

Chapter 2

Pathways for the Degradation of Styrene

Abstract The monomer styrene can be degraded by various microorganisms under aerobic and anaerobic conditions. Therefore, several peripheral pathways are employed yielding few central intermediates as 3-vinylcatechol, phenylacetic acid, benzoic acid, or 2-ethylhexanol. However, the anaerobic breakdown of styrene is less extensively described compared to the aerobic metabolization, and for the latter mainly Pseudomonads and Actinobacteria have been studied. There is only one styrene-specific pathway, designated side-chain oxidation, reported so far, while all other routes can be considered as unspecific. Thus microorganisms possessing pathways for toluene and biphenyl via direct ring cleavage, for example, can breakdown styrene as well. Besides these degradation capabilities, the partial metabolic activity of higher organisms is mentioned which often yields marker compounds, mandelic acid and phenylglyoxylic acid.

Keywords Aromatic degradation • Xenobiotic • Side-chain oxygenation • *Ortho*- and *meta*-cleavage • Oxygenase • Phenylacetic acid

2.1 Styrene-Degrading Microorganisms

The distribution of styrene among various environments due to a natural occurrence (Warhurst and Fewson 1994; Shirai and Hisatsuka 1979) or more importantly by industrial spillage makes the compound to a potential carbon source for microorganisms. For that, either unspecific routes may be used or even specific ones can evolve and allow microorganisms to use styrene as sole source of carbon and energy. Respective metabolisms for styrene have been found among several prokaryotic and eukaryotic organisms (Table 2.1). However, in the first studies regarding the bioavailability and mineralization of styrene mainly mixed cultures have been

Table 2.1 Microorganisms capable to mineralize styrene

Class	Genus	References
<i>Bacteria</i>		
Actinobacteria	<i>Brevibacterium</i>	Hou et al. (1983)
	<i>Corynebacterium</i>	Itoh et al. (1996)
	<i>Gordonia</i>	Alexandrino et al. (2001), Oelschlägel et al. (2014a, b)
	<i>Mycobacterium</i>	Burback and Perry (1993)
	<i>Nocardia</i>	Furuhashi et al. (1986), Hartmans et al. (1990)
	<i>Rhodococcus</i>	Hartmans et al. (1990), Jung and Park (2005), Oelschlägel et al. (2012, 2014a, b), Patrauchan et al. (2008), Tischler et al. (2009), Toda and Itoh (2012), Warhurst et al. (1994a), Zilli et al. (2003)
	<i>Streptomyces</i>	Przybulewska et al. (2006)
	<i>Tsukamurella</i>	Arnold et al. (1997)
Bacilli	<i>Bacillus</i>	Przybulewska et al. (2006)
Clostridia	<i>Clostridium</i>	Grbić-Galić et al. (1990)
α -Proteobacteria	<i>Methylosinus</i>	Higgins et al. (1979)
	<i>Sphingobium</i>	Oelschlägel et al. (2014b)
	<i>Sphingomonas</i>	Arnold et al. (1997)
	<i>Sphingopyxis</i>	Oelschlägel et al. (2014b)
	<i>Xanthobacter</i>	Hartmans et al. (1989, 1990)
β -Proteobacteria	<i>Nitrosomonas</i>	Keener and Arp (1994)
γ -Proteobacteria	<i>Enterobacter</i>	Grbić-Galić et al. (1990)
	<i>Methylococcus</i>	Colby et al. (1977)
	<i>Pseudomonas</i>	Alexandrino et al. (2001), Baggi et al. (1983), Beltrametti et al. (1997), Bestetti et al. (1984), Gąszczak et al. (2012), Ikura et al. (1997), Kim et al. (2005), Lin et al. (2010), Marconi et al. (1996), O'Conner et al. (1995), Oelschlägel et al. (2014a), Panke et al. (1998), Park et al. (2006), Rustemov et al. (1992), Shirai and Hisatsuka (1979), Utkin et al. (1991), Velasco et al. (1998)
	<i>Xanthomonas</i>	Arnold et al. (1997)
Sphingobacteria	<i>Sphinogobacterium</i>	Przybulewska et al. (2006)
<i>Fungi</i>		
Agaricomycetes	<i>Bjerkandera</i>	Braun-Lüllemann et al. (1997)
	<i>Phanerochaete</i>	Braun-Lüllemann et al. (1997)
	<i>Pleurotus</i>	Braun-Lüllemann et al. (1997)
	<i>Trametes</i>	Braun-Lüllemann et al. (1997)
Dothideomycetes	<i>Cladosporium</i>	Weber (1995), Weber et al. (1995)
	<i>Caldariomyces</i>	Geigert et al. (1986)
Eurotiomycetes	<i>Aspergillus</i>	Paca et al. (2001)
	<i>Exophiala</i>	Cox et al. (1993, 1996)
	<i>Penicillium</i>	Cox (1995), de Jong et al. (1990), Paca et al. (2001)
Sordariomycetes	<i>Gliocladium</i>	Cox (1995)
	<i>Sporothrix</i>	Cox (1995), René et al. (2010)

investigated (Alexandrino et al. 2001; Araya et al. 2000; Arnold et al. 1997; Cox et al. 1993; Grbić-Galić et al. 1990; Lu et al. 2001; Weigner et al. 2001). Initially, Pseudomonads were investigated as well because of their well-known potential in breakdown of aromatic compounds (Baggi et al. 1983; Bestetti et al. 1984; Shirai and Hisatsuka 1979). Later, further pure strains as, for example, from the genus *Rhodococcus* were isolated and studied in detail (Table 2.1; see Sects. 2.2–2.4).

As already indicated, most of the studies describe the aerobic breakdown of styrene via phenylacetic acid by microorganisms (Tischler and Kaschabek 2012) in which especially the genus *Pseudomonas* was frequently investigated (O’Leary et al. 2002). However, in the meantime, it is reported that several Gram-negative and Gram-positive bacteria as well as fungi are able to mineralize styrene completely. Therefore, the monomer styrene seems to be readily bioavailable and may enter the cell via diffusion or by means of a specially evolved transport system (Mooney et al. 2006a; Nikodinovic-Runic et al. 2009). When investigated in dependence of the organism, styrene shows inhibitory effects on the growth behavior (Cox et al. 1993, 1997; Gąszczak et al. 2012; Tischler et al. 2009). However, *Pseudomonas* sp. E-93486 could utilize styrene as a carbon source even from media containing a styrene concentration of up to 90 g m^{-3} (Gąszczak et al. 2012). In comparison, the white-rot fungi *Pleurotus ostreatus* was able to degrade $37 \text{ g styrene m}^{-3}$ within 48 h, whereas related fungi were not capable to utilize such styrene concentrations (Braun-Lüllemann et al. 1997).

Despite the presence or absence of molecular oxygen, two major strategies to activate and further degrade the monomer styrene have been identified (Mooney et al. 2006b; O’Leary et al. 2002). In both cases oxygen (molecular or from water) is used by microorganisms to activate styrene. This initial attack either targets the aromatic nucleus or the vinyl side-chain. The latter one seems to be styrene-specific since epoxidation (aerobic) or hydratation (anaerobic) of the vinyl side-chain has frequently been described for various microorganisms (Tischler and Kaschabek 2012). Whereas the initial mono- or dihydroxylation of the aromatic ring seems to be a product of unspecific pathways which are commonly responsible for the degradation of structurally related aromatic compounds such as biphenyl, ethylbenzene, or toluene (Cho et al. 2000; Patrauchan et al. 2008; Warhurst et al. 1994a). That is reasonable, since usually these routes can be induced by various compounds. And they can be passed through the same enzymatic machinery. This is possible due to relaxed substrate specificity of the respective enzymes and a similar reaction pattern (e.g. *meta*-cleavage of the aromatic nucleus). Analogous intermediates can often be observed for the substrates mentioned (Carmona et al. 2009; Patrauchan et al. 2008).

2.2 Aerobic Styrene Metabolism

The monomer styrene is either attacked at the vinyl side-chain or at its aromatic nucleus by means of mono- or dioxygenases, respectively. So far, most studies have revealed a route via side-chain oxidation which can therefore be supposed

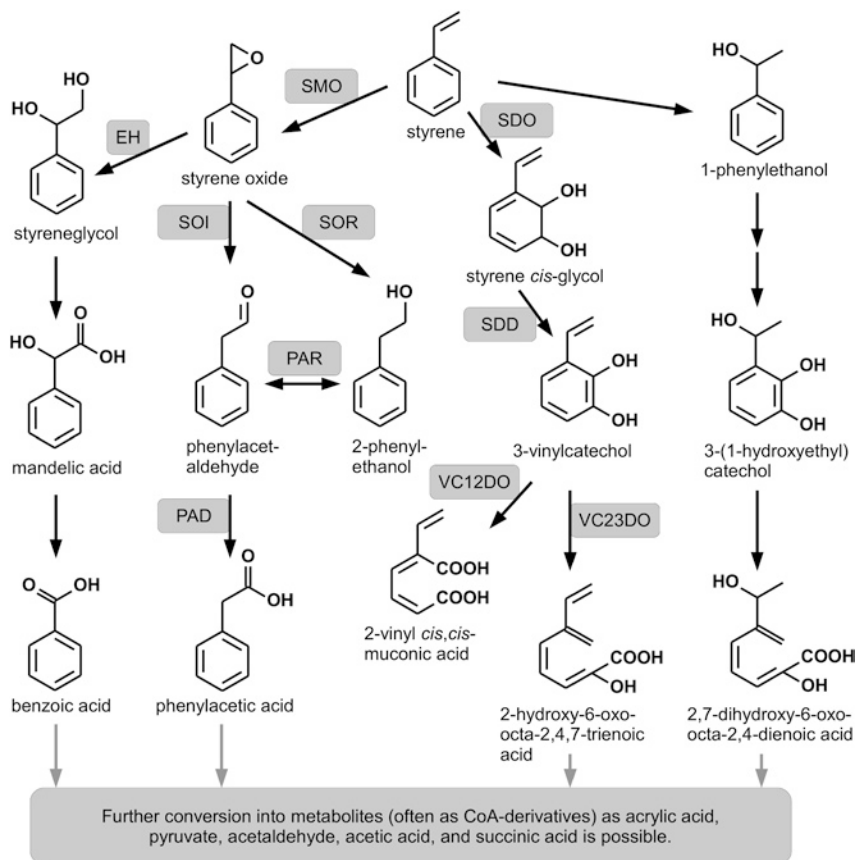


Fig. 2.1 Aerobic styrene metabolism

as the favored mechanism in nature (Beltrametti et al. 1997; Cox et al. 1996; Hartmans et al. 1990; Itoh et al. 1996; Oelschlägel et al. 2012, 2014b; Panke et al. 1998; Park et al. 2006; Toda and Itoh 2012; Velasco et al. 1998). In the following sections both pathways and, in addition, some modified routes are discussed (Fig. 2.1).

2.2.1 Vinyl Side-Chain Oxidation (Upper Styrene-Degrading Pathway)

The upper styrene pathway starts with an activation of the vinyl side-chain via oxidation in order to produce phenylacetic acid as a key product (Fig. 2.1). Numerous strains have been reported following this route, for example, *Corynebacterium* sp. AC-5 (Itoh et al. 1996), *Exophiala jeanselmei* (Cox et al. 1996), *Xanthobacter*

sp. strain 124X (Hartmans et al. 1989), *Sphingopyxis fribergensis* Kp5.2 (Oelschlägel et al. 2014b, 2015) *Rhodococcus* sp. ST-5 (Toda and Itoh 2012), *Rhodococcus opacus* ICP (Tischler et al. 2009; Oelschlägel et al. 2012), and several *Pseudomonas* strains (Kantz et al. 2005; Lin et al. 2010; Marconi et al. 1996; O'Connor et al. 1995, 1997; Panke et al. 1998; Park et al. 2006; Utkin et al. 1991; Velasco et al. 1998). In addition, some white-rot fungi also follow a side-chain oxidation route, but employing other enzymes and therefore yielding different intermediates (see Sect. 2.2.4) (Braun-Lüllemann et al. 1997).

First, styrene monooxygenases (SMO) use molecular oxygen to perform styrene epoxidation. Interestingly, bacteria have an FAD-dependent enzyme (reviewed by Ceccoli et al. 2014; Huijbers et al. 2014; Monterisino et al. 2011), whereas fungal species contain a heme dependent cytochrome P450 monooxygenase (Cox et al. 1996). In the case of bacterial SMOs, the conversion is highly enantioselective and yields the respective *S*-enantiomer of styrene oxide. The epoxide formed gets further converted by styrene oxide isomerase (SOI) into phenylacetaldehyde while the chiral information introduced gets lost again. Typically, the SOI is a transmembrane protein and cofactor independent (Itoh et al. 1997; Oelschlägel et al. 2012). Some preference of the SOI for the *S*-enantiomer of styrene oxide has also been shown (Itoh et al. 1997; Miyamoto et al. 2007; Oelschlägel et al. 2012). Next, the NAD-consuming phenylacetaldehyde dehydrogenase (PAD) catalyzes the reaction of phenylacetaldehyde into phenylacetic acid. As side-reaction unspecific dehydrogenases and/or reductases might convert phenylacetaldehyde into 2-phenylethanol and vice versa (Beltrametti et al. 1997; Marconi et al. 1996).

Finally, this peripheral route to transform styrene into phenylacetic acid can be seen as the only styrene-specific pathway identified so far (Mooney et al. 2006b; Tischler and Kaschabek 2012). That gets obvious from the following points: (i) the pathway is positively regulated by styrene (Velasco et al. 1998), (ii) it comprises enzymes which are highly specific for styrene(s) and corresponding metabolites, (iii) knockout mutants for key enzymes lost ability to grow on styrene as sole carbon source (Han et al. 2006), (iv) only styrene derivatives can be converted to respective acids but at poor rates (Oelschlägel et al. 2014a), and (v) neither such styrene derivatives nor other aromatic compounds serve as sole carbon sources and could not be degraded via this peripheral route which was exemplarily shown for a key enzyme of this pathway (Itoh et al. 1997) or the co-metabolic turnover of styrene derivatives (Oelschlägel et al. 2015).

2.2.2 Phenylacetic Acid Catabolism (Lower Styrene-Degrading Pathway)

The side-chain oxidation of styrene can be seen as a peripheral catabolic route yielding the central intermediate phenylacetic acid (Navarro-Llorens et al. 2005; Olivera et al. 1998; Teufel et al. 2010). Besides styrene, also phenylalanine,

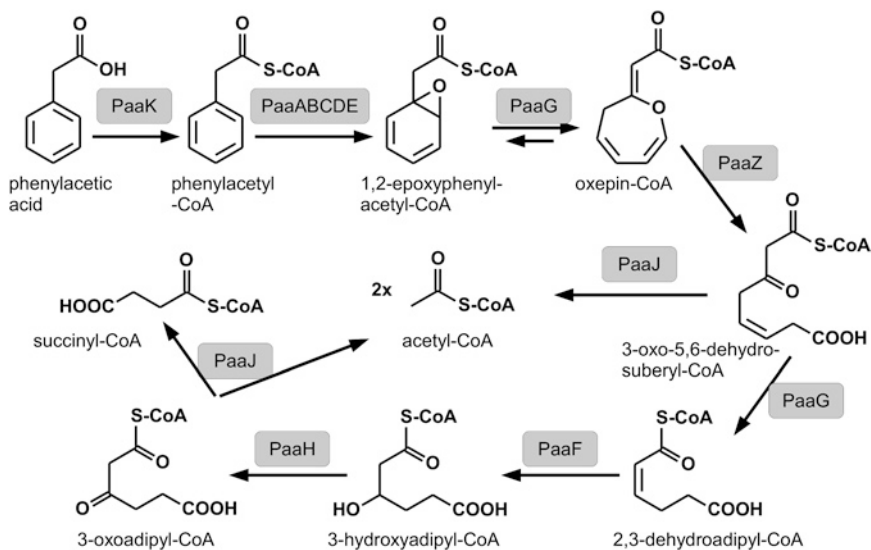


Fig. 2.2 Aerobic catabolism of phenylacetic acid

2-phenylethanol, phenylethylamine, *trans*-styrylacetic acid, phenylalkanoic acids, and tropic acid also lead to this intermediate. However, the upper and lower styrene-degrading pathways are connected via regulatory elements (Peso-Santos et al. 2006) and therefore the phenylacetic acid catabolism will be outlined herein. Further, it should be mentioned that this pathway is the only one elucidated for aerobic phenylacetic acid so far, while present in about 16 % of all genome-sequenced microorganisms (Teufel et al. 2010). The key enzymes and reactions (Fig. 2.2) are conserved but different types of regulators (PaaR and PaaX) have been described, respectively (Chen et al. 2012).

Phenylacetic acid can be actively imported by transporter proteins (PaaL, PaaP) (Peso-Santos et al. 2006) or produced from styrene as mentioned above. Then an ATP-consuming reaction of a phenylacetate-CoA ligase (PaaK) yields phenylacetyl-CoA. The thioester obtained gets activated at the aromatic nucleus to the 1,2-epoxide by means of a multicomponent epoxidase (PaaABCDE) (Teufel et al. 2010). Next, the ring 1,2-epoxyphenylacetyl-CoA isomerase (PaaG) isomerizes the reactive epoxide to an oxepin-CoA intermediate which undergoes a hydrolytic ring cleavage. During the latter step NADPH is formed by an oxepin-CoA hydrolase (PaaZ) and β -oxidation steps follow to yield acetyl-CoA and succinyl-CoA (by PaaJ, PaaG, PaaF, PaaH) (Navarro-Llorens et al. 2005; Teufel et al. 2010).

2.2.3 Direct Ring Cleavage of Styrene

A direct attack of the aromatic nucleus by mono- or dioxygenases is another possibility to activate styrene for a further breakdown (Fig. 2.1). However, a

dioxygenation and a later *meta*-cleavage was found to be prominent among several microorganisms as, for example, *Rhodococcus rhodochrous* NCIMB 13259 (Warhurst et al. 1994a), *Pseudomonas* sp. Y2 (Utkin et al. 1991), *Xantobacter* sp. strain 124X (Hartmans et al. 1989), *Pseudomonas putida* MST (Bestetti et al. 1989), and *Rhodococcus jostii* RHA1 (Patrauchan et al. 2008). Also the contemporary presence of side-chain oxygenation and direct ring cleavage in a single microorganism in an active form seems possible (Hartmans et al. 1989; Utkin et al. 1991).

Warhurst and coworkers (1994a) have described in detail a probable styrene-catabolic pathway via ring hydroxylation and *meta*-cleavage in detail. The sequential activity of styrene 2,3-dioxygenase (SDO) and styrene 2,3-dihydrodiol dehydrogenase (SDD) initially dihydroxylate styrene to styrene *cis*-glycol and then catalyze the rearomatization of this glycol to yield 3-vinylcatechol. Considering catechol-like compounds as the key intermediates, these two peripheral steps are similar to those which are involved in the degradation of benzene-, ethylbenzene-, and toluene, respectively (Mars et al. 1997; Smith 1990; Warhurst et al. 1994b). The 3-vinylcatechol obtained can then undergo *ortho*- or *meta*-cleavage. Both have been found in strain NCIMB 13259, in which, however, the activity of the vinylcatechol 1,2-dioxygenase (VC12DO) leads to the *ortho*-product 2-vinyl-*cis,cis*-muconic acid which represents a dead-end product (Warhurst et al. 1994a, b). Respectively, the *meta*-route including the vinylcatechol 2,3-dioxygenase (VC23DO) resembles the catabolically relevant one leading to 2-hydroxy-6-oxo-octa-2,4,7-trienoic acid. The latter product is further transformed into acrylic acid, acetaldehyde, and pyruvate. These observations are in congruence with results obtained from the degradation of various alkylcatechols (Knackmuss et al. 1976; Marín et al. 2010; Patrauchan et al. 2008).

After converting styrene to 1-phenylethanol, *Pseudomonas* sp. Y2 also showed the potential to directly attack the aromatic nucleus and to degrade the intermediate via a *meta*-pathway (Utkin et al. 1991). The enzymes involved were not studied, but a similar route as described above can be suggested leading to complete styrene mineralization via analogous intermediates.

However, it should be mentioned that this described styrene pathway is supposed to be rather unspecific (Tischler and Kaschabek 2012). Styrene itself seems not to be the original substrate, but funneled through the route due to a relaxed substrate specificity of involved enzymes. This is also shown by both *Rhodococcus* strains reported (Patrauchan et al. 2008; Warhurst et al. 1994a) which were able to utilize besides styrene benzene, ethylbenzene, toluene, and other aromatic compounds. In most cases a clear indication for a *meta*-cleavage route was found. Most striking is that strain RHA1 utilizes biphenyl as carbon source and even activates this compound faster than the others (Patrauchan et al. 2008). Genomic studies revealed the respective biphenyl operon and a knockout of the corresponding biphenyl dioxygenase abolished the growth of strain RHA1 on styrene. Respectively, the biphenyl catabolic operon enables this strain to co-metabolize styrene via a *meta*-cleavage pathway.

2.2.4 Alternative Routes to Mineralize or Transform Styrene

Marconi and coworkers (1996) have reported 2-phenylethanol as a minor metabolite obtained during styrene degradation activity of *Pseudomonas fluorescens* ST which usually performs side-chain oxygenation. Therefore, additional activities of styrene oxide reductase (SOR) and phenylacetaldehyde reductase (PAR) have been predicted by the above-mentioned study (Fig. 2.1). But, the compound 2-phenylethanol was determined to be a major metabolite for other bacteria, such as, *Pseudomonas* sp. 305-STR-1-4 (Shirai and Hisatsuka 1979), *Pseudomonas* sp. Y2 (Utkin et al. 1991), and *Xanthobacter* strain 124X (Hartmans et al. 1989). Furthermore, this styrene biotransformation might belong to an unspecific route which has been supposed to be the ethylbenzene pathway (Tischler and Kaschabek 2012).

Rhodococcus sp. ST-10 has been reported to harbor an incomplete styrene degradation route since no enzymatic SOI activity was determined which was suggested to be overcome by some other microbial activity from ecosystem or chemical isomerization (Toda and Itoh 2012). Respectively, no *styC*-gene has been identified for strain ST-10 yet. It was speculated that due to the chemical conversion of styrene oxide into the respective phenylacetaldehyde the strain might utilize styrene as carbon source. But, this assumption is rather unlikely since under conditions as occurring inside a cell, the epoxide isomerization probably does not occur (Han et al. 2006; Oelschlägel et al. 2012). Further, strain ST-10 yielded acetophenone and styreneglycol as dead-end metabolites (Toda and Itoh 2012). With respect to other possible routes (Fig. 2.1), the activity of SOR and PAR might in concert overcome the missing SOI activity. However, the latter activities have not been shown for strain ST-10 so far. Several other strains have been found to co-metabolize styrene yielding growth or enrichment of dead-end products, respectively. Thus, styrene is often epoxidized and not further catabolized as shown, for example, for *Nitrosomonas* or *Nocardia* (Furuhashi et al. 1986; Keener and Arp 1994). Otherwise *R. jostii* RHA1 was found to funnel styrene through its biphenyl pathway and can use so styrene as a sole carbon source (Patrauchan et al. 2008).

The difference between the bacterial and the fungal side-chain oxygenation route has already been mentioned (Sect. 2.2.1). In dependence of the microorganism studied, different enzymes for initial styrene epoxidation are employed. However, for a few white-rot fungi also the further catabolism differs significantly (Fig. 2.1) (Braun-Lüllemann et al. 1997). Herein, the epoxide formed is further converted to styreneglycol by means of an epoxide hydrolase (EH). The diol obtained is further oxidized to mandelic acid by a dehydrogenase and thereafter decarboxylated to yield benzoic acid. This route shows high similarity to the detoxification metabolism of human (Rueff et al. 2009). In addition, side products like 2-phenylethanol were also determined.

2.3 Anaerobic Routes for Styrene Degradation

Anaerobic mineralization of aromatic compounds is already known for some time, but the detailed investigations on pathways, genetics, enzymes, and the general capacity have just recently been reported and further work is still necessary (Carmona et al. 2009). Microbial degradation of aromatic compounds under anaerobic conditions is handicapped due to the delocalized electrons and therewith stabilized aromatic structure. Further, the absence of molecular oxygen does not allow hydroxylations and oxygenolytic ring cleavage reactions which are main processes in aerobic breakdown of such compounds. Respectively, reductive reactions are mainly recruited to attack and activate aromatic compounds. As for aerobic pathways described, a variety of peripheral routes lead to central intermediates of the anaerobic catabolism, for example, benzoyl-CoA and resorcinol. These pathways depend on the redox potential and electron acceptors applicable for the microorganism (Carmona et al. 2009).

However, not much information is available on the anaerobic metabolism of styrene. Pure cultures and microbial consortia obtained from anaerobic sludge were investigated for their potential in styrene mineralization (Grbić-Galić et al. 1990; Araya et al. 2000). The styrene breakdown was determined and respective metabolites were identified. Based on these intermediates pathways have been assigned (Fig. 2.3). Here two main metabolites have to be mentioned: phenylacetic acid and ethylphenol. Both intermediates can funnel via benzoyl-CoA into the central metabolism (Carmona et al. 2009). And interestingly, both consortia and

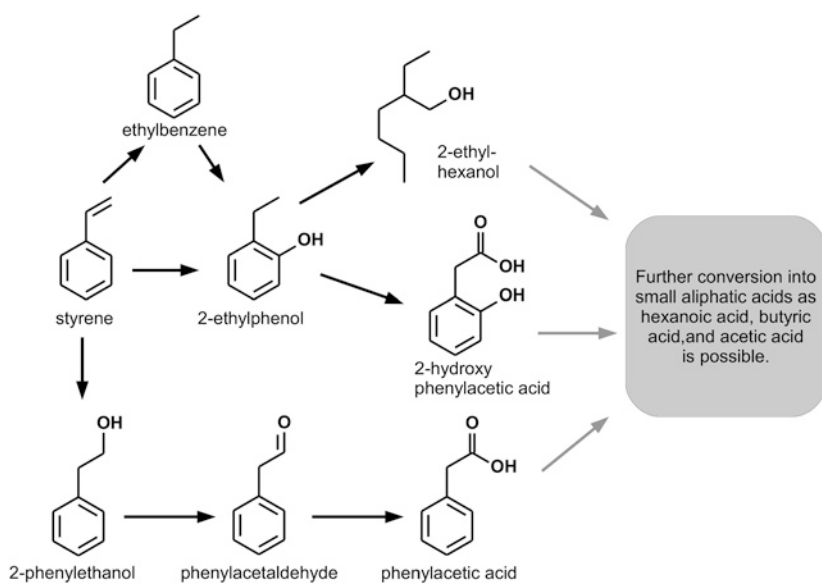


Fig. 2.3 Anaerobic styrene degradation routes based on metabolites determined (adapted from Grbić-Galić et al. 1990; Tischler and Kaschabek 2012)

pure cultures were able to almost completely mineralize styrene via those proposed routes. Carbon dioxide has been supposed to be the final product and only traces of aromatic or alicyclic intermediates remained in the media. But, respective gene clusters, regulatory elements, and enzymes have not been studied, so far, and therefore the anaerobic styrene breakdown remains uncovered.

Anaerobic consortia and *Enterobacter* have been found to degrade styrene via a peripheral route into phenylacetic acid (Grbić-Galić et al. 1990). It is similar to the aerobic styrene conversion via side-chain oxygenation. As the authors state, styrene is first transformed into 2-phenylethanol by addition of water catalyzed by a hydratase. Steps following are catalyzed by different dehydrogenases which transform 2-phenylethanol via phenylacetaldehyde into phenylacetic acid (Grbić-Galić et al. 1990). Alternatively, the aromatic ring of styrene can be directly attacked and the vinyl side-chain can be reduced yielding 2-ethylphenol. The latter compound can then be converted into 2-ethylhexanol by direct ring cleavage or oxidized into 2-hydroxyphenylacetic acid. However, both routes assigned for anaerobic styrene metabolism seem to be important and occurring contemporarily in the microbial consortia (Tischler and Kaschabek 2012).

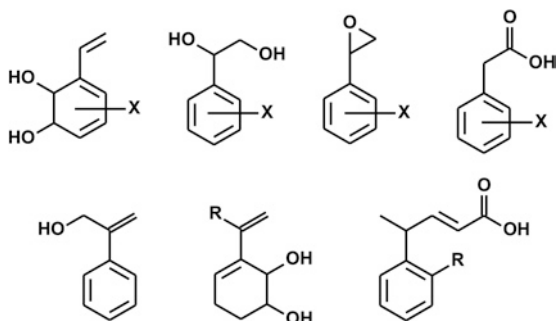
2.4 Biotransformation of Substituted Styrene Compounds

Besides styrene itself, several microorganisms are capable to metabolize or even use substituted styrene compounds or oligomers as sole source of carbon and energy (Higashimura et al. 1983; Oelschlägel et al. 2014a, b; Omori et al. 1974; Tuschii et al. 1977; Warhurst et al. 1994a, b).

For example, α -methyl and β -methylstyrene can serve as substrates for bacteria (Omori et al. 1974; Warhurst et al. 1994a). Bestetti and coworkers (1989) were able to confirm observations earlier made on these biotransformations. *P. putida* strain MST was also isolated with α -methylstyrene as sole source of carbon and energy. Thus two different initial attacks on the styrene derivative have been postulated since 2-phenyl-2-propen-1-ol and 1,2-dihydroxy-3-isopropenyl-3-cyclohexene were determined from the culture broth (Fig. 2.4). No evidence for the side-chain oxidation and respective enzymes were determined as had been described earlier for another *Pseudomonas* strain (Baggi et al. 1983). But, the intermediates detected, which were hydroxylated at the aromatic ring, indicate the presence of a more unspecific degradation pathway (compare with Sect. 2.2.3). Furthermore, *R. rhodochrous* NCIMB 13259 is able to grow on α -methylstyrene as a sole carbon source but not on β -methylstyrene (Warhurst et al. 1994a). As for styrene, the strain might possess a direct ring cleavage route in order to activate and transform α -methylstyrene.

The biodegradation of halogenated styrenes has not been described so far. But, the bacterial conversion of, especially, chlorinated styrene's into various intermediates is possible (Hudlicky et al. 1993; Oelschlägel et al. 2014a). First, *P. putida* 39D and its toluene dioxygenase which is probably involved in the direct ring hydroxylation have been employed to convert styrene and chlorostyrenes into corresponding

Fig. 2.4 Metabolites determined from biotransformations of styrene, its derivatives, and oligomers. *X* halogen atom or hydrogen, *R* methyl or hydrogen



diols (Fig. 2.4) (Hudlicky et al. 1993). No further breakdown has been described and therefore the chlorostyrenes serve not as carbon source for strain 39D. Another possible biotransformation has been revealed by several other studies which demonstrated that SMOs are able to epoxidize halogenated styrenes (reviewed by Montersino et al. 2011; see Sects. 4.1 and 5.1). Additionally, the *p*-cymene monooxygenase obtained of *P. putida* F1 converts 4-chlorostyrene into the 4-chlorostyrene oxide (Nishio et al. 2001). More recently, it has been shown to take advantage of the whole side-chain oxidation pathway in order to convert halogenated styrenes into corresponding phenylacetic acids (Oelschlägel et al. 2014a). Several microorganisms (e.g., *R. opacus* 1CP and *P. fluorescens* ST) can be used to convert styrene and substituted derivatives. Surprisingly, it has been found that even phenylacetic acid was overproduced from styrene and released into the fermentation medium. But, if halogenated styrenes were applied, no further utilization of respective halogenated phenylacetic acids was observed. Therefore, halogenated styrene derivatives were not biodegraded, but metabolites of bioconversions were enriched (Fig. 2.4).

Styrene oligomers can be degraded by soil microorganisms to some extent as reported by Tuschii and coworkers (1977). The dimer disappears rapidly from mixed cultures and a degradation of up to 70 % was determined. But, already the trimer appeared to be a poor substrate since less than 15 % could be degraded, whereas higher oligomers were not transformed by the soil microorganisms. During this study, the strain *Alcaligenes* sp. 559 was isolated from the consortium mentioned and found to utilize such styrene oligomers as sole carbon source. Further, the strain 559 was able to use biphenyl and *trans*-stilbene, but not the monomer styrene. The latter is a clear indication that the microbial degradation of styrene oligomers does not involve enzymes of the styrene-catabolic pathways described above. Later, another strain described as *Pseudomonas* sp. 419 was also found to degrade styrene dimers (Higashimura et al. 1983).

2.5 Styrene Transformation in Humans

The monomer styrene is mainly taken up via lungs, skin, or rarely by an oral application (Hartmans 1995; Khaksar and Ghazi-Khansaris 2009; Rueff et al. 2009; Warhurst and Fewson 1994). It is rapidly distributed among the organs

and usually attacked at the vinyl side-chain to yield styrene oxide prior further transformation (Rueff et al. 2009). The respective enzyme involved in epoxidation is a cytochrome P450 monooxygenase (CYP). And it is supposed that the reactive epoxide formed is mainly causing the (geno)toxic effects related to styrene exposure. This metabolite can be hydrolyzed by EH yielding styreneglycol which can be further oxidized by means of dehydrogenases. Thus mandelic acid and phenylglyoxylic acid are produced and represent the main urinary metabolites after styrene transformation takes place in human body. Also the transamination of these metabolites is possible which finally yields corresponding amino acids and phenylglycine. A similar biotransformation of styrene is carried out by some fungi (Fig. 2.1) (Braun-Lüllemann et al. 1997).

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