P450 active in alkane and NADPH-cytochrome (P450) reductase hydroxylation [11]; induction of enzymes involved in oxidation of fatty alcohols and their aldehides; peroxisomes proliferation, induction of the characteristic *beta*-oxidative pathway and of the enzymes involved in glyoxalic acid cycle and gluconeogenesis [12].

The alkanes are mainly degraded using the monoterminal oxidation pathway [13], and transformed into fatty acids with the same length of the carbon branch (**Figure 1**).

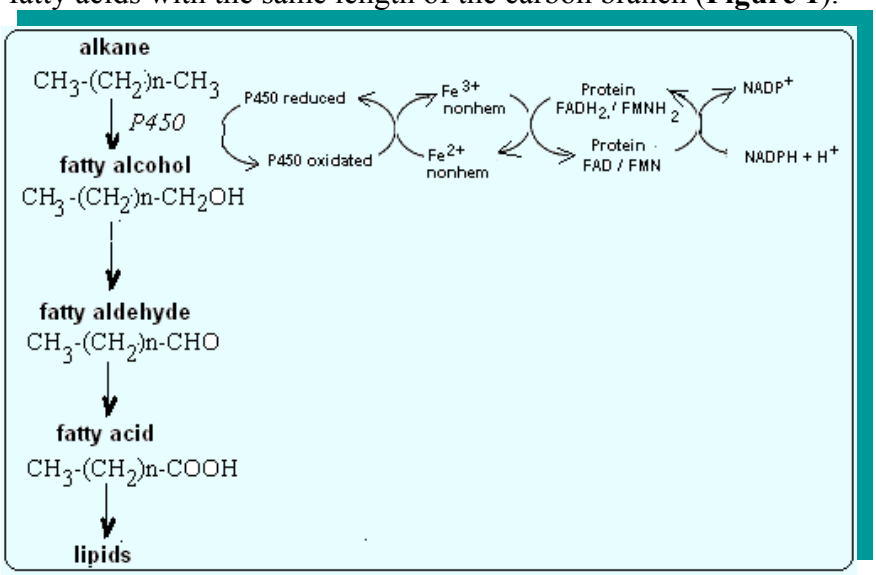

Figure 1. Monoterminal oxidation pathway in yeasts and cytochrome P450 action in the first step of alkane oxidation

Diterminal oxidation pathway takes place more rarely and it results in dicarboxylic fatty acids production, the short chain ones being secreted in the growth medium [14, 15, 16]. The first step takes place in the endoplasmatic reticulum, with the formation of a dicarboxylic acid which is transported into the peroxisome. The acetyl-coenzyme A obtained is then transferred to the mitochondria through carnitine acetyltransferase system (**Figure 2**).

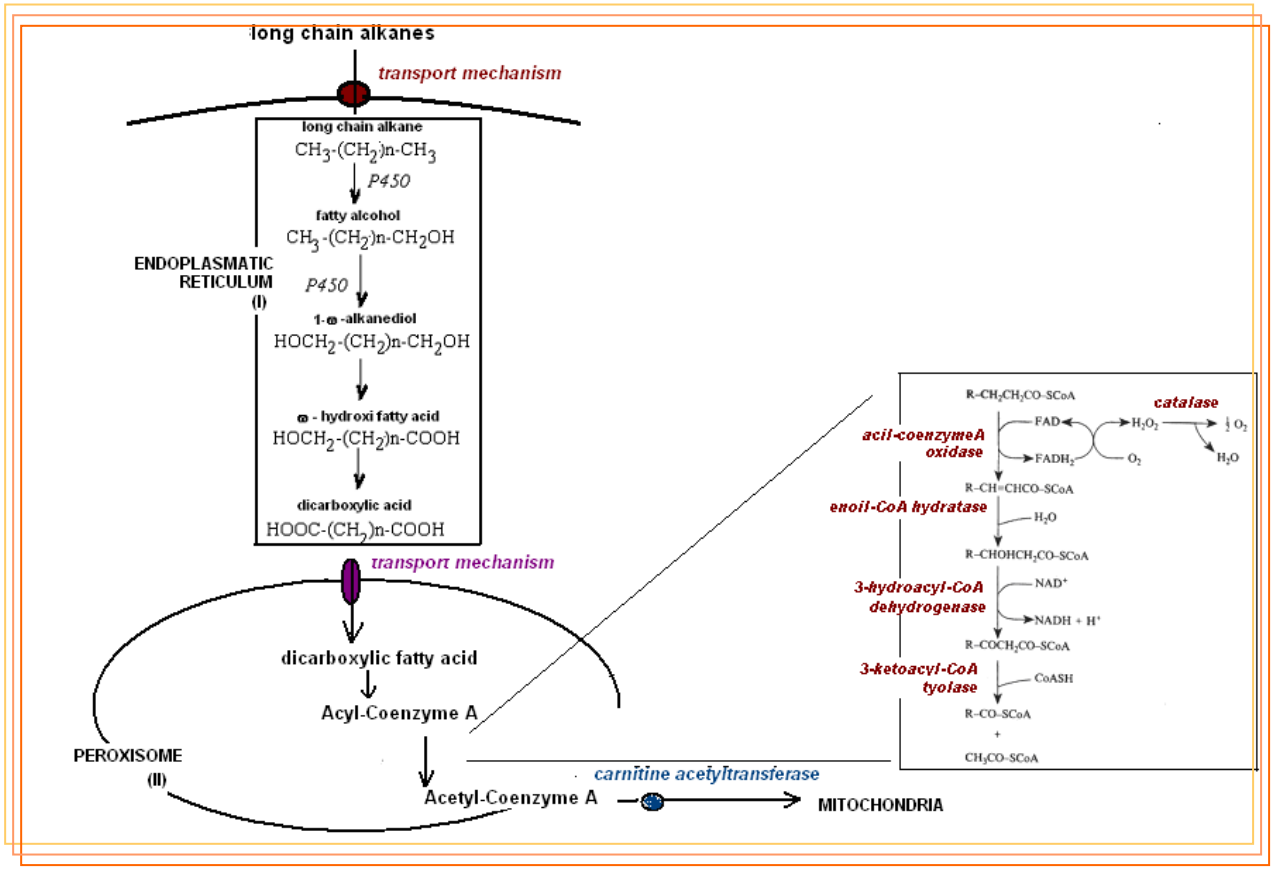

Figure 2. Diterminal oxidation pathway for long branched alkanes (I – ω-oxidation; II – □-oxidation)

Extensive studies showed that there are more than 80 genes involved in obtaining the alkane specific phenotype, and at least 26 genes are responsible for alkane prelevation from the culture medium and in their oxidation to fatty acids [17, 18,19].

Alkane–assimilating yeasts such as *C. maltosa* present several forms for cytochrome P450, encoded by eight structural similar genes named *P450 alk*, belonging to *CYP52* gene family. Together with NADPH-cytochrome P450 reductase, these forms are responsible for catalysing the terminal hydroxylation of n-alkanes as first step in alkane metabolism [20, 21]. Six of the isoforms belong to *CYP52A* subfamily, the others to *CYP52C2* and *CYP52D1*. The activity of most genes depends on the presence of aliphatic hydrocarbons in the growth medium. Thus, *CYP52A - CYP52A3, 52A4, 52A5* and *52A6* are highly induced by alkanes, while *CYP52C2* and *CYP52D1* are only weakly induced.

In *C. tropicalis* cells there have been identified ten *CYP52* genes. Comparative studies on the aminoacid sequence of the encoded proteins, revealed the following pairs of allele genes: *CYP52A13* and *CYP52A14*, *CYP52A15* and *CYP52A16*, *CYP52A17* and *CYP52A18*, *CYP52A19* and *CYP52A20*. No alleles have been identified for *CYP52A12* and *CYP52D2*. Further studies determined aminoacid sequence similarity between *CYP52A13* and *CYP52A17* with *CYP52A5* and, respectively, *CYP52A9* from *C. maltosa*.

The yeast species *Yarrowia lipolytica*, presents eight genes named *ALK1* to *ALK8*, showing 30 – 44% similarity with *P450 alk* genes from *C. maltosa* [22].

Bioremediation technologies

In order to choose an effective bioremediation technology, there are various factors that need to be considered: the nature of the contaminant, the size and characteristics of the contaminated surface, the final goal of the process and the costs. Since bioremediation cannot be applied as a singular technology, the geological, hydrological and microbiological structure of the contaminated area may represent important limiting parameters.

Bioremediation technologies may be applied: *ex situ* – the polluted soil is subject to various treatments in bioreactors; *in situ* – the metabolic activity of the indigenous microbial communities is augmented by supplementing some of the limiting growth factors, e.g. oxygen, phosphorus and nitrogen [23, 24].

In present, the most used bioremediation strategies include:

➢ *bioaugmentation* – mostly used for soil remediation by addition of microorganisms or specific enzymes with degrading effects on the polluted substrate;

➢ *bioventing* – air is ventilated through soil in order to augment the growth of indigenous or exogenous microorganisms;

➢ *in situ biodegradation* – aqueous solutions containing nutrients and oxygen are circulated through contaminated soil, improving the growth rate of microbial communities;

➢ *bioreactors* – are used for soil as well as water remediation, by creating mixing conditions aimed to increase the bioremediation of soil-bound or water-soluble contaminants;

➢ *biofilters* – used for volatile organic compounds which are eliminated by air circulation through compost or soil containing microorganisms with xenodegrading abilities;

➢ *landfarming* – oil spills effects are diminished by spreading the contaminated soil over a prepared bed, and the microbial biodegradative activity is stimulated through aeration;

➢ *composting* – the polluted soil is mixed with non-toxic organic compounds required for the development of a rich microbial population.

Bioremediation has many advantages compared to the conventional decontamination techniques, such as: maintaining the ecological equilibrium; the contaminants are eliminated through microbial metabolic processes; biological systems transport costs are relatively low and use less energy; bioremediation may be used in combination with other treatment technologies.

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