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Microbial Hydrocarbon Formation from Biomass

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Abstract Fossil carbon sources mainly contain hydrocarbons, and these are used on a huge scale as fuel and chemicals. Producing hydrocarbons from biomass instead is receiving increased attention. Achievable yields are modest because oxygen atoms need to be removed from biomass, keeping only the lighter carbon and hydrogen atoms. Microorganisms can perform the required conversions, potentially with high selectivity, using metabolic pathways that often end with decarboxylation. Metabolic and protein engineering are used successfully to achieve hydrocarbon production levels that are relevant in a biorefinery context. This has led to pilot or demo processes for hydrocarbons such as isobutene, isoprene, and farnesene. In addition, some non-hydrocarbon fermentation products are being further converted into hydrocarbons using a final chemical step, for example, ethanol into ethene. The main advantage of direct microbial production of hydrocarbons, however, is their potentially easy recovery because they do not dissolve in fermentation broth.

Keywords Yields, Product recovery, Gaseous products, Isoprenoids

Contents

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1 Introduction

Hydrocarbons are organic compounds consisting entirely of hydrogen and carbon. They are mostly used as combustion fuels, usually in hydrocarbon mixtures such as gasoline, diesel, and jet fuel. They can also be used for the synthesis of other chemicals, for the synthesis of polymers, as lubricants, as solvents, or as propellants for aerosol sprays, for example.

The present industrial production of hydrocarbons and their mixtures is almost entirely based on fossil resources such as natural gas, petroleum, and coal, which largely consist of hydrocarbons. These can be used as fuel. Oil refineries are used on a huge scale to split petroleum into more valuable and less valuable fractions, and to convert the latter as much as possible into more useful components. Highly selective catalysts are used to obtain transportation fuels, such as gasoline, kerosene, and diesel, which are mixtures of hydrocarbons with properties in a certain range. Similarly, pure hydrocarbons are obtained, such as ethene, propene, and styrene, the monomers of the most important synthetic polymers. Natural gas and coal can also be processed instead of being directly combusted.

Hydrocarbons can also be produced from biomass. Currently, such renewable production is only in exceptional cases competitive with petrochemical production, but it can be assumed that the competitiveness will increase in the future.

This chapter treats the (potential) microbial production of hydrocarbons in a biorefinery context, thus using biomass or one of its components as feed material. For the most common types of biomass and upstream processing, the main microbial feed components are carbohydrates, particularly glucose. However, microorganisms might also convert lipids and proteins into hydrocarbons. Microbial lignin conversion is more difficult, requiring aerobic conditions and leading to degradation into CO_2 and water rather than formation of hydrocarbons. Instead, products from thermochemical processing of lignin-containing biomass, such as syngas and pyrolysis oil, may also be funneled into the central carbon metabolism of microorganisms and subsequently be converted into products such as hydrocarbons. Phototrophic organisms can do the same, using CO_2 and water as feed components. However, the metabolic pathways shown in this chapter are limited to the actual formation reactions of hydrocarbons from central metabolites, assuming fermentable carbohydrates as the available feed material.

Many non-hydrocarbon products of microbial metabolism can be converted into hydrocarbons using follow-up chemistry. This is largely left outside the scope of this chapter, but a few such chemical conversions are mentioned.

2 Achievable Reaction Yields

In the context of developing a biorefinery concept, upper limits for achievable product yields should be made early on to determine the economic potential of such a biorefinery. The price of fermentable biomass ($/kg) divided by the achievable hydrocarbon mass yield on this biomass gives the minimum feedstock contribution to the bio-hydrocarbon production costs, and this should be significantly lower than the price of petrochemical hydrocarbon.

Biomass-based production, either (thermo)chemically or microbially, requires conversions that remove oxygen and trace elements such as present in biomass components. Biomass pretreatment and hydrolysis leads, for example, to glucose ($C_6H_{12}O_6$). This consists of 0.53 g/g of oxygen, which obviously limits the maximum yield of hydrocarbon to 0.47 g/g. However, oxygen removal in the form of O_2 is thermodynamically unfavorable at fermentation conditions if no sunlight (photosynthesis) or other external energy source is used. A situation that may be thermodynamically feasible (depending on the hydrocarbon) is that oxygen atoms are removed from glucose in the form of CO_2 and H_2O. Then, stoichiometric calculations [1] lead to maximum yields ranging from 0.27 g/g for methane (CH_4; the least oxidized hydrocarbon) to 0.36 g/g for naphthalene ($C_{10}H_8$; an example of a more oxidized hydrocarbon). For oxygen-containing fermentation products, the maximum yields achievable with ideal stoichiometries are less modest [1].

Real yields of hydrocarbon on glucose are even lower, because at least a small portion of the glucose is used for cell growth, and because enzymatic reactions to achieve stoichiometrically ideal metabolic pathways from glucose to hydrocarbon are unknown or not yet in place, or such pathways are thermodynamically constrained. A reaction that consumes O_2, such as shown for some entries in Table 1, may lead to a thermodynamically favorable pathway, but at the expense of the maximum achievable yield.

If glucose is to be converted to transportation fuel, it is also important to consider the fraction of the fuel value of glucose that can be retained in the hydrocarbon products. As the co-products CO_2 and H_2O have no fuel value, the fuel value of glucose may be largely transferred to the hydrocarbon products, with some side-product formation and small entropic losses [12].

3 Product Recovery

In an industrial processing context, extracellular production is often preferred, because it eliminates the need for cell disruption, hence enabling cell reuse and product recovery during fermentation. Even recovery of extracellular microbial products is often challenging because they need to be separated from a large amount of water which contains numerous other solutes. For hydrocarbons the situation can

Table 1 Enzymatic reactions leading to hydrocarbons

Enzymes	Substrates	Products	Hydrocarbon product examples	References
Decarboxylase	R-COOH	R-H; CO_2	Styrene	McKenna and Nielsen [2]
Aldehyde deformylase (ADO)	Aldehyde; O_2; NADPH	Alkane; Formate; H_2O	Hydrocarbons down to propane	Schirmer et al. [3] and Menon et al. [4]
P450 fatty acid decarboxylase (OleT)	Fatty acid; H_2O_2	Terminal alkene; CO_2; 2 H_2O	Heptadec-1-ene	Rude et al. [5]
Non-heme Fe^{II} oxidase (UndA)	Fatty acid; O_2; 2 e^-; 2 H^+	Terminal alkene; CO_2; 2 H_2O	C_9–C_{13} terminal alkenes	Rui et al. [6]
P450 oxidative decarbonylase (CYP4G)	Fatty aldehyde; O_2; NADPH	Hydrocarbon; CO_2; H_2O	Heptadecane	Qiu et al. [7]
Synthases	Branched 1-pyrophospho-2-alkene; Water	Branched-1,3-alkadiene; Pyrophosphate	Isoprene, farnesene	Whited et al. [8] and George et al. [9]
Mevalonate diphosphate decarboxylase[a]	3-Hydroxy-3-methylalkanoic acid; ATP	2-Methylalkene; CO_2; ADP; Phosphate	Isobutene	Gogerty and Bobik [10] and Rossoni et al. [11]

[a]Actually a kinase phosphorylating the substrate to 3-methyl-3-phosphocarboxylic acid followed by spontaneous decarboxylation [11]

Table 2 Aqueous solubilities of hydrocarbons at 25 °C, 1 atm

Hydrocarbon	Solubility (g/kg)	Reference
Methane	0.023	Clever and Young [13]
Ethene	0.13	Hayduk [14]
Isoprene	0.61	Shaw [15]
Styrene	0.25	Shaw [16]
Dodecane	3.4×10^{-6}	Shaw et al. [17]

be much simpler because the formed concentrations can easily surpass the aqueous solubilities shown in Table 2 if a reasonable production organism is available.

Gaseous hydrocarbons escape from fermentation broth together with the formed CO_2, water vapor and other (trace) impurities, in many cases requiring further processing steps. In the well-established biogas process, for example, H_2S is typically removed by (reactive) absorption, eventually followed by CO_2 removal by absorption or pressure swing adsorption [18]. In the last few years, product recovery through the gas phase has been showcased as one of the key features of DuPont's isoprene process [8].

If liquid hydrocarbons are formed by fermentation, the hydrocarbon amount exceeding its solubility forms a (light) organic phase if the hydrocarbon is excreted by the cells. Product extraction during fermentation by means of solvent addition

has been of almost standard use – at least at laboratory scale – to overcome product toxicity and volatility in the production of monoterpenes and short-chain alkanes [19]. With or without solvent addition, however, emulsion formation is likely to occur [20] as has been reported in the production of farnesene [21].

In the subsequent sections, the status of microbial production of specific hydrocarbons is treated. This builds on previous reviews [1, 19, 22], but some significant new developments have taken place.

4 Methane

Methane is the only hydrocarbon that is produced as primary metabolite by natural microorganisms. During anaerobic digestion, mixed cultures of microorganisms convert in several steps various biomass components into carbon dioxide, hydrogen, and organic acids, mainly acetic acid. Finally, acetic acid is converted into equimolar amounts of methane and carbon dioxide during methanogenesis by methanogenic archea. On the basis of theoretical stoichiometry, the maximum yield of methane per glucose equivalent is 0.27 g/g, but a somewhat different yield is obtained with biomass. The produced biogas also does not contain equimolar amounts of methane and carbon dioxide because some of the CO_2 dissolves in the liquid effluent.

Anaerobic digestion can be used for converting biomass under nonsterile conditions, whereas the produced gas can be easily recovered. Commercial operation requires relatively simple equipment and operations, but the low productivity, typically below 0.03 g/L/h), leads to large vessels. Biogas can be directly used as fuel, or used for heat and electricity generation in a combined heat and power plant, or upgraded to natural gas quality, that is, to a methane concentration of at least 90%. The state-of-the-art has recently been described [18].

5 Ethene

In a biorefinery context, producing ethene (ethylene) from biomass at commercial levels would require a route that approaches the theoretical yield limit of ethene on glucose of 2 mol/mol (0.31 g/g). Currently, this is achieved on a commercial scale by using ethanol fermentation and subsequent acid-catalyzed dehydration of ethanol to ethene [23]. Such a process requires low biomass prices to be competitive [24].

It has been claimed that the ethanol dehydration might be performed enzymatically instead [25, 26]. Ideally, such an enzyme activity would be incorporated in an ethanol-producing microorganism, potentially leading to a direct conversion of 1 mol glucose into 2 mol ethene. However, it is not clear whether the equilibrium of the dehydration reaction would be favorable at fermentation conditions, and if the pathway would be usable.

Another hypothetical pathway leading potentially to the desired stoichiometry would be via acrylic acid. Fermentative production of acrylic acid has been studied [1], and enzymatic decarboxylation of acrylic acid to ethene has been suggested. In some organisms this would be because of a side activity of pyruvate decarboxylase Enzymatic decarboxylation of acrylic acid to ethene has been suggested to occur in some organisms because of a side activity of pyruvate decarboxylase [27]. The proof for this has been considered to be weak [28]. Besides, acrylic acid is more valuable than ethene, which makes the decarboxylation unattractive.

Proven pathways for biological ethene formation rely on the natural formation of ethene by plants in small amounts for signaling functions such as stimulation of fruit-ripening [29]. Three such pathways are known, but none are useful for reaching yields of ethene on glucose above 0.12 g/g [30], and they can be considered unattractive for large-scale ethene synthesis. The final enzymatic steps of the natural pathways might be considered for finding higher-yield synthetic pathways, and are given here. 1-Aminocyclopropane-1-carboxylate is converted by an oxygenase into ethene, cyanide, CO_2, and water, using oxidation of L-ascorbate to L-dehydroascorbate [31]. *(S)*-2-Oxo-4-thiomethylbutyric acid is decomposed into ethene, methanethiol, and CO_2 by an NADH-Fe(III) oxidoreductase, which activates O_2 [32]. The ethene-forming enzyme (EFE) occurring in *Penicillium digitatum* and *Pseudomonas syringae* catalyzes several reactions, amongst others a conversion of 2-oxoglutarate with O_2 into ethene, CO_2, and water [33]. Heterologous expression of the *efe* gene from *P. syringae* has led to ethene production in a number of hosts [34].

6 Other Gaseous Hydrocarbons

Many gaseous hydrocarbons have been found to be formed by microorganisms [22, 35]. For example, ethanogenesis can be carried out by methanogenic archaea under the conditions required for methanogenesis, using enrichment cultures from some deep lake sediments [36]. Small amounts of ethane have been detected. The mechanisms of biogenic ethane formation and the biochemistry of the microorganisms involved in this process have to be elucidated before any reasonable conversion can be developed.

Traces of propane have been found with ethane under the same conditions [36], and the propane metabolic pathways for natural biosynthesis are also not known. However, cyanobacteria contain aldehyde deformylating oxygenases (ADOs; formerly aldehyde decarbonylases), which can be used to engineer metabolic pathways for alkane biosynthesis. ADO catalyzes O_2-dependent conversion of aldehyde into alkane and formate in the presence of an electron donor. Native aldehyde carbon chain lengths range from C_{16} to C_{18}, but with the shorter chain aldehydes that are not encountered in native cyanobacteria, activity has also been observed [3, 37]. Co-expression of ADO with a butyraldehyde-producing pathway in

Escherichia coli led to accumulation of up to 32 mg/L of propane [4, 37]. This proof-of-principle probably leads to significant follow-up activities on this topic.

Traces of propene (propylene) are formed in aerobic cultures of *Rhizopus* strains of many different types of organisms. The responsible enzyme was not identified [38, 39]. Aerobic formation of propene from isobutyraldehyde by rabbit cytochrome P-450 has been demonstrated however [40]. It is assumed that monooxygenase activity with NADPH as electron donor leads to propene and formic acid [41]. The required isobutyraldehyde might be produced from glucose [1], but no such propene pathway seems to be pursued.

Biological formation of isobutene (isobutylene; 2-methylpropene) has been studied since the 1970s using strains such as the yeast *Rhodotorula minuta* [42]. Isobutene is formed by reductive decarboxylation of isovalerate, which is catabolically derived from L-leucine [43, 44]. Studies on the responsible enzyme point to a cytochrome P450 monooxygenase that is involved in hydroxylating benzoate [45]. The highest observed production was merely 0.45 mg/L/h, and it is not clear how a pathway via isovalerate can be used to obtain a commercially interesting yield of isobutene on glucose.

Dehydrative decarboxylation of 3-hydroxyisovalerate into isobutene is more useful [46]. This conversion was reported as side-activity of mevalonate diphosphate decarboxylase, MVD [10, 47]. Variation of precursor and enzyme might lead to various alkenes. The putative MVD from *Picrophilus torridus* is one of the most efficient wild-type enzymes in the patent applications in this field. It turned out to be no decarboxylase but a kinase, which uses ATP to phosphorylate mevalonate to mevalonate-3-phosphate. At a much lower rate it can convert 3-hydroxyisovalerate to an unstable 3-phosphate intermediate that undergoes consequent spontaneous decarboxylation to form isobutene [11]. 3-Hydroxybutyrate was similarly phosphorylated, but the phosphorylated product seems too stable to decarboxylate into propene. 3-Hydroxypropanoate was not converted by the kinase [11].

Using metabolic and protein engineering, such enzymatic activities are used to obtain commercially interesting production of isobutene and other alkenes [47, 48]. Other patent applications of Global Bioenergies describe isobutanol dehydration to isobutene as a side activity of engineered oleate hydratase and other hydratases [25, 26]. If feasible, an attractive metabolic pathway might be obtained [46]. Similarly, isopropanol might be dehydrated to propene and but-3-en-1-ol and but-3-en-2-ol might be dehydrated to butadiene.

Metabolic pathways to 1,3-butadiene have also been formulated in other patents [49, 50]. The final reaction should again be dehydration of a butenol or butanediol isomer, potentially via a phosphate intermediate. Pathways to the required precursors have been described in the same patent applications.

In November 2014, Global Bioenergies produced isobutene by direct fermentation for the first time in pilot scale (www.global-bioenergies.com). Moreover, Global Bioenergies has announced successful lab-scale production of butadiene by direct fermentation of glucose (www.global-bioenergies.com/communiques/141126_pr_en.pdf). Other companies, however, focus on fermentative production of alcohols, which can be very efficient [1], followed by acid-catalyzed dehydration

to the corresponding alkenes. Acid-catalyzed dehydration of isopropanol to propene, for example [51], is easy. Braskem pursues commercial bio-based propene production using fermentative ethanol production, followed by chemical conversion into ethene, dimerization, and metathesis [52].

7 Isoprene

Isoprene, or 2-methyl-1,3-butadiene, is naturally formed by various microorganisms, plants, and animals. Massive amounts, estimated at 600 million tons/year, are emitted by plants into the atmosphere [53]. Formation occurs via elimination of pyrophosphate from 3,3-dimethylallyl pyrophosphate by the key enzyme isoprene synthase. The precursor is formed in the mevalonate (MEV) and the methylerythritol phosphate (MEP) pathways. The MEV pathway is used by archaea, some bacteria, and most eukaryotes (including the yeast *Saccharomyces cerevisiae*), whereas the MEP pathway is used in most bacteria (including *E. coli*) and green algae. Both pathways occur in plants. Genencor (now DuPont) and Goodyear have genetically engineered *E. coli* for the production of isoprene through fermentation of glucose [8, 54]. The MEP pathway might have an isoprene yield on glucose of up to 0.30 g/g, whereas the MEV pathway is limited to 0.25 g/g according to the theoretical net overall reaction [8]:

$$1.5\ \text{Glucose} + 2O_2 \rightarrow \text{Isoprene} + 4CO_2 + 5\ H_2O$$

Still, the better known MEV pathway was selected for strain development. Isoprene has an atmospheric boiling point of 34 °C and it is hardly water soluble, so it was emitted with the fermentor off-gas at a concentration of around 18%, together with the formed CO_2, and potentially with unconverted O_2. Further downstream processing was required for recovering isoprene with 99.5% purity for polymerization to rubber [55]. The amount of isoprene that was collected corresponded to 60 g/L in the fermentation broth, at a productivity of 2 g/L/h and yield on glucose was 0.11 g/g [8]. Isoprene from such fermentation has been used by Goodyear in the production of prototype tires. Various other companies are active in this field [56]. A calculation indicated that costs for the bio-isoprene would be slightly higher than the actual market price of its fossil counterpart, but might become competitive [56].

8 Isoprenoids

Isoprenoids are a highly diverse set of compounds that are built from at least one C_5 isoprene unit via head-to-tail addition of the key intermediate isopentenyl diphosphate (IPP) [57], and hence their biosynthesis resembles that of isoprene. The last

decade has seen fast developments in the metabolic engineering of this pathway. This interest originated from medical applications, in particular through the development of the antimalarial drug precursor artemisinic acid, leading to production on industrial scale by Sanofi [58]. Currently, the pathway receives enormous attention for its potential in the generation of replacements for diesel and jet fuel, as well as in replacing plant based flavors and fragrances. The focus has been mainly on monoterpenes (C_{10}), sesquiterpenes (C_{15}), and a few higher terpenoids ($>C_{20}$). The state-of-the-art has recently been comprehensively reviewed, for example, by Cuellar and van de Wielen [19] and Schrader and Bohlmann [59]. Monoterpenes such as pinene and limonene have been shown to have, after hydrogenation, properties similar to the light end of traditional kerosene aviation fuel, making them suitable as drop-in replacements or as enrichment for hydrogenated sesquiterpenes such as farnesane [9, 60]. Limonene is also an important precursor to several pharmaceutical and commodity chemicals. For example, hydroxylation of limonene results in perillyl alcohol, a potential anti-cancer agent [61]. Monoterpenes have been reported to be highly toxic to the microbial cell, interacting with cellular and mitochondrial membranes and dismantling membrane integrity. This is currently being overcome by engineered cell export systems and through extractive fermentations.

Sesquiterpenes have seen important developments in the last few years. The farnesene isomers, a group of natural sesquiterpenes including β-farnesene (7,11-dimethyl-3-methylene-1,6,10-dodecatriene), lead to farnesane upon catalytic hydrogenation. Farnesane is already being produced at commercial scale by Amyris, and it has been certified as a diesel and jet fuel replacement in blends up to 35% and 10%, respectively (www.amyris.com; [9]). In *S. cerevisiae*, the mevalonate pathway enzymes, converting acetyl-CoA into farnesyl diphosphate, are overexpressed, and the latter intermediate is converted into *(E)*-β-farnesene and diphosphate. This final reaction is catalyzed by a farnesene synthase, because of expression of the corresponding gene sequence from *Artesemia annua*. Improvement of the *S. cerevisiae* strain and the fermentation conditions has led to titers of 104.3 g/L with a productivity of 0.70 g/L/h as disclosed by Amyris in 2010 [9]. Further developments are being made downstream in the pathway, broadening the product spectrum. Several companies are currently active, in particular for flavor and fragrance applications (e.g., Evolva, Firmenich, Amyris, and Isobionics). Production of valencene, its derivative nootkatone, and santalene in the milligrams per liter range has been reported [62, 63].

Higher isoprenoids or terpenoids ($>C_{20}$) are currently applied in cosmetics, pharmaceuticals, and nutraceuticals. Their (over)production has been demonstrated in several microorganisms, resulting mostly in intracellular accumulation. Microbial production of the triterpenoid ($C_{30}H_{50}$) squalene has reached commercial scale by Amyris and their first skin-care product was launched in May 2015 (www.amirys.com).

9 Liquid Linear Alkanes and Alkenes

Microbial formation of linear alkanes and alkenes often involves metabolic pathways to fatty acids. Such biosynthesis has been well-studied in bacteria and yeast, in particular for the production of free fatty acids (FFAs), fatty acid alkyl esters, and hydrocarbons. Recent advances in this area have been reviewed [19].

Fatty aldehydes are often the direct precursor of long-chain alkanes or alkenes. Depending on the enzyme type, the carbonyl group can be released as formate, CO, or CO_2.

Aldehyde-deformylating oxygenases (ADOs) are ferritin-like nonheme dimetal-carboxylate enzymes that catalyze alkane formation from aldehyde in many cyanobacteria under O_2 consumption and formate formation [64, 65]. Incorporating an alkane biosynthesis pathway from cyanobacteria in *E. coli* led to a mixture of uneven C_{13} to C_{17} alkanes and alkenes [3]. The pathway coexpresses genes for acyl-ACP (acyl carrier protein) reductase and an ADO enzyme from the cyanobacterium *Synechococcus elongatus* converting aldehyde to alkane, up to 0.3 g/L, mostly extracellular. The process is currently under optimization by REG Life Sciences (formerly LS9; www.reglifesciences.com), and pilot-plant fermentations (1,000 L scale) have already been performed [66]. By altering the FFA pool – either by pathway engineering or medium supplementation – more recent studies [67, 68] have resulted, respectively, in larger fractions of even alkanes (mostly C_{14} and C_{16}) and a broader product spectrum, including linear and branched alkanes and alkenes. The titers are, however, still in the order of a few milligrams per liter.

Decarbonylases that release CO from aldehydes, forming alkanes, have been shown in vertebrates, insects, plants, and algae [69]. *Arabidopsis thaliana* fatty aldehyde decarbonylase potentially releases CO [20]. Recently, it was engineered with the pathway for fatty acid biosynthesis and fatty aldehyde formation in *E. coli*. This led to titers up to 0.3 g/L of FFAs, ranging from C_8 to C_{16}, and to up to 0.6 g/L alkanes, mostly nonane and dodecane [70]. According to the authors, this mixture is suitable for petrol replacement.

In some insects, hydrocarbons are formed from fatty aldehydes using cytochrome P450 enzymes that consume NADPH and O_2, and release $NADP^+$, CO_2, and water [7]. Unsaturation in the fatty aldehyde chain leads to alkenes rather than alkanes.

Terminal linear alkenes (α-olefins, very useful as chemical intermediates) are formed from fatty acids in some eukaryotes and bacteria. The enzyme from *Jeotgalicoccus* sp. ATCC 8456, OleT, is a cytochrome P450, and consumes H_2O_2. It forms CO_2 and 2 equiv. of H_2O when abstracting hydrogens from the α and β positions of the fatty acid [5]. Light-driven in situ generation of H_2O_2 improves the conversion [71]. In *Pseudomonas aeruginosa*, a non-heme oxidase has been found that decarboxylates fatty acid to alkene (1-undecene). In this UndA enzyme, O_2 forms an $Fe^{IV}{=}O$ species that needs to be regenerated to Fe^{II} using a reducing agent [6].

In many bacteria, linear alkene production occurs via condensation of two carboxylic acids to a dione, followed by reductions and dehydrations. This yields nonterminal alkenes such as 14-heptacosene [72, 73]. It is not yet clear how the final step to alkene proceeds.

10 Aromatic Hydrocarbons

The aromatic hydrocarbon for which fermentative production from biomass is best developed is styrene, also known as phenylethene [2]. In an L-phenylalanine overproducing *E. coli* host, glucose conversion into styrene was achieved by the co-expression of phenylalanine ammonia lyase from *Arabidopsis thaliana* and *trans*-cinnamate decarboxylase from *S. cerevisiae*. In shake flask cultures, up to 0.26 g/L styrene accumulated, close to the styrene toxicity threshold (determined as 0.3 g/L). Upon periodic stripping, the equivalent of 0.56 g/L styrene was produced, whereas 0.84 g/L was produced by in situ solvent extraction [74]. Genetic engineering approaches are required to obtain commercially attractive productivities and yields. The potential to use engineered *S. cerevisiae* instead of *E coli* for styrene production has been shown [75]. A technoeconomic evaluation showed that styrene production from sugars might be competitive in the case where styrene would form its own organic phase which could be decanted [76]. Formation of traces of styrene from forest waste biomass has been shown using wild type *Penicillium expansum* [77].

Biosynthesis of other aromatic hydrocarbons might be possible. For example, toluene is formed during anaerobic degradation of phenylalanine by bacteria such as *Tolumonas auensis* [78]. Phenylalanine is assumed to be converted into phenylacetate, which is then decarboxylated [79]. The responsible enzymes are not known, and fermentative production of toluene from glucose does not seem to be pursued.

Naphthalene, another aromatic hydrocarbon, is used by termites as fumigant [80], and traces of naphthalene are emitted by the endophytic fungus *Muscodor vitigenus* when grown on agar plates with glucose [81]. In neither case is it clear how this naphthalene might be formed. Compounds such as benzene, toluene, and *o*- and *m*-xylene are also excreted in traces by endophytic fungi [82, 83], and by plants [84]. This has led to advocating the use of fungi for producing so-called mycodiesel [85].

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