Appl Microbiol Biotechnol (2006) 72:223–243
DOI 10.1007/s00253-006-0476-5

MINI REVIEW

Microbial degradation of nonylphenol and other alkylphenols—our evolving view

P. F. X. Corvini · A. Schäffer · D. Schlosser

Received: 2 March 2006 / Revised: 11 April 2006 / Accepted: 19 April 2006 / Published online: 7 July 2006

Abstract Because the endocrine disrupting effects of nonylphenol (NP) and octylphenol became evident, the degradation of long-chain alkylphenols (AP) by microorganisms was intensively studied. Most NP-degrading bacteria belong to the sphingomonads and closely related genera, while NP metabolism is not restricted to defined fungal taxa. Growth on NP and its mineralization was demonstrated for bacterial isolates, whereas ultimate degradation by fungi still remains unclear. While both bacterial and fungal degradation of short-chain AP, such as cresols, and the bacterial degradation of long-chain branched AP involves aromatic ring hydroxylation, alkyl chain oxidation and the formation of phenolic polymers seem to be preferential elimination pathways of long-chain branched AP in fungi, whereby both intracellular and extracellular oxidative enzymes may be involved. The degradation of NP by sphingomonads does not proceed via the common degradation mechanisms reported for short-chain AP, rather, via an unusual *ipso*-substitution mechanism. This fact underlies the peculiarity of long-chain AP such as NP isomers, which possess highly branched alkyl groups mostly containing a quaternary α-carbon. In addition to physicochemical parameters influencing degradation rates, this structural characteristic confers to branched isomers of NP a biodegradability different to that of the widely used linear isomer of NP. Potential biotechnological applications for the removal of AP from contaminated media and the difficulties of analysis and application inherent to the hydrophobic NP, in particular, are also discussed. The combination of bacteria and fungi, attacking NP at both the phenolic and alkylic moiety, represents a promising perspective.

Keywords Bacteria · Degradation · Fungi · Laccase · Nonylphenol isomers · Sphingomonas

P. F. X. Corvini · A. Schäffer
Department of Environmental Research,
RWTH Aachen University,
52074 Aachen, Germany

A. Schäffer
Fraunhofer Institute for Molecularbiology and Applied Ecology,
57392 Schmallenberg, Germany

D. Schlosser (✉)
Department of Environmental Microbiology,
UFZ Centre for Environmental Research Leipzig-Halle,
Permoserstr. 15,
04318 Leipzig, Germany
e-mail: dietmar.schlosser@ufz.de

Introduction

Alkylphenols (AP) include many compounds ranging from cresols to dodecylphenol. AP consist of a phenol ring, which is mono- or polysubstituted by alkyl chains of variable length. AP and their polyethoxylated derivatives are used directly as intermediates or as additives (emulsifiers, detergents, and flotation and dispersing agents) for a wide range of industrial products and processes (Saito et al. 2004a,b; Guenther et al. 2005). Nonylphenol (NP) is by far the most commercially prevalent member of the AP family, representing approximately 85% of the total AP market. Because studies in the early eighties reported NP as a persistent pollutant in sewage sludge (Stephanou and Giger 1982; Giger et al. 1984, 1987), the presence of NP was widely reported in surface water, groundwater, atmosphere, pristine and sludge-amended soils, and food (Sundaram and Szeto 1981; Ekelund et al. 1993; Espadaler et al. 1997; Dachs et al. 1999; Liber et al. 1999; Moeder et al. 2000; Saito et al. 2004a,b). In the last decade, reports on adverse effects of NP on the endocrine system increased its concern,

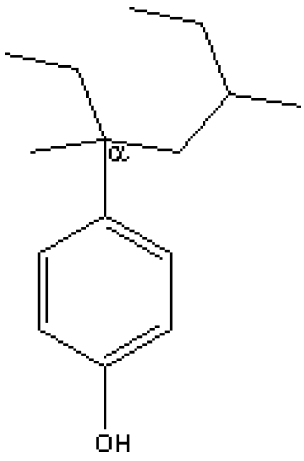

Fig. 1 Chemical structure of 4-[1-ethyl-1,3-dimethylpentyl] phenol possessing a quaternary α-carbon on the branched alkyl chain. This compound was found to be a major constituent of commercial tNP mixtures (Ruß et al. 2005)

although NP toxicity and bioaccumulation in aquatic species were already known (Granmo et al. 1989; Ekelund et al. 1990; Soto et al. 1991). For these reasons and due to the many studies referring to a higher xenoestrogenic potential of NP and octylphenol (OP), environmental studies have focussed on mainly NP and, to a lesser extent, on OP (Routledge and Sumpter 1997).

High NP concentrations in the environment are obviously correlated to anthropogenic activities and it is mainly found as a highly persistent microbial degradation product of NP polyethoxylate (NPnEO) surfactants (Giger et al. 1984, 1987; Tanghe et al. 1998, 1999a,b). NPnEOs are produced by the ethoxylation of technical grade NP (tNP). The latter is obtained by the alkylation of phenol with branched nonenes, resulting in a mixture of isomers of *para*-substituted derivatives (*p*-NP) and minute amounts of *ortho*-substituted NP (*o*-NP) (Kim et al. 2004). Up to now, 22 isomers of tNP were identified (Wheeler et al. 1997; Thiele et al. 2004), but a recent report cites the detection of about 80 isomers (Guenther et al. 2005). tNP is recalcitrant to degradation because more than 85% of the isomers possess a quaternary α-carbon on the branched alkyl chain (Wheeler et al. 1997) (Fig. 1). Such a stable structure is resistant to ω- and β-oxidation of the nonyl chain (Van Ginkel 1996). For this reason, tNP biodegradability is different to that of the linear alkyl chain NP (4-*n*-NP), which was not reported to be present in tNP.

Due to the nonyl chain, NP is hydrophobic (log K_{OW}=4.48) and tends to sorb to various materials (Ahel et al. 1993; Vinken et al. 2004). Such strong sorption makes the analysis of NP in environmental samples and even in mineral media difficult, and can lead to an overestimation of the degradation rates. Furthermore, many studies on NP degradation are impaired by the fact that the isomer mixture of tNP gives rise to a complex metabolic pattern. To overcome this difficulty, some authors have investigated the metabolism of the AP *tert*-OP, which consists of only one isomer (Tanghe et al. 2000; Pedersen and Hill 2000a,b, 2002; Ferreira-Leach and Hill 2001). Recently, methods for the synthesis of single isomers of NP were developed (Meldahl et al. 1996; Lalah et al. 2001; Vinken et al. 2002; Ruß et al. 2005). This has led to advances in the identification of the isomers contained in tNP, the elucidation of their degradation pathways and their mechanisms of action as hormonal disrupters (Meldahl et al. 1996; Lalah et al. 2003a–c; Corvini et al. 2004a–2006a; Schmidt et al. 2004; Gabriel et al. 2005a,b; Preuss et al. 2004; Ruß et al. 2005). Most studies have focussed on one of the most abundant and effective isomers of tNP, i.e., the 4(3′,5′-dimethyl-3′-heptyl)-phenol (4-[1-ethyl-1,3-dimethylpentyl] phenol according to International Union of Pure and Applied Chemistry (IUPAC) nomenclature) (Fig. 1).

The present paper focuses on NP biodegradation by axenic cultures of microorganisms and complex microbial communities in various environmental compartments. Attention is paid to the structure of the alkyl chain, i.e., branched isomers or the less relevant 4-*n*-NP, as a possible decisive feature for the catabolic pathways. To simplify the comparison between the various studies, IUPAC nomenclature of the various isomers and metabolites is used. Information concerning the degradation of other AP are also compiled where appropriate, e.g., for the comparison of the degradation pathways. Physicochemical and biological parameters affecting the microbial degradation of NP are discussed and biotechnological solutions for an improved elimination of NP are presented.

Degradation of NP by microbial consortia

In the aquatic environment, a rapid diminution of the NP concentration is observed in streams, static waterbodies, and sea water as a consequence of dilution with water flow, surface evaporation, codistillation, and degradation (Sundaram and Szeto 1981; Ekelund et al. 1993; Liber et al. 1999). However, NP dissipation is mostly due to sorption of the compound to sediments where it may subsequently accumulate in fauna and flora or undergo biodegradation. Ultimate NP biodegradation was reported in sediments, in soils, sewage treatment plants, and sludge-amended soils (Ekelund et al. 1993; Fujii et al. 2000a; Topp and Starratt 2000; Hesselsøe et al. 2001; Staples et al. 2001; Telscher et al. 2005). These studies suggest that NP degradation by microbial consortia is ubiquitous. The extent of mineralization of NP into CO_2 in sludge amended-soil containing NP can reach 40% and the corresponding half-lives range between 2 and 10 days (Topp and Starratt 2000; Staples et al. 2001).

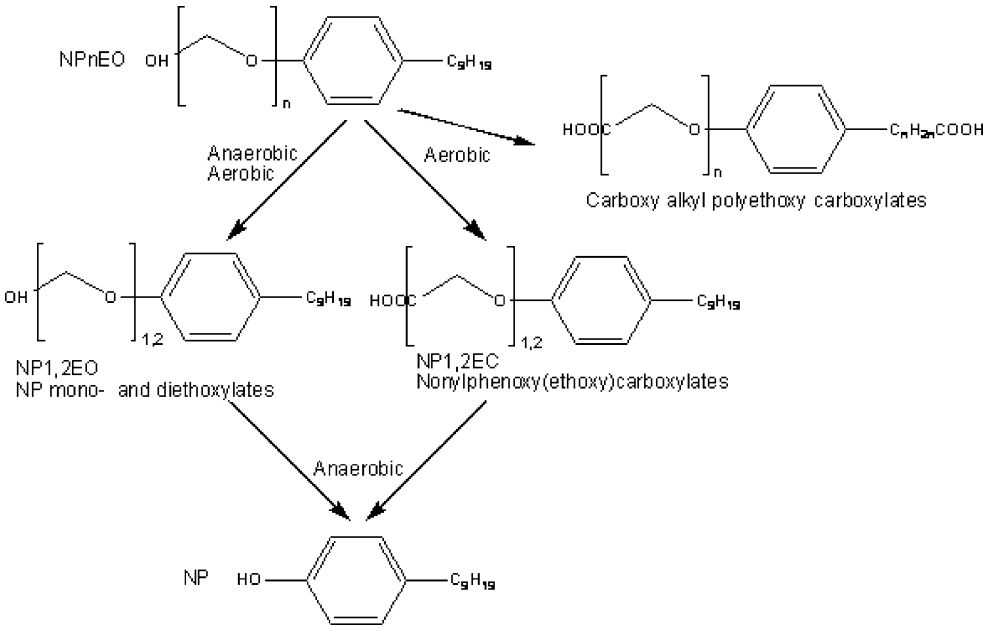

Fig. 2 Degradation of NPnEO in the wastewater treatment plant (adapted from Giger et al. 1984 and Di Corcia et al. 1998)

Almost no information on the degradation pathways of NP in the environment is available, while metabolites of NPnEO were reported (Fig. 2). Hydroxylated side-chain products of NPnEO containing non-highly branched NP were reported in wastewater treatment plants and environmental samples (Di Corcia et al. 1998; Jonkers et al. 2001; Montgomery-Brown et al. 2003). These compounds were oxidized at both the alkyl and the ethoxylate part of the NPnEO molecules, forming carboxyalkyl polyethoxy carboxylates. When tNP was applied in a bioreactor inoculated with culture enrichments from NP-contaminated soil, carboxylic acids, branched alkanes, and short-chain AP (three and four carbons) were detected in the effluent (Soares et al. 2005b). However, almost no chromatographic data were provided in this study. Recently, 4-[1-ethyl-1,3-dimethylpentyl]-2-nitrophenol was identified as a metabolite of 4-[1-ethyl-1,3-dimethylpentyl]phenol in sandy loam soil/sludge systems (Telscher et al. 2005). To date, this is the first report of a metabolite of NP in environmental samples and it points to the possibility of a rather unexpected degradation pathway.

AP-degrading microorganisms

For a long time, it was thought that a complex microflora is a prerequisite for tNP degradation (van Ginkel 1996). Since the pioneering work of Tanghe et al. (1999b) who isolated a novel *Sphingomonas* strain designated as *Sphingomonas* sp. strain TTNP3, there was an increasing number of reports on bacteria, yeast, and fungi with the ability to degrade NP.

Bacterial isolates

Most reported tNP-degrading bacteria belong to the sphingomonads. All the strains were found in various environments affected by humans. *Sphingomonas* sp. TTNP3, *Sphingomonas cloacae* and *Sphingomonas xenophaga* strain Bayram were isolated from municipal wastewater treatment plants (Tanghe et al. 1999b; Fujii et al. 2001; Gabriel et al. 2005a). *Sphingobium amiense*, formerly reported as *Sphingomonas* sp. YT, was isolated from river sediment and is also capable of degrading tNP (deVries et al. 2001; Ushiba et al. 2003). *Stenotrophomonas* sp., *Pseudomonas mandelii* and *Pseudomonas veronii* were isolated from NP-contaminated soil (Soares et al. 2003a). These cold adapted bacteria degrade tNP, and the use of this as a source of carbon for growth was demonstrated by microrespirometry.

A total of 37 bacterial strains degrading NP were isolated by a single working group from various environments (sediment, sewage sludge, and activated sludge) under aerobic and anaerobic conditions (Yuan et al. 2004; Chang et al. 2004a,b, 2005a,b). *Acinetobacter baumannii*, *Arthrobacter nicotianae*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus horikoshii*, *Bacillus sphaericus*, *Corynebacterium* sp., and *Pseudomonas* sp. strain JC1 belong to these isolates. However, the experimental procedures used for the isolation, the identification of the bacteria, and the measurement of the NP concentration have left some doubts to the real capacity of the isolates to degrade and use NP as a sole source of carbon.

From all the NP-degrading bacterial isolates, sphingomonads display the highest degradation capacity, reaching

averaged volumetric degradation rates of up to 100 mg l^{-1} day^{-1} (Tanghe et al. 1999a,b; Fujii et al. 2000a,b; Corvini et al. 2004c; Gabriel et al. 2005a). Sphingomonads can grow on NP as a sole carbon and energy source. With *Sphingomonas* species, turbidity, total biomass, and respiration activity were well correlated with a concomitant NP degradation during incubation on minimal medium (Tanghe et al. 1999b; Fujii et al. 2000a,b; Gabriel et al. 2005a). In the case of *Sphingomonas* sp. TTNP3, it was unequivocally demonstrated by using a radioactively labeled single-branched isomer of NP that the aromatic ring was mineralized (approx. 70%) and a lesser extent (about 15%) was integrated into the biomass (Corvini et al. 2004c).

The degradation of short-chain AP such as cresols and xylenols was described in a wide range of bacteria, including *Nocardia* sp., *Achromobacter* sp., *Thauera aromatica*, *Desulfobacterium cetonicum*, *Burkholderia* sp., *Alcaligenes eutrophus*, *Bacillus stearothermophilus*, and many *Pseudomonas* species (Chapman and Hopper 1968; Buswell 1975; Poh and Bayly 1980; Hughes and Bayly 1983; Tschech and Fuchs 1987; Hanne et al. 1993; Kahng et al. 2001; Müller et al. 2001; Song et al. 2000). In addition, *Pseudomonas putida* was reported to degrade ethylphenol (McIntire et al. 1984). *P. veronii* INA06 and *Pseudomonas* sp. KL28 were isolated from activated sludge (Ajithkumar et al. 2003; Jeong et al. 2003). The former strain uses *p*-cresol, 4-*n*-propylphenol, 4-*n*-butylphenol, *n*-hexylphenol, and 4-*n*-amylphenol as a sole carbon source, but can only degrade 4-*n*-NP cometabolically with phenol (Ajithkumar et al. 2003). *Pseudomonas* sp. KL28 was able to utilize 4-*n*-AP with an alkyl chain length of up to five C atoms as a carbon source (Jeong et al. 2003).

Filamentous fungi and yeast isolates

Degradation of NP by several groups of filamentous fungi and also one yeast isolate was described. Among terrestrial filamentous fungi, tNP degradation was reported for several white-rot basidiomycetes, including *Bjerkandera* sp., *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, and *Trametes versicolor* (Dubroca et al. 2005; Soares et al. 2005a, 2006). In the presence of glucose as a cosubstrate, within 25 days *Bjerkandera* sp. and *T. versicolor* cultures removed approximately 99 and 97%, respectively, of tNP initially added at 100 mg l^{-1} (Soares et al. 2005a). Another study described the removal of tNP applied at 11 mg l^{-1} by *P. chrysosporium* and *T. versicolor* in the presence of sugars as cosubstrates (Dubroca et al. 2005). Other terrestrial species with a reported capacity for oxidizing tNP are micromycetes belonging to the zygomycete genera *Cunninghamella* and *Mucor*, and three *Fusarium* species representing mitosporic fungi (Dubroca et al. 2005). As was the case for the white-rot fungi, sugars were employed as cosubstrates. No organic degradation metabolites were described in the aforementioned studies. The white-rot fungi *P. chrysosporium* and *T. versicolor*, and *Cunninghamella*, *Fusarium*, and *Mucor* strains were also capable of oxidizing 4-*n*-NP (Dubroca et al. 2005).

Microfungi isolated from surface waters, such as a strain of the aquatic hyphomycete (Ingoldian fungus) *Clavariopsis aquatica*, a strictly aquatic mitosporic species with a known teleomorph belonging to the ascomycete genus *Massarina* (Webster 1992), and the unidentified mitosporic fungal isolate UHH 1-6-18-4, degraded both tNP and 4-*n*-NP (Junghanns et al. 2005). Under cometabolic conditions using malt extract as a growth substrate, strain UHH 1-6-18-4 and *C. aquatica* removed 81.5 and 63%, respectively, of the tNP initially applied at 55 mg l^{-1} (250 μM) within 30 days. Biological oxidation of tNP was verified by the detection of organic degradation metabolites. No degradation products were detectable after complete 4-*n*-NP removal, possibly due to the rapid further oxidation of such compounds.

So far, the only yeast isolate reported to degrade NP, a *Candida aquaetextoris* strain originally isolated from sludge from a textile industry wastewater treatment plant, was reported to grow on 4-*n*-NP (Corti et al. 1995; Vallini et al. 1997, 2001). In the absence of any other carbon source, 100 mg l^{-1} of 4-*n*-NP essentially completely disappeared within 14 days. This was accompanied by the formation of degradation intermediates (Vallini et al. 2001). *C. aquaetextoris* is the only known example of a fungal organism capable of growing on 4-*n*-NP, whereas neither filamentous fungi nor yeasts have been demonstrated to utilize tNP as a growth substrate.

Most studies addressing the fungal degradation of other AP focused on short-chain compounds. Cometabolic degradation was described for *p*-cresol in *P. chrysosporium* (Kennes and Lema 1994) and for both *o*-cresol and 2,6-dimethylphenol in a *Penicillium frequentans* strain (Hofrichter et al. 1995). Several mitosporic molds and yeasts are able to utilize *m*-cresol, *p*-cresol, and 4-ethylphenol as growth substrates, and yeasts were also reported to grow on *o*-cresol (Hofrichter and Scheibner 1993; Jones et al. 1993, 1994; Middelhoven and Spaaij 1997; Middelhoven et al. 2000, 2004; Garcia-Pena et al. 2005).

Degradation pathways of NP by sphingomonads

While detoxification metabolites and catabolites of branched isomers of NP were described in invertebrates, fishes, and plants (Pedersen and Hill 2000a,b; Lalah et al. 2003b; Schmidt et al. 2004), degradation and transformation pathways in bacteria were only documented in sphingomonads, i.e., *Sphingomonas* sp. TTNP3, *S. cloacae*,

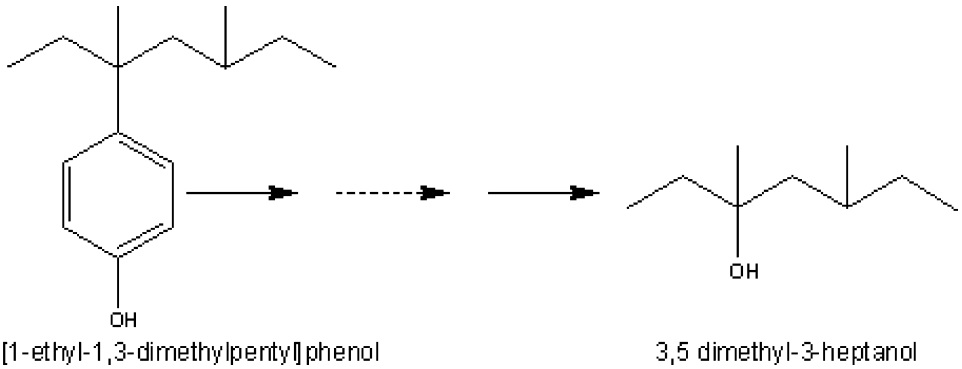

Fig. 3 Production of 3,5-dimethyl-3-heptanol by *Sphingomonas* sp. strain TTNP3 as the dead-end product of 4-[1-ethyl-1,3-dimethylpentyl]phenol (adapted from Corvini et al. 2004c)

and *S. xenophaga* Bayram (Tanghe et al. 1999a,b; Fujii et al. 2000b; Corvini et al. 2004a–2006b; Gabriel et al. 2005a,b).

The NP degradation pathways of *Sphingomonas* sp. TTNP3, *S. xenophaga*, and *S. cloacae* share similarities as the corresponding alcohols of the alkyl side-chains of NP (nonanols) are metabolites produced by these three strains (Fujii et al. 2000b; Tanghe et al. 2000; Gabriel et al. 2005a,b). Experiments with *Sphingomonas* sp. TTNP3 and 4-[1-ethyl-1,3-dimethylpentyl]phenol led to the identification of 3,5-dimethyl-3-heptanol as the hydroxylated alkyl chain of this NP isomer at the α-C position (Corvini et al. 2004c; Fig. 3). The high volatility and the high degree of branching of nonanol congeners make them dead-end products that are volatilized, and only the ring moiety of tNP is assimilated (Corvini et al. 2004c; Fujii et al. 2000b; Gabriel et al. 2005a).

Some authors have assumed that degradation of *p*-NP starts with fission of the aromatic ring (Tanghe et al. 1999b; deVries et al. 2001; Fujii et al. 2001). The initial detachment of the alkyl chain and subsequent cleavage of all ring carbon atoms (e.g., as C_2 units) with possible decarboxylation was proposed as an alternative to an attack on the aromatic ring. This would imply the oxidation of the tetravalent α-C of the nonyl chain (Corvini et al. 2004c).

Beyond a report on metabolites that were possibly hydroxylated at the ring in *S. amiense* (de Vries et al. 2001), the decisive step in the elucidation of NP metabolism was achieved with *Sphingomonas* sp. TTNP3 and 4-[1-ethyl-1,3-dimethylpentyl]phenol. Metabolites were detected in intracellular extracts of *Sphingomonas* sp. TTNP3 and were identified as 2-[1-ethyl-1,3-dimethylpentyl]-1,4-benzenediol. They result from a (NIH)-shift mechanism where hydroxylation of atom C-4 of the aromatic ring is followed by the migration of the alkyl chain to an adjacent C atom of the ring (Corvini et al. 2004a). In a study with 4-[1,1,5-trimethylhexyl]-phenol, it was confirmed in the same bacteria that NP undergoes hydroxylation at the *ipso* position (atom C-4) (Corvini et al. 2005). A NIH-shift product, i.e., 2-[1,1,5-trimethylhexyl]-1,4-benzenediol and the 4-[(1,1,5-trimethylhexyl) oxy]phenol were identified as catabolites of this NP isomer.

The degradation of 4-(1-methyl-octyl)-phenol), a NP isomer with a tertiary α-C, which was cometabolized with isomers containing a quaternary α-C was studied in *S. xenophaga* (Gabriel et al. 2005a). The degradation of this isomer led to the formation of 4-hydroxy-4-(1-methyl-octyl)-cyclohexa-2,5-dienone, 4-hydroxy-4-(1-methyl-octyl)-cyclohex-2-enone, and 2-(1-methyl-octyl)-benzene-1, 4-diol (Gabriel et al. 2005b). These authors hypothesized that the degradation pathway occurred via the formation of an alkyloxyphenol as a consequence of a (1,2-C,O) shift after an *ipso*-hydroxylation of NP and would lead to *p*-benzoquinone.

A further study carried out with *Sphingomonas* sp. TTNP3 provided evidence that hydroquinone is the central metabolite in the degradation pathway of NP isomers (Corvini et al. 2006a; Fig. 4). The mechanism is a type II *ipso* substitution requiring a free hydroxy group at the *para* position as corroborated by previous studies where the methylated form of 4-[1-ethyl-1,3-dimethylpentyl]phenol was not degraded (Corvini et al. 2004b). Consequently, hydroxylation at position C-4 of the alkyl chain leaves the alkylated quinol as a carbocation or radical species, and hydroquinone is formed (Corvini et al. 2006a). The alkylic intermediate mainly reacts with water to form nonanol. To a much smaller extent, it reacts with the de novo formed hydroquinone and leads to 2-alkyl benzenediol or to alkyloxyphenols according to the steric constraints of the alkyl chain. Hydroquinone is further degraded into organic acids, such as succinate and 3,4-dihydroxy butanedioic acid, while benzenediol and alkyloxy derivatives are dead-end products.

Degradation pathways of other AP in bacteria

Most of the studies deal with the degradation of cresols (as parent compounds or as intermediaries in the degradation of toluene) and xylenols. Depending on the position of the

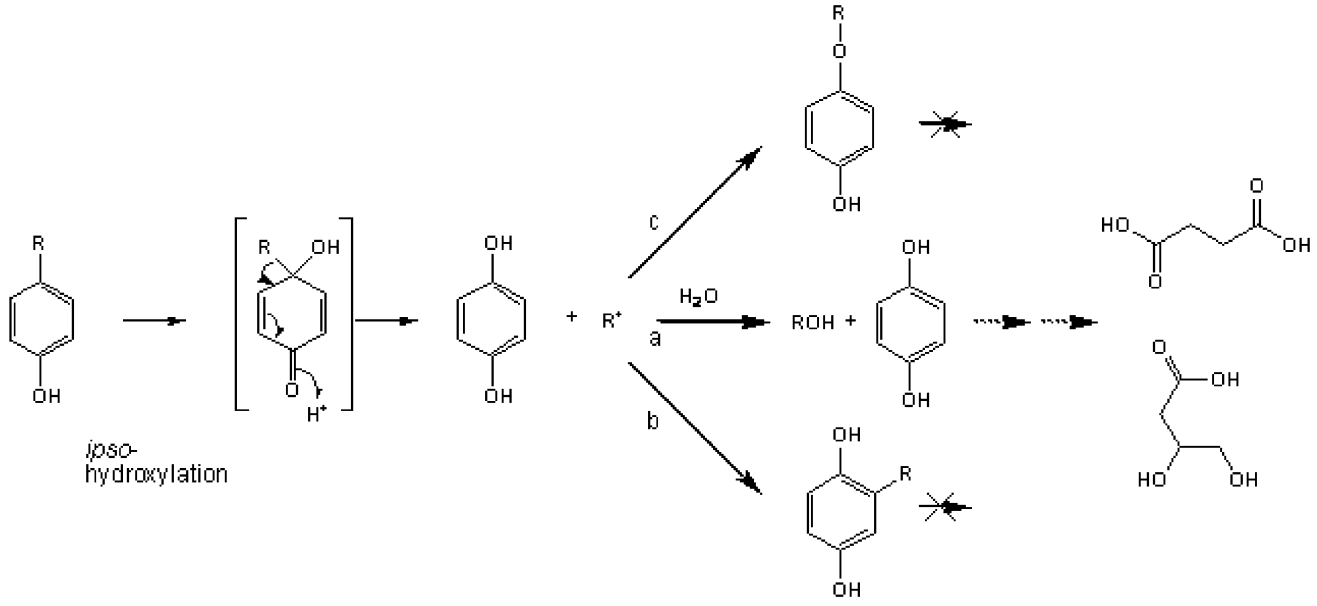

Fig. 4 Degradation pathways of NP isomers by *Sphingomonas* sp. strain TTNP3 as the dead-end product of 4-[1-ethyl-1,3-dimethylpentyl]phenol (adapted from Corvini et al. 2006a,b)

alkyl group(s) on the phenol ring (*o*-, *m*- and *p*-substitution), the metabolism of cresols and xylenols occurs via two main routes, i.e., oxidation of the ring or the alkyl substituent(s).

Hydroxylation of the ring as an initial step in the degradation of AP was reported to proceed only by *ortho*- and *meta*-hydroxylation, and in contrast to NP degradation, no *ipso*-hydroxylation mechanism was described. Phenol and methylated phenol hydroxylases and their genetic organization were extensively reviewed (Watanabe et al. 2002; Leahy et al. 2003; Powlowski and Shingler 1994). The degradation of *o*-cresol in *B. stearothermophilus* PH24, *Burkholderia* sp. JS150, and *P. cepacia* G4 leads to the formation of 3-methylcatechol, which is further degraded via *meta*-cleavage and production of 2-hydroxy-6-oxo-hepta-2,4-dienoic acid (Buswell 1975; Shields et al. 1989; Shields et al. 1995; Kahng et al. 2001). In a similar way, the degradation of 2-propylphenol by a mutant of *Pseudomonasazelaica* involves a monooxygenase, which catalyses the production of 3-propylcatechol (Kohler et al. 1993). 3-Propylcatechol is also degraded via a *meta*-fission before hydrolysis to butyric acid and 2-hydroxypenta-2,4-dienoic acid. Moreover, this monooxygenase has a broad substrate specificity and is able to oxidize various 2-alkylphenols, i.e., methyl-, ethyl-, propyl-, *i*-propyl-, butyl-, *sec*-butyl-, and *tert*-butylphenol (Jaspers et al. 2000; Meyer et al. 2002, 2003). The degradation of *m*-cresol in *Ralstonia pickettii* PKO1, *Burkholderia* sp. JS150, and *B. stearothermophilus* PH24 leads to the production of 4-methylcatechol, which is further degraded via *meta*-cleavage to yield 2-hydroxy-5-methyl-muconic semialdehyde (Buswell 1975; Kahng et al. 2001; Fishman et al. 2004). In *A. eutrophus*, *B. stearothermophilus* PH24, *Burkholderia* sp. JS150, and *R. pickettii* PKO1, *p*-cresol metabolism also leads to 4-methyl catechol, which undergoes subsequent *meta*-cleavage (Buswell 1975; Hughes and Bayly 1983; Hughes et al. 1984; Kahng et al. 2001; Fishman et al. 2004). In *P. veronii*, the initial degradation of *p*-hexyl and *p*-amylphenol also involves a phenol hydroxylase and a 2,3-catechol dioxygenase (Ajithkumar et al. 2003). A similar mechanism was described for *Pseudomonas* sp. KL28 possessing a gene cluster encoding for these enzymes, which enable it to degrade substrates with linear alkyl chains having three carbon units (Jeong et al. 2003). It was demonstrated that this bacterium did not transform octyl- and nonylphenol. Most of the studied strains possess the ability to convert many cresols via the formation of catechol derivatives and in some cases, this also applies to dialkylphenolic compounds like in *P. stutzeri* OX1, which degrades 2,3-dimethylphenol via the production of 3,4-dimethylcatechol (Bertoni et al. 1996). Similarly, *Pseudomonas pickettii* PK01 and *Pseudomonas* sp. strain CF600 degrade 3,4-dimethylphenol via *meta*-cleavage of the alkylated catechols (Kukor and Olsen 1991; Olsen et al. 1994; Pavel et al. 1994; Powlowski and Shingler 1994). In the case of 2,6-xylenol, *Mycobacterium* sp. strain DM1 was shown to hydroxylate the substrate at the *para* position (Ewers et al. 1989). The 2,6-dimethyl-hydroxyquinone was further oxidized into 2,6-dimethyl-3-hydroxyquinone, the latter being the substrate for intradiol cleavage.

Alternatively, degradation can be initialized by sequential oxidations of the alkyl group(s). The degradation of *p*-cresol in *Pseudomonas* sp. NCIB 9866 occurs through the hydroxylation of the methyl group by *p*-cresol methyl

hydroxylase (PCMH) by means of an oxygen atom derived from water, which is incorporated in the quinone methide intermediate (Hopper 1978). In a second reaction catalyzed by the same enzyme, *p*-hydroxybenzyl alcohol is converted into aldehyde, which is further oxidized to benzoic acid and then to protocatechuic acid. Similarly, *Pseudomonas mendocina* KR1 and *Bacillus pumilis* degrade *p*-cresol to protocatechuate, which is metabolized through an *ortho*-cleavage pathway. Protocatechuate may also be degraded via *meta*-cleavage in other *p*-cresol degrading strains (Whited and Gibson 1991; Günther et al. 1995; Heinaru et al. 2000). *Sphingomonas aromaticivorans* F199 was reported to also possess homologous genes to those of *P. mendocina* KR1 encoding for PCMH and *p*-hydroxybenzaldehyde dehydrogenase (Romine et al. 1999). In the case of dialkylphenols, one of the alkyl groups is oxidized first. In the degradation of 2,4-xylenol by *Pseudomonas* sp. NCIB 9866, the methyl group situated at C-4 of the ring is oxidized first (Chapman and Hopper 1968). Then, the second methyl group is oxidized, leading to 4-hydroxyisophthalic acid, which is decarboxylated to protocatechuate and degraded by *ortho*-fission of the benzene nucleus. In *Pseudomonas* sp. NCIB 9867 and NCIB 9869, the degradation of *m*-cresol, 2,5-xylenol, and 3,5-xylenol starts with the oxidation of the methyl group situated at the *meta* position (Hopper and Chapman 1970). Alkyl-substituted 3-hydroxybenzoic acids and alkyl-substituted gentisic acids are formed from 2,5-xylenol, 3,5-xylenol, 3-ethyl-5-methylphenol, and 2,3,5-trimethylphenol in *Pseudomonas* sp. NCIB 9867, and the gentisic acid derivatives undergo breakdown via the gentisate degradation pathway. PCMH of *P. putida* strain NCIB 9869 was characterized well, and it was shown that it can catalyze the dehydrogenation of the α-C of 4-ethylphenol and rehydrate the quinone methide into the corresponding alcohol, which is further oxidized to form the 4-hydroxyacetophenone (McIntire et al. 1984). In Pseudomonads, the oxygenation of hydroxyacetophenone by a Baeyer–Villiger type reaction and the consecutive cleavage of 4-phenyl acetate leads to the production of acetate and hydroquinone (Darby et al. 1987; Reeve et al. 1989; Jones et al. 1994; Tanner and Hopper 2000; Kamerbeek et al. 2001). Nevertheless, this pathway is not effective for AP with quaternary α-carbon atoms as found in NP isomers. In *P. putida* JD1, 4-ethylphenol methylene hydroxylase catalyzes AP biotransformations in a similar way (Reeve et al. 1989, 1990; Hopper and Cottrell 2003). Unlike PCMH, 4-ethylphenol methylene hydroxylase can oxidize 4-*n*-propylphenol and 4-*n*-butylphenol to the corresponding alcohols. Other authors reported the formation of a 4-amino derivative in *Pseudomonas* sp. cultivated with NP, although little information concerning metabolite identification was provided (Yuan et al. 2004).

The two main pathways are not exclusive in some microorganisms. For instance *P. putida* NCIB 9869 degrades *m*-cresol preferentially via the formation of a methylated catechol and a extradiol cleavage, while *p*-cresol and 3,5-xylenol undergo oxidation of the methyl group before the production of gentisate (Hopper and Taylor 1975). Furthermore, enzymes such as 3,5-xylenol methylhydroxylase and PCMH of *P. putida* NCIB 9869 possess broad substrate specificity oxidizing both 3,5-xylenol and on *p*-cresol (Keat and Hopper 1978a). This bacterium also possesses distinct alcohol dehydrogenases, which are active on both *m*- and *p*-hydroxybenzyl alcohols (Keat and Hopper 1978b). *Pseudomonas* sp. NCIB 9867 (*Pseudomonas alcaligenes*) possesses a set of isoenzymes for activities of the gentisate pathway, i.e., maleylpyruvate hydrolase, gentisate 1,2-dioxygenase, and 6-hydroxylation of 3-hydroxybenzoate. Other enzymes such as xylenol methyl hydroxylase, benzylalcohol dehydrogenase, and benzyldehyde dehydrogenase have broad substrate specificity (Poh and Bayly 1980).

Under anaerobic conditions, nitrate-reducing bacteria usually degrade *p*-cresol via the sequential oxidation to 4-hydroxybenzyl alcohol, 4-hydroxybenzaldehyde, and 4-hydroxybenzoic acid (Bossert and Young 1986; Kuhn et al. 1988; Bossert et al. 1989). The PCMH of *Achromobacter* sp. is similar to that of the pseudomonads, which were also shown to initiate the oxidation of *p*-cresol to 4-hydroxybenzoic acid under denitrifying conditions (Tschech and Fuchs 1987; Hopper et al. 1991). *T. aromatica* is also able to degrade *p*-cresol under denitrifying conditions (Song et al. 2000). The use of a different degradation strategy was described in a sulfate-reducing bacterium. *D. cetonicum* degrades *p*-cresol and *m*-cresol in the presence of sulfate as the electron acceptor via an activation of the methyl group by addition of fumarate. 4-Hydroxybenzylsuccinate is further degraded, probably via β-oxidation to 4-hydroxybenzoyl-CoA, which is reductively dehydroxylated to benzoyl-CoA (Müller et al. 2001).

NP degradation pathways in filamentous fungi and yeasts

Fungi degrade AP exclusively under aerobic conditions. However, less information is available about the pathways involved in NP degradation than for bacteria. Also, the question whether fungal attack on NP finally results in mineralization of the compound to CO_2 or represents a cometabolic biotransformation leading to the accumulation of organic metabolites has not yet been adequately answered. Such processes may even take different forms in different fungal groups.

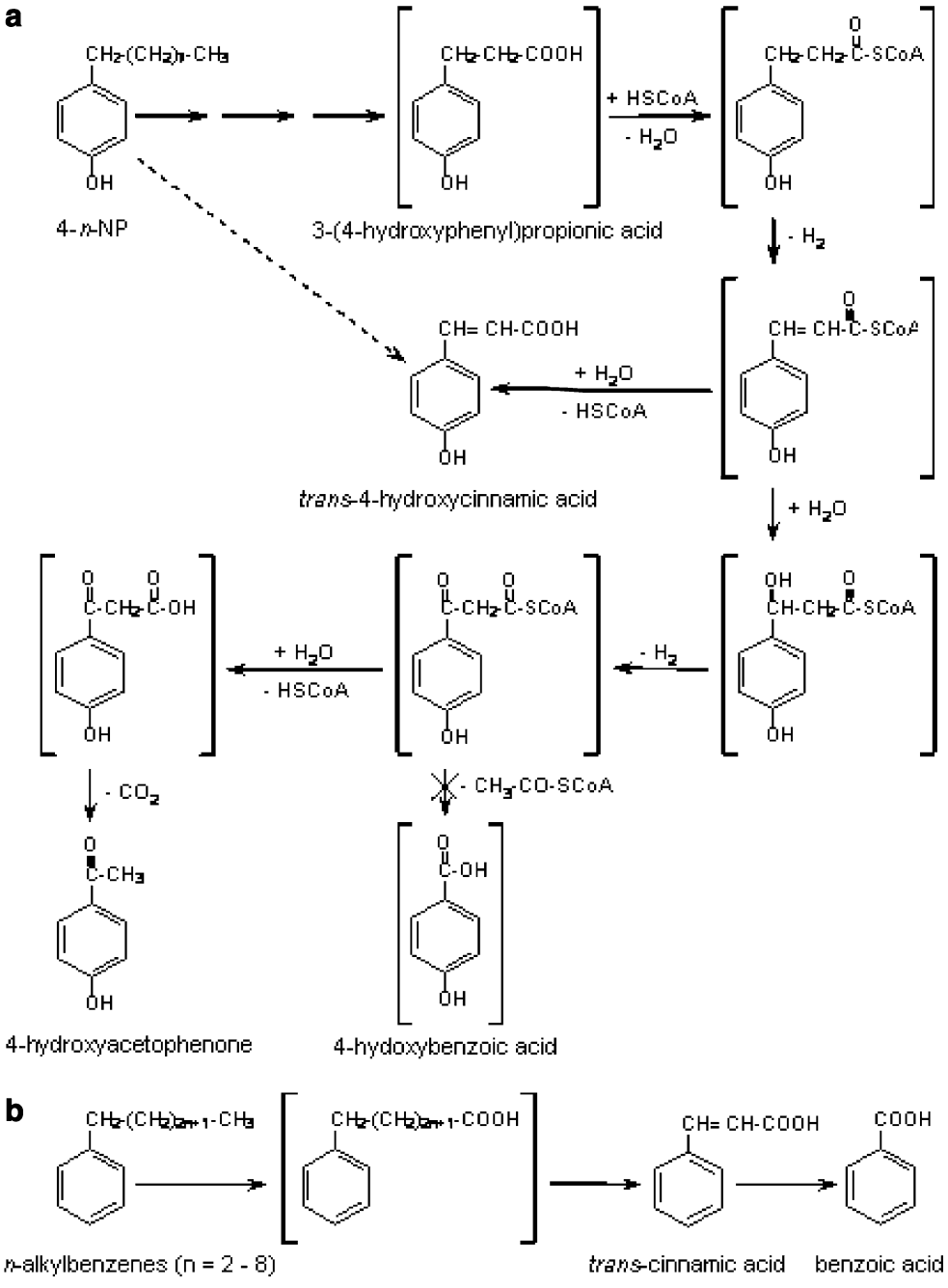

Fig. 5 Proposed metabolic routes for the degradation of **a** 4-*n*-NP in the yeast *C. aquaetextoris* (adapted from Vallini et al. 2001) and **b***n*-alkylbenzenes with odd-numbered carbon chains ranging from C_7 to C_{19} in *Candida* strains (adapted from Jigami et al. 1974). Compounds in *brackets* were not identified in the aforementioned studies and represent hypothetical intermediates

An earlier study described phenyl-propionic acid, phenyl-propanoic (cinnamic) acid, and benzoic acid as metabolites of 1-phenylnonane degradation in the actinomycete *Nocardia salmonicolor*, which indicates ω- and subsequent β-oxidation of the nonyl chain (Sariaslani et al. 1974). This would suggest that NP degradation might also proceed via breakdown of the alkyl moiety. In the yeast *C. aquaetextoris*, *trans*-4-hydroxycinnamic acid and 4-hydroxyacetophenone (4-acetylphenol) (Fig. 5a) accumulated during growth on 4-*n*-NP (Vallini et al. 2001), indicating oxidative attack at the alkyl chain. This partly parallels 4-*n*-NP metabolism in other eukaryotes such as fish where ω- and subsequent β-oxidation was implicated (Thibaut et al. 1998). Similarly, the metabolism of 4-*n*-NP in rats led to the detection of the glucuronide and sulfate conjugates of 4-hydroxycinnamic acid, 3-(4-hydroxyphenyl)propionic acid, and 4-hydroxybenzoic acid (Zalko et al. 2003). The latter also represents the final product of ω- and β-oxidation of the linear nonyl chain of 4-*n*-NP in fish (Thibaut et al. 1999, 2002b). In *C. aquaetextoris*, *trans*-4-hydroxycinnamic acid and 4-hydroxyacetophenone were also produced from the theoretical β-oxidation intermediate 3-(4-hydroxyphenyl)

propionic acid (Fig. 5a) when employed as a substrate, but at considerably lower rates than from 4-*n*-NP. In contrast to NP metabolism in fish and rat (Thibaut et al. 1999, 2002b; Zalko et al. 2003), neither 3-(4-hydroxyphenyl)propionic acid nor 4-hydroxybenzoic acid were detected in *C. aquaetextoris* (Fig. 5a) (Vallini et al. 2001). 4-Hydroxyacetophenone and 4-hydroxybenzoic acid did not serve as growth substrates for *C. aquaetextoris*, whereas 4-*n*-NP and, to a considerably lesser extent, also 3-(4-hydroxyphenyl)propionic acid supported growth. Growth on *trans*-4-hydroxycinnamic acid could not be unambiguously demonstrated. The complete removal of 4-*n*-NP in *C. aquaetextoris* cultures and the accompanying growth of the organism suggest mineralization of the compound to a certain extent, although this was not shown by Vallini et al. (2001). The metabolism of *n*-alkylbenzenes in yeasts shows similarities to 4-*n*-NP degradation by *C. aquaetextoris* (Fig. 5b). Two *Candida* strains accumulated *trans*-cinnamic acid as the only detectable metabolite during growth on *n*-alkylbenzenes with odd-numbered carbon chains ranging from C_7 to C_{19}. Under equal conditions, two other *Candida* strains accumulated benzoate, but not *trans*-cinnamic acid. Neither benzoate nor *trans*-cinnamic acid served as a growth substrate for any of these strains. The assimilation of *n*-alkylbenzenes was proposed to proceed through β-oxidation of the respective alkyl chain (Jigami et al. 1974).

Degradation of tNP by the aquatic hyphomycete *C. aquatica* and the unidentified aquatic isolate UHH 1-6-18-4 led in both cases to the detection of two main groups of oxidation products that could be partly characterized by gas chromatography–mass spectrometry (Junghanns et al. 2005, Moeder et al. 2006b). A group of several hydroxynonyl phenols, obviously resulting from the simultaneous nonyl chain monohydroxylation of different tNP isomers, indicated subterminal nonyl chain oxidation. Subterminal nonyl chain oxidation was also described for both NP isomers with branched nonyl chains and 4-*n*-NP in other eukaryotic model systems such as fish (Meldahl et al. 1996; Thibaut et al. 1998, 2002a,b), plants (Schmidt et al. 2003, 2004), and human cytochrome P450 (Inui et al. 2001). The involvement of cytochrome P450 systems in the alkyl chain oxidation of NP was also demonstrated in fish (Thibaut et al. 2002a) and was proposed for plants (Schmidt et al. 2004). A second group of degradation products detected in *C. aquatica* and UHH 1-6-18-4 could be attributed to different phenolic compounds with alkyl chains shorter than C_9 and would indicate subsequent shortening of the hydroxylated nonyl chains of at least some of the individual isomers contained in the tNP mixture (Junghanns et al. 2005; Moeder et al. 2006b). The assignment of exact structures was complicated by the uncertainty of oxygen-containing functional groups and the large number of possible positions in the respective alkyl chain. To date, alkyl chain shortening of chain-branched NP isomers was not described and neither in other eukaryotes nor in bacteria (Tanghe et al. 1999a,b; Corvini et al. 2004c). In the aquatic fungi, concentrations of metabolites of both groups increased during the cultivation period, indicating a cometabolic biotransformation rather than mineralization. However, a metabolite matching the mass spectral characteristics of 4-hydroxybenzoic acid transiently appeared in cultures of the fungal isolate UHH 1-6-18-4 with the concentration decreasing during further cultivation (Junghanns et al. 2005). This indicates breakdown of the alkyl chain of at least one isomer of the tNP mixture to 4-hydroxybenzoic acid, which than may undergo further degradation. 4-Hydroxybenzoic acid is degraded by different filamentous fungi and yeasts (Anderson and Dagley 1980; Middelhoven 1993; Wright 1993; Dittmann et al. 2002; Kamada et al. 2002). The enzymes responsible for NP alkyl chain oxidation in fungi remain to be established. As could be deduced from *n*-alkane and fatty acid metabolism involving β-oxidation in filamentous fungi and yeasts, hydroxylases belonging to the cytochrome P450 family would be potential candidates for fungal enzymes catalyzing NP nonyl chain hydroxylation, although experimental evidence for this is still missing (Nakayama and Shoun 1994; Nakayama et al. 1996; Scheller et al. 1998; Kitazume et al. 2000; Yadav and Loper 2000; Craft et al. 2003; van Beilen et al. 2003; Ayala and Torres 2004; Matsuzaki and Wariishi 2004; van Beilen and Funhoff 2005).

It is interesting to note that hydroxylations of the aromatic ring of NP were not detected in filamentous aquatic fungi (Junghanns et al. 2005) or in the yeast *C. aquaetextoris* (Vallini et al. 2001). This is in contrast to the oxidative attack of NP in bacteria (see the previous section) and in eukaryotes other than fungi (Coldham et al. 1998; Inui et al. 2001; Doerge et al. 2002; Lalah et al. 2003a,b; Schmidt et al. 2003; Zalko et al. 2003), and also to the fungal degradation of other AP (see next section). In the aquatic fungi and *C. aquaetextoris*, the detection of aromatic hydroxylation products of NP may have been hampered by their possible transient appearance and rapid further metabolization as implicated in bacteria (Tanghe et al. 1999a,b; Corvini et al. 2004b, 2006a). Hydroxylation of the aromatic ring of NP can also not be ruled out in other fungi. During growth of different filamentous fungi on *n*-alkylbenzenes, both *n*-alkyl chain and aromatic ring metabolization were reported (Fedorak and Westlake 1986).

The degradation of ^{14}C-labeled 4-*n*-NP was followed in liquid cultures of the white-rot fungus *T. versicolor* (Dubroca et al. 2005). After incubation for 12 days, about 29% of the initially applied radioactivity was detected in the culture supernatants, approximately 23% was biomass-associated, and 6% was recovered as CO_2. This points to a certain degree of mineralization of 4-*n*-NP. However, it is

not clear from this study whether 4-*n*-NP was uniformly or selectively ^{14}C-labeled, and no organic intermediates were described. Wood- and soil litter-decomposing white-rot basidiomycetes possess an outstanding potential not only to convert a broad variety of xenobiotic compounds into organic products but also to mineralize them to CO_2. This is attributed to a complex and relatively unspecific cometabolic degradation mechanism also active during lignin degradation (Bumpus et al. 1985; Field et al. 1993; Hammel 1995; Reddy 1995; Sack et al. 1997; Mester and Tien 2000; Steffen et al. 2002). Key components of the ligninolytic system are extracellular radical-generating enzymes such as laccases (EC 1.10.3.2), manganese peroxidases (MnP, EC 1.11.1.13), and lignin peroxidases (LiP, EC 1.11.1.14). Lignin-modifying enzymes are produced by white-rot fungi in individual combinations and amounts and can also oxidatively attack many xenobiotics (Hatakka 1994; Thurston 1994; Vares et al. 1994; Schlosser and Höfer 2002; Mester and Tien 2000; Leonowicz et al. 2001; Hofrichter 2002; Martinez 2002; Mayer and Staples 2002). Several authors have discussed the possibility of the involvement of lignin-modifying enzymes in NP degradation (Kollmann et al. 2003; Dubroca et al. 2005; Junghanns et al. 2005; Soares et al. 2005a, 2006).

A number of studies have addressed NP degradation by isolated lignin-modifying enzymes such as laccase (Tanaka et al. 2001; Tsutsumi et al. 2001; Saito et al. 2003, 2004a,b; Dubroca et al. 2005; Junghanns et al. 2005), MnP (Tsutsumi et al. 2001), and LiP (Kimura et al. 2004). Laccase-catalyzed degradation of tNP and 4-*n*-NP consistently led to the detection of several oligomeric products in the di- to pentamer range (Dubroca et al. 2005; Junghanns et al. 2005). Coupling products resulting from NP oxidation by laccase and MnP were also postulated in another study where enzymatic conversions were accompanied by a removal of the estrogenic activity of NP (Tsutsumi et al. 2001). In contrast to oxidative attack at the alkyl chain, lignin-modifying enzymes act on phenolic OH groups and catalyze the formation of phenoxyl radicals, which subsequently undergo different spontaneous reactions including oxidative coupling (Thurston 1994; Mester and Tien 2000; Leonowicz et al. 2001; Hofrichter 2002; Mayer and Staples 2002; Dec et al. 2003). Besides attack on NP by intracellular enzymes, lignin-modifying enzymes could provide an extracellular route for acting on NP and its excreted oxidation intermediates, which may lead to the removal and detoxification of the compound via oxidative coupling reactions (Tsutsumi et al. 2001; Dubroca et al. 2005; Junghanns et al. 2005). Such a scenario would be especially relevant for laccases because these enzymes are not restricted to ligninolytic basidiomycetes but also occur in environmentally ubiquitous ascomycetes, mitosporic fungi, and yeasts (Thurston 1994; Mayer and Staples 2002; Saito et al. 2003; Liers et al. 2006; Junghanns et al. 2005). In view of the reported formation of hazardous by-products, such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans upon enzymatic oxidative coupling of chlorophenols (Oberg et al. 1990; Morimoto and Tatsumi 1997), oxidative coupling products require a careful assessment with respect to their structures, biological activities, and stabilities.

Degradation pathways of other AP in fungi and yeasts

Degradation of short-chain AP such as *p*-cresol in fungal organisms proceeds via protocatechuic (3,4-dihydroxybenzoic) acid as a central intermediate, either by oxidation of the methyl group to carboxyl and subsequent ring hydroxylation or by ring hydroxylation to 4-methylcatechol, followed by its oxidation to protocatechuate (Powlowski and Dagley 1985; Jones et al. 1993). NADPH-dependent hydroxylases were implicated in the initial reactions (Jones et al. 1993; Wright 1993). Protocatechuic acid undergoes ring fission through protocatechuate-3,4-dioxigenase (EC 1.13.11.3) to β-carboxy-*cis*,*cis*-muconate. Its subsequent cyclization to β-carboxymuconolactone (instead of γ-carboxymuconolactone as found in bacteria) is specific for eukaryotic β-ketoadipate pathways (Harwood and Parales 1996; Wright 1993). Alternative fungal routes involve the conversion of protocatechuic acid into the intradiol ring cleavage substrates 1,2,4-trihydroxybenzene and catechol (Harwood and Parales 1996; Wright 1993), and the direct ring fission of 4-methylcatechol without protocatechuate formation (Powlowski and Dagley 1985). Cometabolic biotransformation of *o*-cresol and 2,6-dimethylphenol by *P. frequentans* led to the formation of several methyl-substituted hydroquinones, hydroxyhydroquinones, *p*-benzoquinones, and hydroxy-*p*-benzoquinones, and also let to the oxidation of methyl to carboxyl groups (Hofrichter et al. 1995). Growth of *Aspergillus fumigatus* on 4-ethylphenol involved its oxidation to 4-hydroxyacetophenone, a subsequent NADPH-dependent Baeyer–Villiger type of oxygenation to 4-hydroxyphenyl acetate, its further hydrolysis to hydroquinone, and NADPH-dependent hydroxylation of the latter to the ring cleavage substrate 1,2,4-trihydroxybenzene (Jones et al. 1994).

In addition to the elucidation of whole pathways, studies have also addressed the substrate specificity of particular fungal enzymes for AP and the respective products formed. Vanillyl alcohol oxidase of *Penicillium simplicissimum* converts 4-ethyl-, 4-*n*-propyl-, 4-isopropyl-, 4-*n*-butyl-, 4-*sec*-butyl-, 4-*n*-pentyl-, and 4-*n*-heptylphenol to the respective mixture of the corresponding benzylic alcohol and alkenylic phenol (Fraaije et al. 1998; van den Heuvel et al. 1998, 2000). Vanillyl alcohol oxidase from *Byssochlamys fulva* V107

hydroxylates *p*-cresol, 4-ethyl-, 4-*n*-propyl-, 4-*iso*-propyl-, 4-*n*-butyl, 4-*sec*-butyl-, and 4-*n*-hexylphenol at the α-carbon of the alkyl group (Furukawa et al. 1999). Phenol hydroxylase from yeast oxidizes *o*-, *m*-, and *p*-cresol to the corresponding methylated catechols (Neujahr and Kjellen 1978). Mushroom tyrosinase (E.C. 1.14.18.1) was reported to catalyze the formation of the corresponding alkylated *o*-benzoquinone from *p*-cresol, ethyl-, 4-*n*-propyl-, 4-*n*-butyl-, and 4-*tert*-butylphenol (Yamada et al. 2005). Laccase was shown to oxidize *o*-cresol, *p*-cresol, 2-ethylphenol, pentadecylphenol, and also phenols substituted with variously unsaturated C_{15} and C_{17} chains (Bollag et al. 1988; Xu 1996; Garzillo et al. 1998; Kobayashi and Higashimura 2003).

Factors influencing degradation of NP

Position and length of alkyl chain

As described for cresols and other short-chain AP, the position of substitution of the phenol ring by the nonyl chain seems to be decisive with regard to the degradation of NP by sphingomonads. *S. amiense* displayed a marked preference for *p*-NP isomers (de Vries et al. 2001). In the case of *Sphingomonas* sp. TTNP3, a residual accumulation of the *o*-NP isomers was observed during the time-course of incubation (Tanghe et al. 1999b; Corvini et al. 2004c). These facts indicate a higher persistence of *o*-NP, although the low endocrine potential and the small proportion of *o*-NP in tNP reduce the significance of this finding. With respect to the *ipso*-hydroxylation mechanism occurring in sphingomonads, the presence of a hydroxyl group adjacent to the alkyl chain could inhibit the reaction for *o*-NP isomers by increasing the steric hindrance at C-2.

In bacteria, the length of the AP alkyl chain seems to be a decisive criterion for the degradation pathway and the microorganisms involved. In pseudomonads, many reports have demonstrated the degradation of short linear alkyl chain AP (Ajithkumar et al. 2003; Hopper and Cottrell 2003; Jeong et al. 2003). In these cases, the degradation pathways involve either catechol derivatives (Jeong et al. 2003) or a quinone methide intermediate (Hopper and Cottrell 2003), and no mechanisms similar to *ipso*-substitution were found. The degradation of various AP derivatives was determined during an incubation period of 2 weeks with *Sphingomonas* sp. TTNP3 (Corvini et al. 2004b). Both isopropyl and *tert*-butylphenol were not degraded, whereas the concentration of *tert*-octylphenol decreased as in the separate study by Tanghe et al. (2000). Although isopropyl and *tert*-butylphenol each possess a highly branched α-C atom, which would allow for the production of secondary and tertiary carbocationic intermediates, no degradation by *Sphingomonas* sp. TTNP3 occurred. These results are also in agreement with the fact that propyl- and hexylphenol are not degraded by *S. cloacae* (Fujii et al. 2000a). These observations indicate that degradation of AP by sphingomonads, known for their capacity to degrade NP, requires substrates having an alkyl chain with a minimal length.

Linking the position and length of alkyl chains to the biodegradability of AP by fungi is complicated by several factors, such as the necessity to differentiate between cometabolic biotransformations and AP utilization for growth, the various enzymes known to oxidize AP but showing different substrate preferences and catalytic mechanisms, and the intracellular enzymes acting on NP, which is yet unknown. A striking observation is that *o*-cresol did not support the growth of filamentous fungi (Hofrichter et al. 1995; Garcia-Pena et al. 2005) and enabled only delayed growth in yeast (Middelhoven and Spaaij 1997). This contrasts with the fungal growth observed on *para*- and *meta*-substituted short-chain AP (Hofrichter and Scheibner 1993; Jones et al. 1993, 1994; Middelhoven and Spaaij 1997; Middelhoven et al. 2000, 2004; Garcia-Pena et al. 2005). Phenol hydroxylase from yeast is able to act on all three cresol isomers (Neujahr and Kjellen 1978). Fungal vanillyl alcohol oxidases oxidize *p*-cresol and other *para*-substituted C_2–C_7 AP (Fraaije et al. 1998; Furukawa et al. 1999; van den Heuvel et al. 1998, 2000), and mushroom tyrosinase was reported to act on *p*-cresol and *para*-substituted C_2–C_4 AP (Yamada et al. 2005). Laccase was shown to oxidize *p*-cresol at higher rates than *o*-cresol and also converts *para*- and *ortho*-substituted AP with larger alkyl substituents including NP (Bollag et al. 1988; Garzillo et al. 1998; Kobayashi and Higashimura 2003; Xu 1996; Junghanns et al. 2005), whereas *m*-cresol oxidation was not reported. This suggests that in laccases, the position of an alkyl chain is a more decisive criterion for AP oxidation than its length.

Influence of the structure of NP alkyl chain on NP degradation

The influence of the alkyl chain structure of NP isomers was considered in studies with sphingomonads. It was shown that 4-*n*-NP was not metabolized as a single source of carbon by *Sphingomonas* sp. TTNP3 and *S. xenophaga* (Corvini et al. 2004b; Gabriel et al. 2005a). These a priori unexpected results can again be explained by the mechanism of type II *ipso*-substitution where a primary carbocationic intermediate from the linear alkyl chain is energetically unlikely. Although the simultaneous decrease of all tNP isomers and the production of many nonanols were indicative of an aspecific degradation of isomers of tNP in *S. cloacae* and *Sphingomonas* sp. TTNP3 (Tanghe et al. 1999b; Fujii et al. 2000b; Corvini et al. 2004c) higher

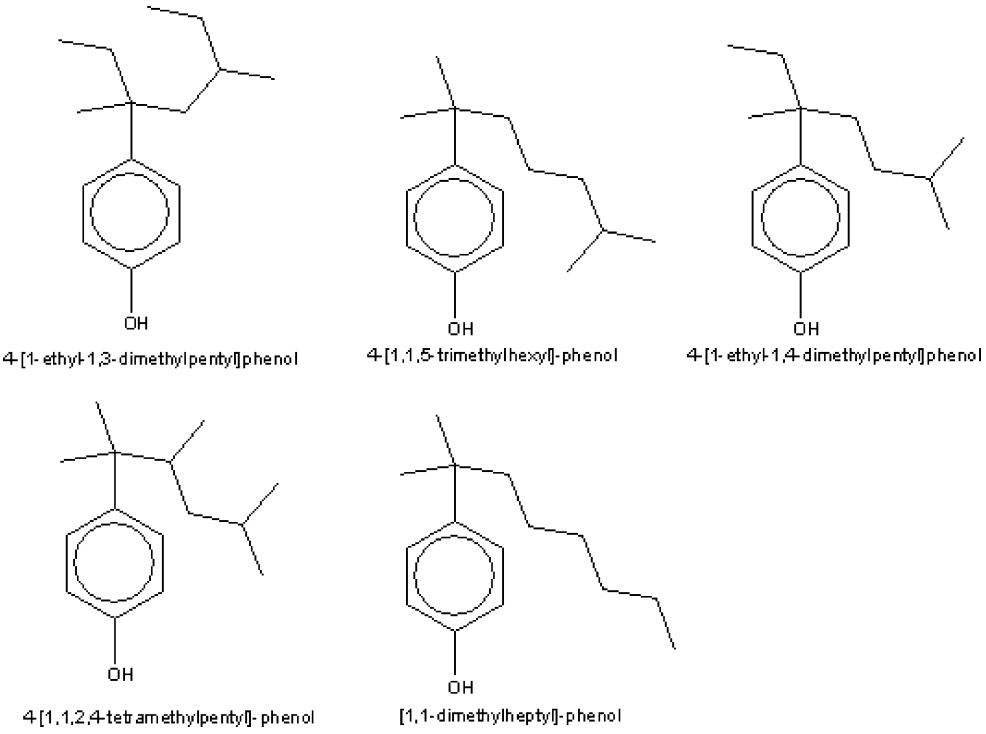

Fig. 6 Chemical structure of various isomers of NP (adapted from Gabriel et al. 2005a and Corvini et al. 2006a,b)

consumption rates were found for 4-[1-ethyl-1,3-dimethylpentyl]phenol than for tNP in *Sphingomonas* sp. TTNP3. Furthermore, slight differences in the biodegradation of the different isomers of NP were reported in *Sphingomonas* sp. TTNP3 and *S. xenophaga*. In the case of *Sphingomonas* sp. TTNP3, differences in the dead-end metabolite trace pattern were reported. Benzenediol derivatives were found for a lot of NP isomers in intracellular extracts of *Sphingomonas* sp. TTNP3 grown on tNP, whereas alkyloxyphenols were not detected for all of these isomers (Corvini et al. 2005). Resting cell assays over short incubation periods demonstrated that despite single isomers of NP, all being degraded via hydroquinone, a comparison of the degradation rates showed that 4-[1,1,5-trimethylhexyl]-phenol is degraded more slowly than 4-[1-ethyl-1,3-dimethylpentyl]phenol and 4-[1-ethyl-1,4-dimethylpentyl]phenol. 4-[1-ethyl-1,3-dimethylpentyl]phenol and 4-[1-ethyl-1,4-dimethylpentyl]phenol possess the same substitution pattern at the quaternary α-C atom (methyl, pentyl, and ethyl), whereas 4-[1,1,5-trimethylhexyl]-phenol possesses two methyl and one hexyl substituents (Fig. 6). These results are in agreement with those obtained after long incubation periods with *S. xenophaga*. This strain degrades 4-[1-ethyl-1,4-dimethylpentyl]phenol slightly more rapidly than 4-[1,1,2,4-tetramethylpentyl]-phenol, which has a dimethylated α-C atom with a highly branched pentyl group, and much faster than 4-[1,1-dimethylheptyl]-phenol, which also has a dimethylated α-C atom but a longer unbranched chain (Gabriel et al. 2005a). Thus, the substitution pattern of the α-C and the branching pattern of the largest alkylic substituent of the α-carbon may influence the degradation rate of the various isomers of NP and govern the pattern of the metabolites produced consecutively in this type II *ipso* substitution.

Concerning the isomerism of the alkyl chain, to date, no effects on the biodegradation rates of the various *p*-NP isomers were clearly shown in the environment. Studies with soil microbial communities suggest that the *p*-NP isomers of tNP were similarly degraded (Topp and Starratt 2000). These results are corroborated by a recent report describing the formation of nitro metabolites when tNP is added to sludge-amended soil (Telscher et al. 2005). Nevertheless, the lack of studies demonstrating, on the one hand, the preferential degradation in environmental samples and, on the other hand, the specific persistence of defined isomers may be related to the difficulties of performing high quality chromatographic analysis of such complex mixtures. Furthermore, considering the various pathways, for instance the alkyl-chain oxidation by fungi, the structure of the alkyl-chain could also play a decisive role in the degradation of NP. The fact that both diastereomers of the 4-[1-ethyl-1,3-dimethylpentyl]phenol isomer were degraded at equal rates indicates that the stereochemistry of the alkyl side-chain is not crucial for the metabolism of the diastereomers by *Sphingomonas* sp. TTNP3. Stereospecificity apparently does not play a decisive role for *ipso*-substitution degradation pathways, but it would be interesting to assess whether this also

applies to pathways requiring, for example, the hydroxylation of alkyl chains.

No study so far has addressed degradation of single isomers of tNP by fungal organisms. However, effects of nonyl chain branching on fungal degradation would be expected from the reported differences in tNP and 4-*n*-NP removal by whole fungal cultures and isolated enzymes. *Cunninghamella*, *Fusarium*, and *Mucor* species removed 4-*n*-NP more efficiently than tNP, whereas the opposite was observed for *P. chrysosporium* and *T. versicolor* (Dubroca et al. 2005). Pertinent literatures indicate alkyl chain oxidation of at least some isomers of tNP by intracellular fungal enzymes (Junghanns et al. 2005; Moeder et al. 2006b), but the exact structures of nonyl chain branched tNP isomers that are susceptible to alkyl chain activation through intracellular enzymes and further breakdown remain to be elucidated. Otherwise, β-oxidation is thought to be restricted to linear or at least not highly branched alkyl chains (Osburn and Benedict 1966; van Ginkel 1996; Tanghe et al. 1999a,b). More insights into such processes are expected from studies applying the single nonyl chain-branched isomers contained in tNP, which are now available (Ruß et al. 2005). Laccase from the aquatic isolate UHH 1-6-18-4 oxidized tNP faster than 4-*n*-NP. This is in contrast to laccase from the aquatic hyphomycete *C. aquatica*, which converted 4-*n*-NP more efficiently than tNP (Junghanns et al. 2005). Laccases act on the phenolic OH group and therefore, branching of the nonyl chains located in the *p*-position should be less important for laccases than for enzymes directly attacking the alkyl chain or adjacent positions of the aromatic ring. Little steric hindrance of bulky *p*-substituents was shown for the laccase-catalyzed oxidation of phenols (Xu 1996).

Other factors governing the degradation of NP

Because high NP concentrations can be toxic to microorganisms, the persistence of NP and other micropollutants may result from their trace concentrations in the environment (Kollmann et al. 2003). The growth and survival of cometabolic NP degraders independent from NP could be advantageous for NP removal when concentrations are well below the K_m or K_s of the degrading enzymatic systems or specialist microorganisms. Enzyme affinity for xenobiotics is often low. For NP oxidation catalyzed by a laccase from a soil-derived ascomycete, a K_m value of 5 mM (1.1 g l^{-1}) was reported (Saito et al. 2003). Such high K_m values would be rather unfavorable for an efficient removal of NP at the concentrations commonly found in water, which are found up to the microgram per liter range (Ying et al. 2002). Nevertheless, laccases from other organisms may differ in their kinetic features. Higher volumetric rates of 4-[1-ethyl-1,3-dimethylpentyl]-phenol degradation were reported in *Sphingomonas* sp. TTNP3 when the NP isomer was supplied above the hydrosolubility limit (25 μM) (Corvini et al. 2006a). For whole cells of this miroorganism, apparent K_m and V_{max} values were 230 μM and 0.050 μmol min^{-1} mg^{-1}, respectively. Nevertheless, immobilized cells of *S. cloacae* were able to degrade NP in trace amounts, i.e., below 40 μg l^{-1} (Fujii et al. 2003). This high performance was attributed to the sorption of NP to the hydrophobic immobilization material leading to local higher concentrations in the vicinity of the bacteria. Similarly, kinetic limitations of laccase activity caused by low NP concentrations in aqueous systems may be diminished in sewage sludge and also in sediments where mean concentrations of up to approximately 1.5 g kg^{-1} and 2.1 mg kg^{-1}, respectively, were reported (Pryor et al. 2002; Ying et al. 2003). The low biodegradability of NP may be explained by its strong sorption to, e.g., sediments and humic acids. This, in turn, should drastically decrease its bioavailability (Wheeler et al. 1997; Tanghe et al. 1998; Vinken et al. 2004). However, despite the high sorption coefficient of NP on humic acids, the degradation rates of NP in an axenic culture of *Sphingomonas* sp. TTNP3 were not affected (Vinken et al. 2004). Another study demonstrated that 4-*n*-NP was degraded within 40 days of incubation with organic material containing sediments, whereas the branched OP remained undegraded despite the higher sorption coefficient of 4-*n*-NP than that of 4-*tert*-OP (K_{OC} 38,900 and 18,200, respectively) (Ying et al. 2003). In other words, the structure of alkyl chain prevailed over the effects of sorption. In the presence of natural organic matter, enzymatic coupling reactions catalyzed by fungal lignin-modifying enzymes may also contribute to humification processes through the formation of stable bound residues (Sarkar et al. 1988; Park et al. 2000; Ahn et al. 2002; Dec et al. 2003). Hydrosolubility of NP is increased at alkaline pH values when NP is deprotonated (pKa estimated between 10 and 12) and becomes less sorbed to organic materials and thus potentially more bioavailable (Ivashechkin et al. 2004). Nevertheless, extreme pH values were demonstrated to exert a negative effect on NP degradation by *S. cloacae* and very probably on its growth (Fujii et al. 2003). NP was not degraded at pH 9, while pH 6 or 8 led to an almost complete removal of 200 ppb NP.

Among the critical parameters for the biodegradation of NP, oxygen appears to be particularly important. Anoxic periods led to a decrease in NP elimination in activated sludge and NP was more rapidly mineralized when oxygen diffusion into the sludge particles was facilitated in sludge–soil systems (Tanghe et al. 1998; Hesselsøe et al. 2001). Furthermore, the NP concentration profile increased with depth in anaerobic sediment layers (Espadaler et al. 1997). Few data concerning the biodegradation of NP in anaerobic

environments are available. It was demonstrated that AP are not degraded during methanogenic treatment of sludge (Razo-Flores et al. 1996). However, the degradation of NP in sediments and in sewage sludge under anaerobic conditions was recently reported (Chang et al. 2004b, 2005a). Sulfate-reducing bacteria would be the major microbial component for the anaerobic degradation of NP in both environments, but the methanogen and eubacterial populations would also be involved. Nevertheless, doubts about the use of 4-*n*-NP remain because in the NP1EO (monoethoxylated tNP) degradation tests under the same incubation conditions, NP was accumulated and no information was provided concerning its further degradation. The easy degradation of linear alkane such as linear alkylbenzene sulfonate under anaerobic conditions would provide a rational explanation of these isolated cases of anaerobic degradation of NP (Mogensen et al. 2003).

With regard to temperature, the data are quite heterogeneous. Decreasing temperature from 28 to 10 °C was shown to affect NP degradation by microflora in activated sludge (Tanghe et al. 1998). Nevertheless, inoculation of bioreactors with enriched bacterial consortia isolated from Swedish contaminated soil displayed satisfactory degradation rates for temperatures ranging from 15 down to 5.5 °C (Soares et al. 2005b). Further experiments were carried out with cold-adapted bacteria isolated from treatment plant in Sweden (Soares et al. 2003a). *P. veronii* was able to grow at temperatures near to 0 °C and it was able to degrade NP at 14 °C. On the whole, the results depend on the type of bacteria and the capability of extremophiles to carry out such reactions.

Biotechnical applications

Particularly interesting is the apparent constitutive expression of this NP-degrading activity in *Sphingomonas* sp. TTNP3 and *S. amiense* (de Vries et al. 2001; Corvini et al. 2006a). Resting cells of both strains are able to directly and rapidly degrade NP without having been precultivated with NP. Such a property may be advantageous for bioremediation strategies. Experiments aimed at improving the degradation of NP were carried out with *Sphingomonas* sp. TTNP3 and *S. cloacae* (Fujii et al. 2000b, 2003; Soares et al. 2003b). *S. cloacae* was used in a bioreactor as chitosan-encapsulated cells to treat NP-contaminated wastewater and was found to degrade NP in parts per million range as efficiently as the free suspended cells (Fujii et al. 2000b). The beads could be used several times and prevented the cells from being washed out of the reactor. The immobilization of *S. cloacae* on hydrophobic polypropylene carriers allowed for the removal of NP in the parts per billion range over a 1-month period in the stabilized system (Fujii et al. 2003).

Other studies showed that repeated "bioaugmentation" by periodically applying *inocula* of effluent to bioreactors improved its biodegradation (Tanghe et al. 1998). *Sphingomonas* sp. TTNP3 was used for the treatment of leachates of soils, which were either artificially or naturally contaminated with NP. As for *S. cloacae*, cells of *Sphingomonas* sp. TTNP3 were immobilized in the bioreactor system. The packed-bed reactor consisted of foamed glass beads, and when fed at a flow rate of 69 ml h^{-1}, it led to the removal of 4.9 mg NP day^{-1}. In a further application with microbial consortia, the same fixed-bed reactor was inoculated with an enrichment culture prepared from a NP-contaminated soil (Soares et al. 2005b). The maximal removal performance was approximately 85 mg day^{-1} when the hydraulic retention time was set at 1.38 h.

White-rot fungi and fungal oxidoreductase enzymes were widely proposed for the treatment of AP-contaminated wastewaters, sludges, and soils (Bollag et al. 1988; Gianfreda et al. 1999; Duran and Esposito 2000; Saito et al. 2003, 2004a,b; Gianfreda and Rao 2004; Dubroca et al. 2005; Soares et al. 2005a, 2006; Auriol et al. 2006), offering a so far not fully explored potential for biotechnological decontamination purposes. Examples of such applications are the removal of phenolic compounds from wastewaters through the use of white-rot fungi in airlift reactors (Ryan et al. 2005), the employment of mushroom tyrosinase to oxidize AP in artificial wastewater (Yamada et al. 2005), the removal of NP from organic solvents by LiP entrapped in reverse micelles (Kimura et al. 2004), and the treatment of NP-contaminated soils with laccase in rotating reactors (Tanaka et al. 2001, 2003). A remarkable feature of laccases with relevance for both natural ecosystems and biotechnological applications is the possibility for enhancing degradation rates and to extend their substrate range in the presence of small, diffusible molecules acting as redox mediators. Natural redox mediators were implicated in white-rot fungi (Eggert et al. 1996; Johannes and Majcherczyk 2000). Redox mediators also considerably enhance laccase-catalyzed NP degradation, as was shown for the artificial model redox mediators 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) and 1-hydroxybenzotriazole (Tsutsumi et al. 2001; Junghanns et al. 2005). Combined fungal–bacteriological processes were considered for the treatment of AP-contaminated waters (Perron and Welander 2004).

Though most biotechnical applications describe the possibility to significantly improve the removal of NP, the hydrophobicity and possible volatilization of NP often make interpretations difficult (Soares et al. 2003b; Vinken et al. 2004; Cirja et al. 2005). This is particularly true for processes operated in continuous mode. Even when the NP concentration is well below the theoretical hydrosolubility, the sampling can be inhomogeneous and precise concentrations are difficult to apply in such systems. Usually for

each measurement at a defined incubation time, the whole vessel needs to be sacrificed for each determination of remaining NP (Tanghe et al. 1999b; Gabriel et al. 2005a,b; Corvini et al. 2004c; Fujii et al. 2000b). Other solutions include increasing the NP solubility by adding tensioactive emulsifiers or saturating the feed of the reactors with NP before applying NP into the system (Soares et al. 2003b, 2005b). Ideally, a complete mass balance of NP should be carried out during the process. This is possible by using radiolabeled NP, provided that the reactor design is adapted to work conditions with radioactive NP (Cirja et al. 2005).

Concluding remarks and perspectives

Numerous studies concerning NP degradation were carried out at lab scale and under real conditions. Due to the extremely hydrophobic nature of NP, its possible volatilization, and the complexity of the technical mixture, scientists have to cope with analytical difficulties such as its extraction from complex matrices and subsequent chromatographic separation. A clear distinction between ultimate biodegradation, modifications of NP molecules, stripping, sorption, and binding in aged samples is needed to integrate the degradation mechanisms into environmental fate studies. This will require new method developments to improve the extraction of NP from environmental matrices. In addition, a better distinction between the various isomers of tNP could be achieved through both the development of high-resolution chromatographic methods such as the coupling of various columns in combination with the synthesis and use of single isomers (Guenther et al. 2005; Moeder et al. 2006a,b).

Though many microbial isolates were reported to degrade NP, further research is necessary to ensure that the decrease in concentrations do not result from experimental artifacts such as stripping and sorption processes. One also has to distinguish ultimate degradation from cometabolism or transformation of NP. Particularly in the case of fungi, further studies aimed at demonstrating the mineralization of NP are needed. For studies addressing the metabolism of NP in axenic cultures and in bacterial consortia, the use of radiotracer techniques has proven to be helpful (Corvini et al. 2004c, 2005; Telscher et al. 2005). In fungi, the degradation pathways obviously proceed via oxidation of the alkyl chain and a demonstration of NP mineralization would require the labeling of the alkyl chain moiety. Concerning the degradation of NP and other AP, it can be deduced from numerous studies that the branching, the length, and the position on the ring of the alkyl chain are decisive traits with regard to the pathways of degradation involved. This is particularly true for mechanisms involving hydroxylation at the ring. It is likely that because of the large size of the nonyl chain, the classical *ortho*- and *meta*-hydroxylation reported for short-chain AP were not described for NP to date. Furthermore, the quaternary α-carbon of NP is a structural feature that makes degradation pathways of short-chain AP, such as the attack via PCMH, not applicable to this compound. Though widely used as a model compound, 4-*n*-NP is not really suited for metabolic studies. Most of the degradation pathways reported proceed via the oxidation of the linear alkyl chain and are not compatible with the quaternary α-carbon structure of tNP. Among those bacteria able to degrade NP, sphingomonads are very interesting microorganisms for metabolic studies due to the unusual mechanisms involved in the degradation pathway. Studies with these bacteria support the evidence showing the unique character of NP conferred by the branched nonyl chain and the quaternary α-carbon in comparison to other AP.

Based on the available data so far on metabolic routes employed in tNP degradation, an efficient tNP removal could be expected upon the cooperation of bacteria and fungi because the former assimilate the aromatic ring and the latter attack the nonyl chain. This should be addressed in further investigations. Bacteria and fungi coexist in many ecosystems and their collaboration in the degradation of short-chain AP (Perron and Welander 2004) and polycyclic aromatic hydrocarbons (Kottermann et al. 1998) was demonstrated.

Acknowledgement We greatly acknowledge K. Smith (UFZ Centre for Environmental Research Leipzig-Halle) for help with the English language.

References

Ahel M, McEvoy J, Giger W (1993) Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. Environ Pollut 79:243–248

Ahn M-Y, Dec J, Kim J-E, Bollag J-M (2002) Treatment of 2,4-dichlorophenol polluted soil with free and immobilized laccase. J Environ Qual 31:1509–1515

Ajithkumar B, Ajithkumar VP, Iriye R (2003) Degradation of 4-amylphenol and 4-hexylphenol by a new activated sludge isolate of *Pseudomonas veronii* and proposal for a new subspecies status. Res Microbiol 154:17–23

Anderson JJ, Dagley S (1980) Catabolism of aromatic acids in *Trichosporon cutaneum*. J Bacteriol 141:534–543

Auriol M, Filali-Meknassi Y, Tyagi RD, Adams CD, Surampalli RY (2006) Endocrine disrupting compounds removal from wastewater, a new challenge. Process Biochem 41:525–539

Ayala M, Torres E (2004) Enzymatic activation of alkanes: constraints and prospective. Appl Catal A Gen 272:1–13

Bertoni G, Bolognese F, Galli E, Barbieri P (1996) Cloning of the genes for and characterization of the early stages of toluene and o-xylene catabolism in *Pseudomonas stutzeri* OX1. Appl Environ Microbiol 62:3704–3711

Bollag J-M, Shuttleworth KL, Anderson DH (1988) Laccase-mediated detoxification of phenolic compounds. Appl Environ Microbiol 54:3086–3091

Bossert ID, Young LY (1986) Anaerobic oxidation of p-cresol by a denitrifying bacterium. Appl Environ Microbiol 52:1117–1122

Bossert ID, Whited G, Gibson DT, Young LY (1989) Anaerobic oxidation of *p*-cresol mediated by a partially purified methylhydroxylase from a denitrifying bacterium. J Bacteriol 171:2956–2962

Bumpus J, Tien M, Wright D, Aust S (1985) Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434–1436

Buswell JA (1975) Metabolism of phenol and cresols by *Bacillus stearothermophilus*. J Bacteriol 124:1077–1083

Chang BV, Yang CM, Cheng CH, Yuan SY (2004a) Biodegradation of phthalate esters by two bacteria strains. Chemosphere 55:533–538

Chang BV, Yu CH, Yuan SY (2004b) Degradation of nonylphenol by anaerobic microorganisms from river sediment. Chemosphere 55:493–500

Chang BV, Chiang F, Yuan SY (2005a) Anaerobic degradation of nonylphenol in sludge. Chemosphere 59:1415–1420

Chang BV, Chiang F, Yuan SY (2005b) Biodegradation of nonylphenol in sewage sludge. Chemosphere 60:1652–1659

Chapman PJ, Hopper DJ (1968) The bacterial metabolism of 2,4-xylenol. Biochem J 110:491–498

Cirja M, Ivashechkin P, Schäffer A, Pinnekamp J, Ostrowski R, Corvini PFX (2005) Optimization of a lab-scale membrane bioreactor designed for fate studies of radio-labelled hydrophobic micropollutants during the wastewater treatment. E-proceedings of the emerging pollutants and emerging technologies—National Young Researchers Conference of the International Water Association, 27–28 October 2005, Aachen, Germany

Coldham NG, Sivapathasundaram S, Dave M, Ashfield LA, Pottinger TG, Goodall C, Sauer MJ (1998) Biotransformation, tissue distribution, and persistence of 4-nonylphenol residues in juvenile rainbow trout (*Oncorhynchus mykiss*). Drug Metab Dispos 26:347–354

Corti A, Frassinetti S, Vallini G, D'Antone S, Fichi C, Solaro R (1995) Biodegradation of nonionic surfactants. I. Biotransformation of 4-(1-nonyl)phenol by a *Candida maltosa* isolate. Environ Pollut 90:83–87

Corvini PFX, Meesters RJW, Schäffer A, Schröder HFr, Vinken R, Hollender J (2004a) Degradation of a nonylphenol single isomer by *Sphingomonas* sp. strain TTNP3 leads to a hydroxylation-induced migration product. Appl Environ Microbiol 70:6897–6900

Corvini PFX, Vinken R, Hommes G, Mundt M, Meesters R, Schröder HF, Hollender J, Schmidt B (2004b) Microbial degradation of a single branched isomer of nonylphenol by *Sphingomonas* TTNP3. Water Sci Technol 50:195–202

Corvini PFX, Vinken R, Hommes G, Schmidt B, Dohmann M (2004c) Degradation of the radioactive and non-labelled branched 3′,5′-dimethyl 3′-heptyl-phenol nonylphenol isomer by *Sphingomonas* TTNP3. Biodegradation 15:9–18

Corvini PFX, Elend M, Hollender J, Ji R, Preiss A, Vinken R, Schäffer A (2005) Metabolism of 4(2′,6′-dimethyl-2′-heptyl)-phenol by *Sphingomonas* sp. strain TTNP3. Environ Chem Lett 2:185–189

Corvini PFX, Hollender J, Ji R, Schumacher S, Prell J, Hommes G, Priefer U, Vinken R, Schäffer A (2006a) The degradation of α-quaternary nonylphenol isomers by *Sphingomonas* sp. strain TTNP3 involves a type II ipso-substitution mechanism. Appl Microbiol Biotechnol 70:114–122

Corvini PFX, Meesters R, Mundt M, Schäffer A, Schmidt B, Schröder HFr, Verstraete W, Vinken R, Hollender J (2006b) Contribution to the detection and identification of oxidation metabolites of nonylphenol in *Sphingomonas* sp. strain TTNP3. Biodegradation (in press)

Craft DL, Madduri KM, Eshoo M, Wilson CR (2003) Identification and characterization of the CYP52 family of *Candida tropicalis* ATCC 20336, important for the conversion of fatty acids and alkanes to á,ω-dicarboxylic acids. Appl Environ Microbiol 69:5983–5991

Dachs J, Van Ry DA, Eisenreich SJ (1999) Occurrence of estrogenic nonylphenols in the urban and coastal atmosphere of the lower Hudson River estuary. Environ Sci Technol 33:2676–2679

Darby JM, Taylor DG, Hopper DJ (1987) Hydroquinone as the ring-fission substrate in the catabolism of 4-ethylphenol and 4-hydroxyacetophenone by *Pseudomonas putida* JD1. J Gen Microbiol 133:2137–2146

Dec J, Haider K, Bollag J (2003) Release of substituents from phenolic compounds during oxidative coupling reactions. Chemosphere 52:549–556

de Vries YP, Takahara Y, Ikunaga Y, Ushiba Y, Hasegawa M, Kasahara Y, Shimomura H, Hayashi S, Hirai Y, Ohta H (2001) Organic nutrient-dependent degradation of branched nonylphenol by *Sphingomonas* sp. YT isolated from a river sediment sample. Microbes Environ 16:240–249

Di Corcia A, Costantino A, Crescenzi C, Marinoni E, Samperi R (1998) Characterization of recalcitrant intermediates of the branched alkyl side chain of nonylphenol ethoxylate surfactants. Environ Sci Technol 32:2401–2409

Dittmann J, Heyser W, Bucking H (2002) Biodegradation of aromatic compounds by white rot and ectomycorrhizal fungal species and the accumulation of chlorinated benzoic acid in ectomycorrhizal pine seedlings. Chemosphere 49:297–306

Doerge DR, Twaddle NC, Churchwell MI, Chang HC, Newbold RR, Delclos KB (2002) Mass spectrometric determination of p-nonylphenol metabolism and disposition following oral administration to sprague–dawley rats. Reprod Toxicol 16:45–56

Dubroca J, Brault A, Kollmann A, Touton I, Jolivalt G, Kerhoas L, Mougin C (2005) Biotransformation of nonylphenol surfactants in soils amended with contaminated sewage sludges. In: Lichtfouse E, Dudd S, Robert D (eds) Environmental chemistry: green chemistry and pollutants in ecosystems. Springer, Berlin Heidelberg New York, pp 305–315

Duran N, Esposito E (2000) Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. Appl Catal B Environ 28:83–99

Eggert C, Temp U, Dean J, Eriksson K (1996) A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase. FEBS Lett 391:144–148

Ekelund R, Bergmanb Å, Granmo Å, Berggren M (1990) Bioaccumulation of 4-nonylphenol in marine animals—a re-evaluation. Environ Pollut 64:107–120

Ekelund R, Granmo Å, Magnusson K, Berggren M (1993) Biodegradation of 4-nonylphenol in seawater and sediment. Environ Pollut 79:59–61

Espadaler I, Caixach J, Om J, Ventura F, Cortina M, Pauné F, Rivera J (1997) Identification of organic pollutants in Ter river and its system of reservoirs supplying water to Barcelona (Catalonia, Spain): a study by GC/MS and FAB/MS. Water Res 31:1996–2004

Ewers J, Rubio MA, Knackmuss HJ, Freier-Schröder D (1989) Bacterial metabolism of 2,6-xylenol. Appl Environ Microbiol 55:2904–2908

Fedorak PM, Westlake DWS (1986) Fungal metabolism of *n*-alkylbenzenes. Appl Environ Microbiol 51:435–437

Ferreira-Leach AM, Hill EM (2001) Bioconcentration and distribution of 4-tert-octylphenol residues in tissues of the rainbow trout (*Oncorhynchus mykiss*). Mar Environ Res 51:75–89

Field JA, de Jong E, Feijoo-Costa G, de Bont JAM (1993) Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. Trends Biotechnol 11:44–49

Fishman A, Tao Y, Wood TK (2004) Toluene 3-monooxygenase of *Ralstonia pickettii* PKO1 is a *para*-hydroxylating enzyme. J Bacteriol 186:3117–3123

Fraaije MW, van den Heuvel RHH, Roelofs JCAA, van Berkel WJH (1998) Kinetic mechanism of vanillyl-alcohol oxidase with short-chain 4-alkylphenols. Eur J Biochem 253:712–719

Fujii K, Urano N, Kimura S, Nomura Y, Karube I (2000a) Microbial degradation of nonylphenol in some aquatic environments. Fish Sci 66:44–48

Fujii K, Urano N, Ushio H, Satomi M, Iida, H, Ushio-Sata N, Kimura S (2000b) Profile of a nonylphenol-degrading microflora and its potential for bioremedial applications. J Biochem 128:909–916

Fujii K, Urano N, Ushio H, Satomi M, Kimura S (2001) *Sphingomonas cloacae* sp. Nov., a nonylphenol-degrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo. Int J Syst Evol Microbiol 51:603–610

Fujii K, Yamamoto R, Tanaka T, Hirakawa T, Kikuchi S (2003) Potential of a new biotreatment: *Sphingomonas cloacae* S-3T degrades nonylphenol in industrial wastewater. J Ind Microbiol Biotech 30:531–535

Furukawa H, Wieser M, Morita H, Sugio T, Nagasawa T (1999) Purification and characterization of vanillyl-alcohol oxidase of *Byssochlamys fulva* V107. J Biosci Bioeng 87:285–290

Gabriel FLP, Giger W, Guenther K, Kohler HPE (2005a) Differential degradation of nonylphenol isomers by *Sphingomonas xenophaga* Bayram. Appl Environ Microbiol 71:1123–1129

Gabriel FLP, Heidlberger A, Rentsch D, Giger W, Guenther K, Kohler HPE (2005b) A novel metabolic pathway for degradation of 4-nonylphenol environmental contaminants by *Sphingomonasxenophaga* Bayram. *Ipso*-hydroxylation and intramolecular rearrangement. J Biol Chem 280: 15526–15533

Garcia-Pena I, Hernandez S, Auria R, Revah S (2005) Correlation of biological activity and reactor performance in biofiltration of toluene with the fungus *Paecilomyces variotii* CBS115145. Appl Environ Microbiol 71:4280–4285

Garzillo A, Colao M, Caruso C, Caporale C, Celletti D, Buonocore V (1998) Laccase from the white-rot fungus *Trametes trogii*. Appl Microbiol Biotechnol 49:545–551

Gianfreda L, Rao MA (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme Microb Technol 35:339–354

Gianfreda L, Xu F, Bollag J-M (1999) Laccases: a useful group of enzymes. Bioremediation J 3:1–26

Giger W, Brunner PH, Schaffner C (1984) 4-nonylphenol in sewage-sludge-accumulation of toxic metabolites from nonionic surfactants. Science 225:623–625

Giger W, Ahel M, Koch M, Laubscher HU, Schaffner C, Schneider J (1987) Behaviour of alkylphenolpolyethoxylate surfactants and of nitrilotriacetate in sewage treatment. Water Sci Technol 19:449–460

Granmo A, Ekelund R, Magnusson K et al (1989) Lethal and sublethal toxicity of 4-nonylphenol to the common mussel (*Mytilus-edulis*-l). Environ Pollut 59:115–127

Guenther K, Kleist E, Thiele B (2005) Estrogen-active nonylphenols from an isomer-specific viewpoint: a systematic numbering system and future trends. Anal Bioanal Chem 384 (2):542–546

Günther K, Schlosser D, Fritsche W (1995) Phenol and cresol metabolism in *Bacillus pumilis* isolated from contaminated groundwater. J Basic Microbiol 35:83–92

Hammel KE (1995) Organopollutant degradation by ligninolytic fungi. In: Hammel KE, Young LY, Cerniglia CE (eds) Degradation of toxic organic chemicals. Wiley-Liss, New York, pp 331–346

Hanne LF, Kirk LL, Appel SM, Narayan AD, Bains KK (1993) Degradation and induction specificity in actinomycetes that degrade *p*-nitrophenol. Appl Environ Microbiol 59:3505–3508

Harwood CS, Parales RE (1996) The β-ketoadipiate pathway and the biology of self-identity. Annu Rev Microbiol 50:553–590

Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: Production and role in lignin degradation. FEMS Microbiol Rev 13:125–135

Heinaru E, Truu J, Stottmeister U, Heinaru A (2000) Three types of phenol and p-cresol catabolism in phenol- and p-cresol-degrading bacteria isolated from river water continuously polluted with phenolic compounds. FEMS Microbiol Ecol 31:195–205

Hesselsøe M, Jensen D, Skals K, Olesen T, Moldrup P Roslev P, Krog Mortensen G, Henriksen K (2001) Degradation of 4-nonylphenol in homogeneous and nonhomogeneous mixtures of soil and sewage sludge. Environ Sci Technol 35:3695–3700

Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb Technol 30:454–466

Hofrichter M, Scheibner K (1993) Utilization of aromatic compounds by the *Penicillium* strain Bi 7/2. J Basic Microbiol 33:227–232

Hofrichter M, Bublitz F, Fritsche W (1995) Cometabolic degradation of o-cresol and 2,6-dimethylphenol by *Penicillium frequentans* Bi 7/2. J Basic Microbiol 35:303–313

Hopper DJ (1978) Incorporation of [^{18}O]water in the formation of p-hydroxybenzyl alcohol by the p-cresol methylhydroxylase from *Pseudomonas putida*. Biochem J 175:345–347

Hopper DJ, Chapman PJ (1970) Gentisic acid and its 3- and 4-methyl-substituted homologoues as intermediates in the bacterial degradation of *m*-cresol, 3,5-xylenol and 2,5-xylenol. Biochem J 122:19–28

Hopper DJ, Cottrell L (2003) Alkylphenol biotransformations catalyzed by 4-ethylphenol methylenehydroxylase. Appl Environ Microbiol 69:3650–3652

Hopper DJ, Taylor DG (1975) Pathways for the degradation of *m*-cresol and *p*-cresol by *Pseudomonas putida*. J Bacteriol 122:1–6

Hopper DJ, Bossert ID, Rhodes-Roberts ME (1991) *p*-Cresol methylhydroxylase from a denitrifying bacterium involved in anaerobic degradation of *p*-cresol. J Bacteriol 173:1298–1301

Hughes EJ, Bayly RC (1983) Control of catechol meta-cleavage pathway in *Alcaligenes eutrophus*. J Bacteriol 154:1363–1370

Hughes EJ, Bayly RC, Skurray RA (1984) Evidence for isofunctional enzymes in the degradation of phenol, *m*- and *p*-toluate, and *p*-cresol via catechol meta-cleavage pathways in *Alcaligenes eutrophus*. J Bacteriol 158:79–83

Inui H, Shiota N, Motoi Y, Ido Y, Inoue T, Kodama T, Ohkawa Y, Okhawa H (2001) Metabolism of herbicides and other chemicals in human cytochrome P450 species and in transgenic potato plants co-expressing human CYP1A1, CYP2B6 and CYP2C19. J Pestic Sci 26:28–40

Ivashechkin P, Corvini P, Dohmann M (2004) Behaviour of endocrine disrupting chemicals during the treatment of municipal sewage sludge. Water Sci Technol 50:141–147

Jaspers MCM, Suske WA, Schmid A, Goslings DAM, Kohler HPE, van der Meer JR (2000) HbpR, a new member of the XylR/DmpR subclass within the NtrC family of bacterial transcriptional activators, regulates expression of 2-hydroxybiphenyl metabolism in *Pseudomonas azelaica* HBP1. J Bacteriol 182:405–417

Jeong JJ, JH Kim, Kim CK, Hwang I, Lee K (2003) 3- and 4-alkylphenol degradation pathway in *Pseudomonas* sp. strain KL28: genetic organization of the lap gene cluster and substrate specificities of phenol hydroxylase and catechol 2,3-dioxygenase. Microbiology 149:3265–3277

Jigami Y, Omori T, Minoda Y, Yamada K (1974) Screening of *n*-alkylbenzene assimilating yeasts and identification of oxidation products from *n*-alkylbenzenes. Agric Biol Chem (Tokyo) 38:401–408

Johannes C, Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl Environ Microbiol 66:524–528

Jones KH, Trudgill PW, Hopper DJ (1993) Metabolism of *p*-cresol by the fungus *Aspergillus fumigatus*. Appl Environ Microbiol 59:1125–1130

Jones KH, Trudgill PW, Hopper DJ (1994) 4-Ethylphenol metabolism by *Aspergillus fumigatus*. Appl Environ Microbiol 60:1978–1983

Jonkers N, Knepper TP, de Voogt P (2001) Aerobic biodegradation studies of nonylphenol ethoxylates in river water using liquid chromatography–electrospray tandem mass spectrometry. Environ Sci Technol 35:335–40

Junghanns C, Moeder M, Krauss G, Martin C, Schlosser D (2005) Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. Microbiology 151:45–57. DOI 10.1099/mic.0.27431-0

Kahng HY, Malinverni JC, Majko MM, Kukor JJ (2001) Genetic and functional analysis of the tbc operons for catabolism of alkyl- and chloroaromatic compounds in *Burkholderia* sp. strain JS150. Appl Environ Microbiol 67:4805–4816

Kamada F, Abe S, Hiratsuka N, Wariishi H, Tanaka H (2002) Mineralization of aromatic compounds by brown-rot basidiomycetes-mechanisms involved in initial attack on the aromatic ring. Microbiology 148:1939–1946

Kamerbeek NM, Moonen MJH, van der Ven JGM, van Berkel WJH, Fraaije MW, Janssen DB (2001) 4-Hydroxyacetophenone monooxygenase from *Pseudomonas fluorescens* ACB. A novel flavoprotein catalyzing Baeyer–Villiger oxidation of aromatic compounds. Eur J Biochem 268:2547–2557

Keat MJ, Hopper DJ (1978a) *p*-Cresol and 3,5-xylenol methylhydroxylases in *Pseudomonas putida* N.C.I.B. 9896. Biochem J 175: 649–658

Keat MJ, Hopper DJ (1978b) The aromatic alcohol dehydrogenases in *Pseudomonas putida* N.C.I.B. 9869 grown on 3,5-xylenol and p-cresol. Biochem J 175:659–667

Kennes C, Lema JM (1994) Simultaneous biodegradation of *p*-cresol and phenol by the basidiomycete *Phanerochaete chrysosporium*. J Ind Microbiol 13:311–314

Kim YS, Katase T, Sekine S, Inoue T, Makino M, Uchiyama T, Fujimoto T, Yamashita M (2004) Variation in estrogenic activity among fractions of a commercial nonylphenol by high performance liquid chromatography. Chemosphere 54:1127–1134

Kimura M, Michizoe J, Oakazaki S, Furusaki S, Goto M, Tanaka H, Wariishi H (2004) Activation of lignin peroxidase in organic media by reversed micelles. Biotechnol Bioeng 88: 495–501

Kitazume T, Takaya N, Nakayama N, Shoun H (2000) *Fusarium oxysporum* fatty-acid subterminal hydroxylase (CYP505) is a membrane-bound eukaryotic counterpart of *Bacillus megaterium* cytochrome P450BM3. J Biol Chem 275:39734–39740

Kobayashi S, Higashimura H (2003) Oxidative polymerization of phenols revisited. Prog Polym Sci 28:1015–1048

Kohler HPE, van der Maarel MJEC, Kohler-Staub D (1993) Selection of *Pseudomonas* sp. strain HBP1 Prp for metabolism of 2-propylphenol and elucidation of the degradative pathway. Appl Environ Microbiol 59:860–866

Kollmann A, Brault A, Touton I, Dubroca J, Chaplain V, Mougin C (2003) Effect of nonylphenol surfactants on fungi following the application of sewage sludge on agricultural soils. J Environ Qual 32:1269–1276

Kottermann MJJ, Vis EH, Field JA (1998) Successive mineralization and detoxification of benzo[a]pyrene by the white-rot fungus *Bjerkandera* sp. strain BOS55 and indigenous microflora. Appl Environ Microbiol 64:2853–2858

Kuhn EP, Zeyer J, Eicher P, Schwarzenbach RP (1988) Anaerobic degradation of alkylated benzenes in denitrifying laboratory aquifer columns. Appl Environ Microbiol 54:490–496

Kukor JJ, Olsen RH (1991) Genetic organization and regulation of a *meta* cleavage pathway for catechols produced from catabolism of toluene, benzene, phenol, and cresols by *Pseudomonas pickettii* PKO1. J Bacteriol 173:4587–4594

Lalah J, Schramm KW, Lenoir D, Henkelmann B, Hertkorn N, Matuschek G, Kettrup A, Gunther K (2001) Regioselective synthesis of a branched isomer of nonylphenol,4-(3′,6′-dimethyl-3′-heptyl)phenol, and determination of its important environmental properties. Chemistry 7:4790–4795

Lalah J, Behechti A, Severin G, Lenoir D, Gunther K, Kettrup A, Schramm K (2003a) The bioaccumulation and fate of a branched 14c-p-nonylphenol isomer in *Lymnaea stagnalis* L. Environ Toxicol Chem 22:1428–1436

Lalah J, Schramm K, Severin G, Lenoir D, Henkelmann B, Behechti A, Guenther K, Kettrup A (2003b) In vivo metabolism and organ distribution of a branched ^{14}C-nonylphenol isomer in pond snails, *Lymnaea stagnalis* L. Aquat Toxicol 62:305–319

Lalah JO, Schramm KW, Henkelmann B, Lenoir D, Behechti A, Gunther K, Kettrup A (2003c) The dissipation, distribution and fate of a branched 14C-nonylphenol isomer in lake water/sediment systems. Environ Pollut 122:195–203

Leahy JG, Batchelor PJ, Morcomb SM (2003) Evolution of the soluble diiron monooxygenases. FEMS Microbiol Rev 27:449–479

Leonowicz A, Cho N, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J (2001) Fungal laccase: properties and activity on lignin. J Basic Microbiol 41:185–227

Liber K, Knuth ML, Stay FS (1999) An integrated evaluation of the persistence and effects of 4-nonylphenol in an experimental littoral ecosystem. Environ Toxicol Chem 18:357–362

Liers C, Ullrich R, Steffen K, Hatakka A, Hofrichter M (2006) Mineralization of ^{14}C-labelled synthetic lignin and extracellular enzyme activities of the wood-colonizing ascomycetes *Xylaria hypoxylon* and *Xylaria polymorpha*. Appl Microbiol Biotechnol 69:573–579

Martinez AT (2002) Molecular biology and structure–function of lignin-degrading heme peroxidases. Enzyme Microb Technol 30:425–444

Matsuzaki F, Wariishi H (2004) Functional diversity of cytochrome P450s of the white-rot fungus *Phanerochaete chrysosporium*. Biochem Biophys Res Commun 324:387–393

Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60:551–565

McIntire W, Hopper DJ, Craig JC, Everhart ET, Webster RV, Causer MJ, Singer TP (1984) Stereochemistry of 1-(4′-hydroxyphenyl) ethanol produced by hydroxylation of 4-ethylphenol by *p*-cresol methylhydroxylase. Biochem J 224:617–621

Meldahl AC, Nithipatikom K, Lech JJ (1996) Metabolism of several ^{14}C-nonylphenol isomers by rainbow trout (*Oncorhynchus mykiss*): in vivo and in vitro microsomal metabolites. Xenobiotica 26:1167–1180

Mester T, Tien M (2000) Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. Int Biodeterior Biodegrad 46:51–59

Meyer A, Schmid A, Held M, Westphal AH, Röthlisberger M, Kohler HPE, van Berkel WJH, Witholt B (2002) Changing the substrate reactivity of 2-hydroxybiphenyl 3-monooxygenase from *Pseudomonas azelaica* HBP1 by directed evolution. J Biol Chem 277:5575–5582

Meyer A, Held M, Schmid A, Kohler HPE, Witholt B (2003) Synthesis of 3-*tert*-butylcatechol by an engineered monooxygenase. Biotechnol Bioeng 81:518–524

Middelhoven WJ (1993) Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeastlike fungi. A literature review and an experimental approach. Antonie Van Leeuwenhoek 63:125–144

Middelhoven WJ, Spaaij F (1997) *Rhodotorula cresolica* sp. Nov., a cresol-assimilating yeast species isolated from soil. Int J Syst Bacteriol 47:324–327

Middelhoven WJ, Scorzetti G, Fell JW (2000) *Trichosporon veenhuisii* sp. Nov., an alkane-assimilating anamorphic basidiomycetous yeast. Int J Syst Evol Microbiol 50:381–387

Middelhoven WJ, Scorzetti G, Fell JW (2004) Systematics of the anamorphic basidiomycetous yeast genus *Trichosporon* Behrend with the description of five novel species: *Trichosporon vadense*, T. smithiae, T. dehoogii, T. scarabaeorum and T. gamsii. Int J Syst Evol Microbiol 54:975–986

Moeder M, Schrader S, Winkler M, Popp P (2000) Solid-phase microextraction-gas chromatography-mass spectrometry of biologically active substances in water samples. J Chromatogr A 873:95–106

Moeder M, Martin C, Harynuk J, Górecki T, Vinken R, Corvini PFX (2006a) Identification of isomeric 4-nonylphenol structures by gas chromatography-tandem mass spectrometry combined with cluster analysis. J Chromatogr A 1102:245–255

Moeder M, Martin C, Schlosser D, Harynuk J, Gorecki T (2006b) Separation of technical 4-nonylphenols and their biodegradation products by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. J Chromatogr A 1107:233–239

Mogensen AS, Haagensen F, Ahring BK (2003) Anaerobic degradation of linear alkylbenzene sulfonate. Environ Toxicol Chem 22:706–711

Montgomery-Brown J, Drewes JE, Fox P, Reinhard M (2003) Behavior of alkylphenol polyethoxylate metabolites during soil aquifer treatment. Water Res 37:3672–3681

Morimoto K, Tatsumi K (1997) Effect of humic substances on the enzymatic formation of OCDD from PCP. Chemosphere 34:1277–1283

Müller JA, Galushko AS, Kappler A, Schink B (2001) Initiation of anaerobic degradation of *p*-cresol by formation of 4-hydroxybenzylsuccinate in *Desulfobacterium cetonicum*. J Bacteriol 183:752–757

Nakayama N, Shoun H (1994) Fatty acid hydroxylase of the fungus *Fusarium oxysporum* is possibly a fused protein of cytochrome P-450 and its reductase. Biochem Biophys Res Commun 202:586–590

Nakayama N, Takemae A, Shoun H (1996) Cytochrome P450foxy, a catalytically self-sufficient fatty acid hydroxylase of the fungus *Fusarium oxysporum*. J Biochem (Tokyo) 119:435–440

Neujahr HY, Kjellen KG (1978) Phenol hydroxylase from yeast. Reaction with phenol derivatives. J Biol Chem 253:8835–8841

Öberg LG, Glas B, Swanson SE, Rappe C, Paul KG (1990) Peroxidase-catalyzed oxidation of chlorophenols to polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Arch Environ Contam Toxicol 19:930–938

Olsen RH, Kukor JJ, Kaphammer B (1994) A novel toluene-3-monooxygenase pathway cloned from *Pseudomonas pickettii*. J Bacteriol 176:3749–3756

Osburn QW, Benedict JH (1966) Polyethoxylated alkyl phenols: Relationship of structure to biodegradation mechanism. J Am Oil Chem Soc 43:141–146

Park J, Dec J, Kim J, Bollag J (2000) Dehalogenation of xenobiotics as a consequence of binding to humic materials. Arch Environ Contam Toxicol 38:405–410

Pavel H, Forsman M, Shingler V (1994) An aromatic effector specificity mutant of the transcriptional regulator DmpR overcomes the growth constraints of *Pseudomonas* sp. strain CF600 on para-substituted methylphenols. J Bacteriol 176:7550–7557

Pedersen RT, Hill EM (2000a) Identification of novel metabolites of the xenoestrogen 4-tert-octylphenol in primary rat hepatocytes. Chem Biol Interact 128:189–209

Pedersen RT, Hill EM (2000b) Biotransformation of the xenoestrogen 4-tert-octylphenol in hepatocytes of rainbow trout (*Oncorhynchus mykiss*). Xenobiotica 30:867–879

Pedersen RT, Hill EM (2002) Tissue distribution and depuration of 4-tert-octylphenol residues in the cyprinid fish, *Scardinius erythrophthalmus*. Environ Sci Technol 36:3275–3283

Perron N, Welander U (2004) Degradation of phenol and cresols at low temperatures using a suspended-carrier biofilm process. Chemosphere 55:45–50

Poh CL, Bayly RC (1980) Evidence for isofunctional enzymes used in m-cresol and 2,5-xylenol degradation via the gentisate pathway in *Pseudomonas alcaligenes*. J Bacteriol 143:59–69

Powlowski JB, Dagley S (1985) Beta-ketoadipate pathway in *Trichosporon cutaneum* modified for methyl-substituted metabolites. J Bacteriol 163:1126–1135

Powlowski J, Shingler V (1994) Genetics and biochemistry of phenol degradation by *Pseudomonas* sp. CF600. Biodegradation 5: 219–236

Preuss TG, Coors A, Jones PD, Giesy JP, Ratte HT (2004) Estrogenic potency of defined nonylphenol isomers. 2nd joint annual meeting of the Society of Environmental Toxicology and Chemistry German Language Branch e.V. and the German Chemical Society Division of Environmental Chemistry and Ecotoxicology, 6–8 October 2004, Aachen, Germany

Pryor SW, Hay AG, Walker LP (2002) Nonylphenol in anaerobically digested sewage sludge from New York state. Environ Sci Technol 36:3678–3682

Razo-Flores E, Donlon B, Field J, Lettinga G (1996) Biodegradability of N-substituted aromatics and alkylphenols under methanogenic conditions using granular sludge. Water Sci Technol 33:45–57

Reddy CA (1995) The potential for white-rot fungi in the treatment of pollutants. Curr Opin Biotechnol 6:320–328

Reeve CD, Carver MA, Hopper DJ (1989) The purification and characterisation of 4-ethylphenol methylenehydroxylase, a flavocytochrome from *Pseudomonas putida* JD1. Biochem J 263:431–437

Reeve CD, Carver MA, Hopper DJ (1990) Stereochemical aspects of the oxidation of 4-ethylphenol by the bacterial enzyme 4-ethylphenol methylenehydroxylase. Biochem J 269:815–819

Romine MF, Stillwell LC, Wong KK, Thurston SJ, Sisk EC, Sensen C, Gaasterland T, Fredrickson JK, Saffer JD (1999) Complete sequence of a 184-kilobase catabolic plasmid from *Sphingomonas aromaticivorans* F199. J Bacteriol 181:1585–1602

Routledge EJ, Sumpter JP (1997) Structural features of alkylphenolic chemicals associated with estrogenic activity. J Biol Chem 272:3280–3288

Ruß AS, Vinken R, Schuphan I, Schmidt B (2005) Synthesis of branched *para*-nonylphenol isomers: occurrence and quantification in two commercial mixtures. Chemosphere 60:1624–1635

Ryan DR, Leukes WD, Burton SG (2005) Fungal bioremediation of phenolic wastewaters in an airlift reactor. Biotechnol Prog 21:1068–1074

Sack U, Heinze T, Deck J, Cerniglia C, Martens R, Zadrazil F, Fritsche W (1997) Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. Appl Environ Microbiol 63:3919–3925

Saito T, Hong P, Kato K, Okazaki M, Inagaki H, Maeda S, Yokogawa Y (2003) Purification and characterization of an extracellular laccase of a fungus (family *Chaetomiaceae*) isolated from soil. Enzyme Microb Technol 33:520–526

Saito I, Onuki A, Seto H (2004a) Indoor air pollution by alkylphenols in Tokyo. Indoor Air 14:325–332

Saito T, Kato K, Yokogawa Y, Nishida M, Yamashita N (2004b) Detoxification of bisphenol A and nonylphenol by purified

extracellular laccase from a fungus isolated from soil. J Biosci Bioeng 98:64–66

Sariaslani F, Harper D, Higgins I (1974) Microbial degradation of hydrocarbons. Catabolism of 1-phenylalkanes by *Nocardia salmonicolor*. Biochem J 140:31–45

Sarkar JM, Malcolm RL, Bollag JM (1988) Enzymatic coupling of 2,4-dichlorophenol to stream fulvic acid in the presence of oxidoreductases. Soil Sci Soc Am J 52:688–694

Scheller U, Zimmer T, Becher D, Schauer F, Schunck W-H (1998) Oxygenation cascade in conversion of n-alkanes to alpha,omega-dioic acids catalyzed by cytochrome P450 52A3. J Biol Chem 273:32528–32534

Schlosser D, Höfer C (2002) Laccase-catalyzed oxidation of Mn^{2+} in the presence of natural Mn^{3+} chelators as a novel source of extracellular H_2O_2 production and its impact on manganese peroxidase. Appl Environ Microbiol 68:3514–3521

Schmidt B, Patti H, Niewersch C, Schuphan I (2003) Biotransformation of [*ring*-U-^{14}C]4-*n*-nonylphenol by *Agrostemma githago* cell culture in a two-liquid-phase system. Biotechnol Lett 25:1375–1381

Schmidt B, Patti H, Hommes G, Schuphan I (2004) Metabolism of the nonylphenol isomer [*ring*-U-^{14}C]-4-(3′,5′-dimethyl-3′-heptyl)-phenol by cell suspension cultures of *Agrostemma githago* and soybean. J Environ Sci Health B Pestic Food Contam Agric Wastes 39:533–549

Shields MS, Montgomery SO, Chapman PJ, Cuskey SM, Pritchard PH (1989) Novel pathway of toluene catabolism in the trichloroethylene degrading bacterium G4. Appl Environ Microbiol 55:1624–1629

Shields MS, Reagin MJ, Gerger RR, Campbell R, Somerville C (1995) TOM, a new aromatic degradative plasmid from *Burkholderia* (*Pseudomonas*) *cepacia* G4. Appl Environ Microbiol 61:1352–1356

Soares A, Guieysse B, Delgado O, Mattiasson B (2003a) Aerobic biodegradation of nonylphenol by cold-adapted bacteria. Biotechnol Lett 25:731–738

Soares A, Guieysse B, Mattiasson B (2003b) Biodegradation of nonylphenol in a continuous packed-bed bioreactor. Biotechnol Lett 25:927–933

Soares A, Jonasson K, Terrazas E, Guieysse B, Mattiasson B (2005a) The ability of white-rot fungi to degrade the endocrine-disrupting compound nonylphenol. Appl Microbiol Biotechnol 66:719–725

Soares A, Murto M, Guieysse B, Mattiasson B (2005b) Biodegradation of nonylphenol in a continuous bioreactor at low temperatures and effects on the microbial population. Appl Microbiol Biotechnol 69(5):597–606

Soares A, Guieysse B, Mattiasson B (2006) Influence of agitation on the removal of nonylphenol by the white-rot fungi Trametes versicolor and Bjerkandera sp. BOL 13. Biotechnol Lett 28:139–143

Song B, Palleroni NJ, Haggblom MM (2000) Description of strain 3CB-1, a genomovar of *Thauera aromatica*, capable of degrading 3-chlorobenzoate coupled to nitrate reduction. Int J Syst Evol Microbiol 50:551–558

Soto AM, Justica H, Wray JW, Sonnenschein C (1991) *Para*-nonylphenol: an estrogenic xenobiotic released from polystyrene. Environ Health Perspect 92:167–173

Staples CA, Naylor CG, Williams JB, Gledhill WE (2001) Ultimate biodegradation of alkylphenol ethoxylate surfactants and their biodegradation intermediates. Environ Toxicol Chem 20: 2450–2455

Steffen K, Hatakka A, Hofrichter M (2002) Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. Appl Microbiol Biotechnol 60:212–217

Stephanou E, Giger W (1982) Persistent organic-chemicals in sewage effluents. 2. Quantitative-determinations of nonylphenols and nonylphenol ethoxylates by glass-capillary gas chromatography. Environ Sci Technol 16:800–805

Sundaram KMS, Szeto S (1981) The dissipation of nonylphenol in stream and pond water under stimulated field conditions. J Environ Sci Health B16:767–776

Tanaka T, Tonosaki T, Nose M, Tomidokoro N, Kadomura N, Fujii T, Taniguchi M (2001) Treatment of model soils contaminated with phenolic endocrine-disrupting chemicals with laccase from *Trametes* sp. in a rotating reactor. J Biosci Bioeng 92:312–316

Tanaka T, Nose M, Endo A, Fujii T, Taniguchi M (2003) Treatment of nonylphenol with laccase in a rotating reactor. J Biosci Bioeng 96:541–546

Tanghe T, Devriese G, Verstraete W (1998) Nonylphenol degradation in lab scale activated sludge units is temperature dependent. Water Res 32:2889–2896

Tanghe T, Devriese G, Verstraete W (1999a) Nonylphenol and estrogenic activity in aquatic environmental samples. J Environ Qual 28:702–709

Tanghe T, Dhooge W, Verstraete W (1999b) Isolation of a bacterial strain able to degrade branched nonylphenol. Appl Environ Microbiol 65:746–751

Tanghe T, Dhooge W, Verstraete W (2000) Formation of the metabolic intermediate 2,4,4-trimethyl-2-pentanol during incubation of a *Sphingomonas* sp. strain with the xeno-estrogenic octylphenol. Biodegradation 11:1–19

Tanner A, Hopper DJ (2000) Conversion of 4-hydroxyacetophenone into 4-phenyl acetate by a flavin adenine dinucleotidecontaining Baeyer–Villiger-type monooxygenase. J Bacteriol 182:6565–6569

Telscher MJ, Schuller U, Schmidt B, Schaffer A (2005) Occurrence of a nitro metabolite of a defined nonylphenol isomer in soil/sewage sludge mixtures. Environ Sci Technol 39:7896–7900

Thibaut R, Debrauwer L, Rao D, Cravedi J (1998) Characterization of biliary metabolites of 4-*n*-nonylphenol in rainbow trout (*Oncorhynchus mykiss*). Xenobiotika 28:745–757

Thibaut R, Debrauwer L, Rao D, Cravedi JP (1999) Urinary metabolites of 4-*n*-nonylphenol in rainbow trout (*Oncorhynchus mykiss*). Sci Total Environ 233:193–200

Thibaut R, Debrauwer L, Perdu E, Goksoyr A, Cravedi JP, Arukwe A (2002a) Regio-specific hydroxylation of nonylphenol and the involvement of CYP2K- and CYP2M-like iso-enzymes in Atlantic salmon (*Salmo salar*). Aquat Toxicol 56:177–190

Thibaut R, Monod G, Cravedi JP (2002b) Residues of ^{14}C-4-*n*-nonylphenol in mosquitofish (*Gambusia holbrooki*) oocytes and embryos during dietary exposure of mature females to this xenohormone. Mar Environ Res 54:685–689

Thiele B, Heinke V, Kleist E, Guenther K (2004) Contribution to the structural elucidation of 10 isomers of technical *p*-nonylphenol. Environ Sci Technol 38:3405–3411

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140:19–26

Topp E, Starratt A (2000) Rapid mineralization of the endocrine-disrupting chemical 4-nonylphenol in soil. Environ Toxicol Chem 19:313–318

Tschech A, Fuchs G (1987) Anaerobic degradation of phenol by pure cultures of newly isolated denitrifying pseudomonads. Arch Microbiol 148:213–217

Tsutsumi Y, Haneda T, Nishida T (2001) Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading basidiomycetes. Chemosphere 42:271–276

Ushiba Y, Takahara Y, Ohta H (2003) *Sphingobium amiense* sp. Nov., a novel nonylphenol-degrading bacterium isolated from a river sediment. Int J Syst Evol Microbiol 53:2045–2048

Vallini G, Frassinetti S, Scorzetti G (1997) *Candida aquaetextoris* sp. Nov., a new species of yeast occurring in sludge from a textile industry wastewater treatment plant in Tuscany, Italy. Int J Syst Bacteriol 47:336–340

Vallini G, Frassinetti S, D'Andrea F, Catelani G, Agnolucci M (2001) Biodegradation of 4-(1-nonyl)phenol by axenic cultures of the yeast *Candida aquaetextoris*: identification of microbial breakdown products and proposal of a possible metabolic pathway. Int Biodeterior Biodegrad 47:133–140

van Beilen JB, Funhoff EG (2005) Expanding the alkane oxygenase toolbox: new enzymes and applications. Curr Opin Biotechnol 16:308–314

van Beilen JB, Li Z, Duetz WA, Smits THM, Witholt B (2003) Diversity of alkane hydroxylase systems in the environment. Oil Gas Sci Technol 58:427–440

van den Heuvel RHH, Fraaije MW, Laane C van Berkel WJH (1998) Regio- and stereospecific conversion of 4-alkylphenols by the covalent flavoprotein vanillyl-alcohol oxidase. J Bacteriol 180:5646–5651

van den Heuvel RHH, Fraaije MW, Ferrer M, Mattevi A, van Berkel WJH (2000) Inversion of the stereospecificity of vanillyl-alcohol oxidase. Proc Natl Acad Sci U S A 97:9455–9460

Van Ginkel CG (1996) Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms. Biodegradation 7:151–164

Vares T, Niemenmaa O, Hatakka A (1994) Secretion of ligninolytic enzymes and mineralization of ^{14}C-ring-labelled synthetic lignin by three *Phlebia tremellosa* strains. Appl Environ Microbiol 60:569–575

Vinken R, Schmidt B, Schäffer A (2002) Synthesis of tertiary ^{14}C-labelled nonylphenol isomers. J Label Compd Radiopharm 45:1253–1263

Vinken R, Höllrigl-Rosta A, Schmidt B, Schäffer A, Corvini PFX (2004) Bioavailability of a nonylphenol isomer in dependence on the association to dissolved humic substances. Water Sci Technol 50:285–291

Watanabe K, Futamata H, Harayama S (2002) Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. Antonie Van Leeuwenhoek 81:655–663

Webster J (1992) Anamorph–teleomorph relationships. In: Bärlocher F (ed) The ecology of aquatic hyphomycetes. Springer, Berlin Heidelberg New York, pp 99–117

Wheeler TF, Heim JR, LaTorre MR, Janes B (1997) Mass spectral characterization of p-nonylphenol isomers using high-resolution capillary GC-MS. J Chromatogr Sci 35:19–30

Whited GM, Gibson DT (1991) Toluene-4-monooxygenase, as three-component enzyme system that catalyzes the oxidation of toluene to *p*-cresol in *Pseudomonas mendocina* KR1. J Bacteriol 173:3010–3016

Wright JD (1993) Fungal degradation of benzoic acid and related compounds. World J Microbiol Biotechnol 9:9–16

Xu F (1996) Oxidation of phenols, anilines, and benzenethiols by fungal laccases: correlation between activity and redox potentials as well as halide inhibition. Biochemistry 35:7608–7614

Yadav JS, Loper JC (2000) Cloning and characterization of the cytochrome P450 oxidoreductase gene from the zygomycete fungus *Cunninghamella*. Biochem Biophys Res Commun 268:345–353

Yamada K, Akiba Y, Shibuya T, Kashiwada A, Matsuda K, Hirata M (2005) Water purification through bioconversion of phenol compounds by tyrosinase and chemical adsorption by chitosan beads. Biotechnol Prog 21:823–829

Ying GG, Williams B, Kookana R (2002) Environmental fate of alkylphenols and alkylphenol ethoxylates—a review. Environ Int 28:215–226

Ying GG, Kookana RS, Dillon P (2003) Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Res 37:3785–3791

Yuan SY, CH Yu, Chang BV (2004) Biodegradation of nonylphenol in river sediment. Environ Pollut 127:425–430

Zalko D, Costagliola R, Dorio C, Rathahao E, Cravedi JP (2003) In vivo metabolic fate of the xeno-estrogen 4-*n*-nonylphenol in Wistar rats. Drug Metab Dispos 31:168–178