Chapter 21
Bioremediation and Biodegradation of Hydrocarbons by Cold-Adapted Yeasts

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Abstract Cold-adapted microorganisms play a significant role in the biodegradation of organic pollutants in cold environments, where ambient temperatures often coincide with their growth temperature range. A number of studies demonstrated the potential of cold-adapted yeasts to degrade a broad range of hydrocarbons, including alkanes, aromatic, and polyaromatic hydrocarbons (PAHs), at low temperatures. The high metabolic versatility and the ability to degrade high amounts of organic pollutants at temperatures down to 1 °C point to the important role of yeasts for biodegradation processes in habitats with permanently low temperatures. The contribution of cold-adapted yeasts in the biodegradation and bioremediation of hydrocarbons in cold environments may be much more important than currently recognized.

Keywords Petroleum hydrocarbons · Phenol · Lipase · Biostimulation

Contents

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21.1 Introduction

The environmental contamination with organic pollutants is a widespread problem in all climates. With increasing attention toward the preservation of the environment, the clean-up of contaminated sites gained increasing interest. A number of methods, including physical, chemical, and biological treatments, are available for the treatment of contaminated soils, ground and surface waters (van Hamme et al. 2003; Singh and Ward 2004; Ma and Jiao 2012).

Microorganisms in contaminated cold environments are subjected to a number of special challenges, nonetheless low-temperature biodegradation of many components of petroleum hydrocarbons at low temperatures has been reported in Arctic, Alpine, and Antarctic environments and can be attributed to cold-adapted hydrocarbon-degrading microorganisms. This chapter is a review of the potential of cold-adapted hydrocarbon-degrading yeasts for the decontamination of pollutants in cold areas.

21.2 Bioremediation

Bioremediation attempts to accelerate the natural biodegradation rates through the optimization of limiting environmental conditions and is an ecologically and economically effective cleanup technology. Bioremediation has been defined as "the use of living organisms to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous compounds" (Gibson and Saylor 1992). The development of biological remediation methods is based on the capacity of a broad spectrum of microorganisms to utilize organic compounds as the sole source of carbon and energy (biodegradation); microorganisms transform or mineralize organic contaminants into less harmful, nonhazardous substances, which are then integrated into natural biogeochemical cycles. As reported already very early (ZoBell 1946), biodegradative microorganisms are widely distributed in nature and can be enriched in many, if not most, types of environments where contaminants may serve as organic carbon sources. This is not surprising since hydrocarbons are naturally occurring organic compounds, and consequently, microorganisms have evolved the ability to degrade

these compounds. Accordingly, hydrocarbon-degrading microorganisms have also been described in cold environments, where they may adapt rapidly to the contamination, as demonstrated by significantly increased numbers of hydrocarbon degraders after a pollution event (Kerry 1990; Aislabie et al. 2001; Margesin and Schinner 2001; Bej et al. 2010; Greer et al. 2010).

Hydrocarbon pollution in cold climates is an area of particular importance since contaminated areas are often remote, and thus, the degradation capacity of indigenous microorganisms is required. Cold environments are increasingly exposed to petroleum exploration, production, and transport, and these activities increase the risk of accidental oil release. Such environments include polar and alpine environments. Also in temperate climatic regions, temperatures of subsoils, groundwater, and industrial wastewater can often decrease to temperature levels around or below 10 °C due to seasonal and/or diurnal fluctuations.

Successful bioremediation of hydrocarbon-contaminated aquatic and terrestrial environments in various cold environments, including Arctic, Antarctic, and Alpine areas, has been reported (Brakstad 2008; Filler et al. 2008; Bej et al. 2010; Greer et al. 2010). Several remediation schemes, such as biopiles, landfarming and engineered bioremediation, have been implemented successfully at petroleum-contaminated cold sites (Filler et al. 2008).

21.3 Limiting Factors for Bioremediation in Cold Environments

The intensity of biodegradation is influenced by a number of environmental factors. The composition, concentration and bioavailability of contaminants as well as the pollution history of the contaminated environment and the chemical and physical characteristics (nutrients and nutrient availability, oxygen, pH value, temperature, water content, salinity) of the contaminated area play a major role.

An important factor governing microbial activity, and thus also biodegradation, is temperature. Due to the Q_{10} effect, reaction rates are reduced in the cold, however, local environmental conditions select for populations with high activities at low temperatures. Cold-adapted microorganisms have evolved a series of adaptation strategies that enable them to compensate for the negative effects of low temperatures on biochemical reactions (Feller 2007; Margesin et al. 2008; Buzzini et al. 2012). Substantial growth and metabolic activity (respiration and biosynthesis) of microorganisms, including yeasts (*Leucosporidum* spp., *Mrakia* spp.) at subzero temperatures down to −20 °C and even −35 °C have been demonstrated (Panikov and Sizova 2007; Bakermans 2008; Amato et al. 2010; see also Chap. 15).

Low temperatures induce the formation of ice crystals, which results in low availability of liquid water, i.e., the creation of low water activity (A_w). Low water activity in habitats like snow, sea ice, and glacier ice influences microbial activity to a significant extent (Gunde-Cimerman et al. 2003).

Temperature affects the rates of biodegradation also by its effects on the physical nature and chemical composition of the contaminants (Atlas and Bartha 1992). Bioavailability and solubility of hydrophobic substances, such as some aliphatic and polyaromatic hydrocarbons (PAHs), are temperature-dependent. At low temperatures, the volatilization of toxic short-chain alkanes is reduced and their water solubility is increased, which results in increased toxicity (Walker and Colwell 1974; Atlas and Bartha 1992; Whyte et al. 1998). A temperature decrease also results in a decrease in diffusion rates of organic compounds and in an increase in viscosity, which affects the degree of distribution (Whyte et al. 1998; Rojo 2009). Under cold conditions, the precipitation of certain alkanes (from crude oil) as waxes greatly diminishes their availability to hydrocarbon-degrading microorganisms (McKenzie and Hughes 1976). All these temperature effects delay the onset of biodegradation under cold conditions.

Until recently, frozen soils were considered a practically impermeable barrier to pollutants. Meanwhile, it is known that hydrocarbons can penetrate into frozen soils. Even ice-saturated soils are not an absolute impermeable barrier for oil penetration (Chuvilin et al. 2001; Barnes and Biggar 2008).

Additional limiting factors for biodegradation, such as low-nutrient availability, dryness, large temperature fluctuations and frequent freeze-thaw events, depend on the local environmental conditions in Arctic, Alpine, and Antarctic regions (Margesin 2004).

21.4 Mycoremediation: The Contribution of Cold-Adapted Yeasts to Bioremediation

Mycoremediation is a new and emerging field in bioremediation and involves the use of fungi to degrade (reduce or eliminate) organic compounds including environmental hazards (Singh 2006; Hughes and Bridge 2010; Harms et al. 2011). The important role of filamentous fungi in the degradation of hydrocarbons or their metabolites (Kerry 1990; Aislabie et al. 2001), and the benefits of their use as bioremediative agents in cold habitats have been recognized (Hughes and Bridge 2010). However, there is little information on the role of cold-adapted yeasts.

According to Hughes and Bridge (2010), yeasts may play an important role in facilitating hydrocarbon degradation for other microbial groups, while Singh (2006) emphasized that yeasts and filamentous fungi may contribute significantly to oil degradation under conditions that select against bacterial growth. Ahearn et al. (1971) recognized already early the important role of yeasts in the *in situ* degradation of surface oil deposits in marine environments and listed a number of advantages of yeasts over bacteria: Vegetative yeast cells are more resistant than those of bacteria to stress conditions, including exposure to UV radiation and alterations of osmotic pressure and salinity. A further advantage is the ability of some yeasts, mainly representatives of the genus *Trichosporon*, to penetrate and develop within oil globules, which offers protection from predators. In contrast, bacterial cells remain attached to the surface of oil globules.

Bacteria and filamentous fungi have been claimed to be the main degraders in soil environments, while bacteria and yeasts appear to be the prevalent hydrocarbon degraders in aquatic ecosystems (Atlas 1981; Singh 2006). A higher occurrence of yeasts was observed in rivers and lakes than in the ocean. Yeast populations increased considerably in oil-contaminated estuarine sediments over a 4-month period but declined after an initial increase in open ocean waters in the presence of petroleum hydrocarbons (Ahearn and Meyers 1976).

Walker and Colwell (1974) compared the utilization of model petroleum in water and sediment samples of marine environments at low temperatures (0–10 °C) by bacteria, yeasts, and filamentous fungi. Hydrocarbon degradation at 0 and 5 °C could be attributed mainly to bacteria, while there was a lack of significant growth of yeasts and filamentous fungi. Yeast populations were higher in April than in February and contributed significantly to petroleum utilization at 10 °C, however, to a significantly lower extent than bacteria. Hydrocarbon degradation by filamentous fungi was not detected at any of the temperatures tested.

Ahearn et al. (1971) established the role of yeasts in the removal of hydrocarbons from oil-contaminated marine environments. Despite the widespread occurrence of hydrocarbonoclastic yeasts, strains able to assimilate high amounts of hydrocarbons (>2 % v/v kerosene or hexadecane) and vapors of aromatic compounds were concentrated in oil-polluted habitats.

21.4.1 Soil Biostimulation

The most widely used bioremediation procedure in cold soils is biostimulation of the indigenous microorganisms by supplementation of appropriate nutrients and optimization of other limiting factors, such as oxygen content, pH, and temperature control. Most commonly hydrocarbon degradation in such studies is attributed to soil bacteria, while unfortunately no studies are available on the contribution of the yeast population to bioremediation processes in soils. There is generally little known about the interactions of soil yeasts *in situ* (Botha 2006). Since ascomycetous and basidiomycetous yeasts constitute a considerable proportion of the indigenous soil population (yeast numbers range from less than 10 to as many as 10^6 culturable cells per gram of soil) and play an important role in mineralization processes (Botha 2006, 2011), it has to be assumed that they contribute to a significant extent to biological decontamination.

21.4.2 Soil Bioaugmentation

Bioaugmentation by inoculating allochthonous hydrocarbon degraders (predominantly bacteria) has been used as a bioremediation option to treat petroleum-contaminated cold and temperate sites. However, this strategy generally

underperformed or gave no better results than fertilization (Margesin 2004; Aislabie et al. 2006; Filler et al. 2008). Similar results were obtained when investigating the efficiency of a cold-adapted yeast strain identified as *Yarrowia lipolytica*, which degraded efficiently hydrocarbons (diesel oil) in liquid culture at 10 °C (Margesin and Schinner 1997a), for soil bioaugmentation. Representatives of this yeast species degrade hydrophilic substances very efficiently (Bankar et al. 2009). The inoculation of five diesel oil–contaminated Alpine subsoils resulted only in a small increase (5–7 %) of the total hydrocarbon decontamination at 10 °C. Biostimulation by inorganic nutrients enhanced oil biodegradation to a statistically significantly greater degree than inoculation. In none of the five soils did fertilization plus inoculation result in a higher decontamination than fertilization alone (Margesin and Schinner 1997b).

The observation that this cold-adapted yeast inoculum introduced into soil did not contribute efficiently to the decontamination process led to a further study in order to compare the biodegradation behavior of this cold-adapted yeast in liquid culture and in one of the previously investigated five soils at temperatures between 4 and 30 °C (Margesin and Schinner 1997a). In liquid culture, the inoculum degraded diesel oil over the whole temperature range tested with a maximum activity between 10 and 20 °C (37–41 % biodegradation of the initial oil content); 25–27 % were degraded at 4 and 25 °C, respectively, and still 18 % were utilized at 30 °C. When inoculated into soil, the degradation activity of the inoculum was completely changed: biodegradation ranged from 0 to 3.6 % and was only observed at temperatures $\leq$15 °C, decreased with increasing temperature and time, and was significantly lower in soil than in liquid culture. With increasing incubation time and temperature, hydrocarbon utilization by the inoculum in soil decreased, whereas degradation by the indigenous soil microorganisms increased.

These data clearly showed that the degradation behavior of an inoculum introduced into soil cannot be predicted from liquid culture experiments and that the success of bioaugmentation cannot be predicted from liquid culture experiments. With increasing indigenous biodegradation, the inoculum might have been replaced (Margesin and Schinner 1997a). Introduced microorganisms are subject to various abiotic and biotic stresses. Preconditions of a successful application of bioaugmentation are the expression of the biodegrading activities in the polluted environment and the survival of the inoculated microorganisms at least for the time necessary for remediation (Fritsche et al. 1998). The bioaugmentation of contaminated soils frequently requires a short-term approach since, even under best conditions, the introduced organisms will not survive for extensive periods (Pritchard et al. 1998).

21.4.3 Wastewater Bioaugmentation

The use of cold-adapted microorganisms for low-energy wastewater treatment leads to a significant decrease in operational costs. In cold climates, wastewater

temperature often decreases to 10 °C and below, which requires the activity of cold-adapted degraders for an efficient treatment.

The amount of organic pollutants in surface water (e.g., lakes or rivers) or wastewater is commonly determined indirectly by measuring the chemical oxygen demand (COD), which thus is a measure of water quality. High COD removal rates (64–83 %) were obtained in artificial sewage, initially containing 500 mg COD per liter of sewage, after 6 h at 5 °C with six cold-adapted yeast strains that belonged to the genera *Candida*, *Pichia*, *Rhodotorula* and *Saccharomyces* (Ma et al. 2007). COD removal by these yeast strains was better than that obtained with six cold-adapted bacterial strains (45–65 %). All strains were isolated in winter from activated sludge at temperatures below 10 °C. The mixture of bacterial and yeast populations resulted in a COD removal of 87 %, which was only slightly higher that the removal observed with yeasts alone. When this mixed population was inoculated into domestic sewage, COD removal (83 %) was only slightly lower compared to that obtained in artificial sewage (87 %) (Ma et al. 2007). These data demonstrate that cold-adapted yeast strains represent a promising source as inocula for accelerated wastewater treatment in climates, where the activity of mesophilic degraders is limited.

21.5 Lipase Activity: An Indicator of the Hydrocarbon Biodegradation Potential

Several cold-adapted yeasts are known to produce cold-active lipases (Chap. 17). A number of cold-adapted lipase-producing bacterial (Margesin et al. 2013) and yeast strains (Margesin et al. 2003) were able to degrade aliphatic hydrocarbons. A positive relation between lipase activity and hydrocarbon biodegradation in marine environments (Ahearn et al. 1971) and in soils contaminated with petroleum hydrocarbons (Margesin et al. 1999, 2007a) has been recognized. Therefore, cold-adapted yeasts that produce cold-active lipases might represent an interesting source for low-temperature hydrocarbon biodegradation.

21.6 Hydrocarbon Biodegradation by Pure Cultures of Cold-Adapted Yeasts

A wide variety of microorganisms, including bacteria, fungi, and algae, have the ability to metabolize aliphatic and aromatic hydrocarbons (Alexander 1999). Nonetheless, most studies on biodegradation abilities of cold-adapted microorganisms have focused on bacteria. Despite the fact that yeasts are able to degrade a wide range of hydrocarbons (Harms et al. 2011), comparatively little is known on the biodegradation abilities of cold-adapted yeasts.

21.6.1 Sources of Isolation

Hydrocarbon-degrading culturable cold-adapted yeasts have been isolated from oil-contaminated cold environments, such as Antarctic (Atlas et al. 1978; Kerry 1990; Aislabie et al. 2001) and Alpine soils (Margesin and Schinner 1997a; Bergauer et al. 2005; Margesin et al. 2005). Antarctic oil-contaminated soils in the Ross Sea region contained 10^4–10^6 culturable yeasts per gram soil in soil surface; these numbers decreased with soil depth (Aislabie et al. 2001). Similar levels were reported in contaminated Antarctic soils from the former McMurdo Dump site on Ross Island (Atlas et al. 1978). The enrichment of yeasts, especially of fermentative representatives, after pollution events was also evident in marine environments (Ahearn et al. 1971; Kutty and Philip 2008).

Kerry (1990) isolated bacteria and yeasts from a number of Antarctic petroleum contaminated soils and reported the predominance of bacterial isolates. Yeasts could be isolated from 11 to 40 % of the samples investigated, however, only 7–10 % of the samples contained yeasts were able to utilize distillate (the main petroleum product used in Australian Antarctic operations, containing a wide range of hydrocarbon components) as sole carbon source.

Hydrocarbon-degrading yeasts could also be isolated from pristine (uncontaminated) cold environments, such as Alpine glacier cryoconite (Margesin et al. 2003), which indicates the ubiquity of hydrocarbon degraders. The ability of strains from pristine habitats to degrade phenol and related compounds may be linked with a role in the degradation of litter and humification process (Bergauer et al. 2005).

21.6.2 Growth Temperature Range

Cold-adapted yeasts are characterized by a more restricted growth temperature range than cold-adapted bacteria; this could be demonstrated with strains isolated from contaminated and from pristine environments. For example, 60 % of the investigated yeast strains but only 8 % of the investigated bacterial strains were unable to grow above 20 °C (Margesin et al. 2003). Similarly, all representatives of the group of twelve *Microbotryomycetidae* strains (mainly representatives of the genus *Rhodotorula*) could not growth above 20 °C, and four of these strains were even unable to grow above 15 °C (Bergauer et al. 2005). The restricted growth temperature range of yeasts indicates their potential for low-temperature bioremediation processes in permanently cold environments. The application of degraders that are active over a wide temperature range might be advantageous in environments that undergo large temperature fluctuations.

Adaptation of cold-adapted yeasts strains to their natural cold environment is also apparent from optimized growth at low temperatures. Two cold-adapted yeast

strains, identified as *Leucosporidiella creatinivora* and *Rhodotorula glacialis* and able to grow at 1–20 °C, produced the highest amount of biomass (as determined by measuring OD600, viable counts and dry mass) at 1 °C, i.e., at 20 °C lower than the maximum temperature for growth (Margesin 2009). This demonstrates that cultivation temperatures close to the maximum growth temperature are not appropriate for studying psychrophiles. Highest yields of cold-adapted cells and their biotechnologically important compounds are generally obtained at cultivation temperatures that correspond to those of the natural environment of the strains, which should be considered for applied aspects.

21.6.3 Low-Temperature Biodegradation of Petroleum Hydrocarbon Fractions: A Comparison of Bacterial and Yeast Strains

Yeasts are known for their metabolic versatility, including the biodegradation of organic compounds (Kutty and Philip 2008). The comparison of culturable cold-adapted bacterial and yeast strains to degrade representative fractions of petroleum hydrocarbons (*n*-alkanes, monoaromatic, and polycyclic aromatic hydrocarbons) demonstrated the efficiency and versatility of yeasts (Margesin et al. 2003). The investigated strains were isolated from uncontaminated Alpine glacier habitats (cryoconite and ice caves) and belonged mainly to the bacterial genera *Pseudomonas* and *Arthrobacter*, and to the yeast genus *Rhodotorula* (Margesin et al. 2007b).

While 79 % of the investigated 28 yeast strains utilized n-hexadecane for growth at 10 °C, only 7 % of the studies 61 bacterial strains were able to degrade *n*-hexadecane and none of them degraded *n*-dodecane. Remarkably, only seven yeast strains but no bacterial strain degraded both *n*-dodecane and *n*-hexadecane. The best *n*-alkane degrader was a representative of the species *Y. lipolytica*.

The efficiency of cold-adapted yeasts compared to bacteria was also shown with regard to the biodegradation of aromatic and PAHs: 13 % of the bacterial strains, but 25 % of the yeast strains degraded 2.5 mM phenol at 10 °C. Similarly, 13 % of the bacterial strains, but 21 % (phenanthrene) or 32 % (anthracene) of the investigated yeast strains utilized three-ring PAHs.

The ability to degrade at least one of the tested hydrocarbons (hexadecane, phenol, phenanthrene or anthracene) at 10 °C was restricted to 26 % of the studied bacterial strains, while 89 % of the yeast strains were degraders. Four yeast strains (*Rhodotorula* spp.) but none of the bacterial strains could grow with both aliphatic and aromatic hydrocarbons. The capability to utilize a wide range of hydrocarbons under cold conditions is advantageous for the low-temperature treatment of mixed pollutions.

21.6.4 Low-Temperature Biodegradation of Phenol

Phenol and phenolic compounds are widely distributed in nature and as environmental pollutants. They are common constituents of many industrial wastewaters, such as those produced from crude oil refineries and coal gasification plants. Due to their toxicity to microorganisms, even low concentrations of phenolic compounds (such as 2 mM) can often cause the breakdown of wastewater treatment plants by inhibition of microbial growth, which can lead to decreased effluent quality (Ren and Frymier 2003). Biological treatment of phenolic compounds is preferable to other methods due to its effectiveness and the production of innocuous end products (Aleksieva et al. 2002).

21.6.4.1 Effect of Phenol Concentration on Biodegradation

Low-temperature phenol biodegradation by cold-adapted Alpine yeasts has been studied using fed-batch cultivation with increasing phenol concentrations as the sole carbon source. This cultivation method has been proven to be efficient for the selection and acclimation of phenol-degrading microorganisms (Guieysse et al. 2001). It could be demonstrated that cold-adapted yeasts tolerate and degrade higher amounts of phenol compared to cold-adapted bacteria.

Amounts of phenol as high as 10–12.5 mM were degraded at 10 °C by cold-adapted *Rhodotorula* species (*Rhodotorula psychrophenolica*, *R. glacialis*) isolated from uncontaminated glacier materials (Margesin et al. 2003, 2007b), while two yeast strains from contaminated soils (*Trichosporon dulcitum* and an urediniomycete later classified as *Glaciozyma watsonii*) even degraded up to 15 mM phenol at 10 °C (Margesin et al. 2005). In comparison, cold-adapted Alpine bacterial strains (*Arthrobacter psychrophenolicus*, *A. sulfureus, Rhodococcus* spp.) degraded up to 10–12.5 mM phenol (Margesin et al. 2003, 2005, 2013) at 10 °C. Among mesophilic yeasts, the degradation of amounts up to 27 mM phenol was reported at 30 °C (Krug et al. 1985; dos Santos et al. 2009).

Cold-adapted phenol-degrading yeasts were also shown to degrade phenol faster than bacteria. A concentration of 10 mM phenol was fully degraded after 11–14 days by two rhodococci, but already after 3 days by *G. watsonii*. 12.5 mM phenol was fully degraded after 7 and 10 days by *G. watsonii* and *T. dulcitum*, whereas the rhodococci needed 25 days (Margesin et al. 2005).

21.6.4.2 Effect of Temperature on Phenol Biodegradation

The optimum temperature for phenol degradation has been found to be generally lower for cold-adapted yeasts than for cold-adapted bacteria (Margesin et al. 2003, 2005). The majority of yeast strains preferred a temperature of 10 °C for phenol degradation, and their biodegradation was faster at 1 °C than at 20 or 15 °C. In one

case, biodegradation performance at 1 and 10 °C was comparable. Few yeasts had an optimum temperature of 20 °C for phenol degradation.

Similarly, studies on the effect of temperature on growth and biodegradation of 5 mM phenol showed that *G. watsonii* degraded 5 mM phenol at 1 °C faster than two rhodococci at 10 °C, but no growth occurred at 20 °C in the presence of phenol (Margesin et al. 2005).

Microorganisms that degrade high amounts of organic pollutants within a short time at temperatures down to 1 °C represent a promising source of accelerated wastewater treatment.

21.6.4.3 Toxicity of Phenol and Related Compounds

Low-temperature (10 °C) biodegradation of phenol and phenol-related monoaromatic compounds of serious environmental concern (catechol, resorcinol, hydroquinone, benzoate, salicylate, guaiacol, *o*-cresol, *m*-cresol, *p*-cresol, *p*-nitrophenol, *p*-nitrotoluene) was evaluated using 32 basidiomycetous yeast strains isolated from Alpine soils and glacier cryoconite (Bergauer et al. 2005). The strains were representatives of the Hymenomycetes (*Cryptococcus terreus*, *Cryptococcus terricola*) or Urediniomycetes (*Rhodosporidium lusitaniae*, *Rhodotorula ingeniosa*, *R. glacialis*, *Rhodotorula psychrophila, R. psychrophenolica*, *Mastigobasidium intermedium*, *L. creatinivora*, *Sporobolomyces roseus*, which is the anamorph of *Sporidiobolus metaroseus*).

None of the 32 strains utilized any of the highly volatile mono-aromatic compounds (benzene, toluene, ethylbenzene, nitrobenzene, *o*-xylene, *m*-xylene, and *p*-xylene) as the sole carbon source. Non/low volatile aromatic compounds were degraded in the following order: phenol > hydroquinone > resorcinol > benzoate > catechol > salicylate ≫ *p*-cresol > *m*-cresol. *o*-Cresol, guaiacol, *p*-nitrophenol, or *p*-nitrotoluene were not utilized for growth.

The toxicity of the tested 19 monoaromatic compounds was influenced by the chemical structure (functional groups) of the compounds. Methylated compounds were highly toxic, followed by methoxylated and hydroxylated compounds; carboxylated compounds had the lowest toxicity. Biodegradability of phenol-related compounds was influenced by volatility and water solubility of the compounds. Interestingly, the taxonomic affiliation of the strains seemed to influences toxicity and biodegradability. *L. creatinivora* strains were characterized by higher IC_{50} values (50 % growth inhibition in the presence of nutrients) than all other yeast species, whereas *S. roseus* was the most sensitive species. In addition, representatives of *L. creatinivora* were characterized by a higher metabolic versatility (i.e., ability to utilize a wide spectrum of compounds) than representatives of other species (Bergauer et al. 2005).

Strains such as those characterized in this study could be useful, for example, as inocula for the acceleration of low-energy wastewater treatment. Members of *Rhodotorula* and *Leucosporidiella*, esp. *L. creatinivora,* could be especially important, because these strains produced higher amounts of biomass, degraded

more non/low volatile mono-aromatic compounds and were able to grow in the presence of higher concentrations than other yeast species.

21.6.4.4 Effect of Immobilization on Phenol Degradation

Immobilization of microorganisms may result in better degradation rates. Due to the promotion of biofilm formation by immobilized cells, they are better protected from damage and can maintain continuous cell growth and biodegradation. The production of exopolymeric substances (EPS) is a characteristic feature of many biofilms. EPS assist in the attachment process and also protect the cells from fluctuating environmental conditions (Chandran and Das 2011).

Immobilized cells of mesophilic *Candida tropicalis* showed better diesel oil (Chandran and Das 2011) and phenol degradation rates and could be exposed without loss of viability to higher amounts of phenol compared to free cells (Juares-Ramirez et al. 2001). The same was reported for *Aureobasidium pullulans* at 30 °C (dos Santos et al. 2009) and for three cold-adapted phenol-degrading yeasts (*L. creatinivora*, *C. terreus*) at 10 °C (Krallish et al. 2006). The *C. terreus* strain was more active in biofilm formation on solid carriers than two *L. creatinivora* strains, however, its phenol degradation performance was probably limited due to the formation of a rich EPS layer, which decreased the diffusion of phenol to the cells surface. All three cold-adapted yeast strains accumulated both trehalose and glycogen during growth on glucose or phenol as sole carbon and energy source (Krallish et al. 2006). Yeasts that accumulate high amounts of intracellular storage compounds (trehalose, glycerol, etc. serve as carbon and energy reserve compounds) are tolerant to adverse environmental conditions (Wiemken 1990). Trehalose plays an important role for the preservation of yeast cells under extreme environmental conditions, such as high temperature, dehydration and high osmotic conditions (Hounsa et al. 1998).

21.6.4.5 Enzymes Involved in Phenol Degradation: Catechol Dioxygenases

Catechol dioxygenases are involved in the second step of phenol degradation and catalyze the ring cleavage of catechol (=*o*-hydroxyphenol) either by the *ortho* (catechol-1,2-dioxygenase; C1,2D) or by the *meta* type (catechol-2,3-dioxygenase; C2,3D). Almost all investigated Alpine cold-adapted phenol-degrading yeast strains oxidize catechol predominantly by the *ortho* type of ring cleavage, while the *meta* type of ring cleavage was only observed in one yeast strain (Margesin et al. 2003, 2005). dos Santos et al. (2009) observed an inhibitory effect on C1,2D activity at phenol amounts above 6 mM phenol.

The presence of C1, 2D activity and the absence of C2,3D activity has also been observed frequently with mesophilic phenol-degrading yeasts. In contrast, bacterial representatives display often both types of ring cleavage.

21.7 Conclusions

Cold-adapted microorganisms play a significant role in the biodegradation of organic pollutants in cold environments, where ambient temperatures often coincide with their growth temperature range. A number of studies demonstrated the potential of cold-adapted yeasts to degrade a broad range of hydrocarbons, including alkanes, aromatic, and PAHs, at low temperatures. The capability to utilize a wide range of hydrocarbons is advantageous for the treatment of mixed pollutions.

The apparently more restricted growth temperature range, substantially lower optimum temperatures for growth and activity as well as the great metabolic versatility of cold-adapted yeasts compared to cold-adapted bacteria point to the important role of yeasts for biodegradation processes in habitats with permanently low temperatures. Yeasts that degrade high amounts of organic compounds within a short time at temperatures down to 1 °C (Margesin et al. 2005) represent an especially useful source for a wide range of applications, such as accelerated wastewater treatment. Due to their ability to tolerate and degrade high amounts of phenol, cold-adapted yeast strains as promising candidates for the biological cleaning of phenol-contaminated environments in climates, where low temperatures can otherwise limit microbial degradation.

Most investigations on low-temperature biodegradation have been concentrated on bacteria, whereas the potential of cold-adapted yeasts may have been underestimated. However, yeasts are a largely unexplored source of cold-adapted hydrocarbon degraders; their contribution in the biodegradation of hydrocarbons in cold environments may be much more important than currently assumed.

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